Critical Reviews in Biochemistry and Molecular Biology, 37(6):375–536 (2002)

Biochemistry and Molecular Biology of Gelatinase B or Matrix Metalloproteinase-9 (MMP-9)

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Abbreviations

$A\beta_{1\text{-}40}$, amyloid-β peptide (1-40); **ALS**, amyotrophic lateral sclerosis; **AP-1/2**, activating protein-1/2; **APMA**, 4-aminophenylmercuric acetate; **ARDS**, Acute Respiratory Distress Syndrome; **ASK1**, apoptosis signal-regulating kinase 1 or MEKK5; **ATF**, activating transcription factor; **Bcl-2**, B-cell lymphoma proto-oncogene-2; **bFGF**, basic fibroblast growth factor; **c-**, cellular; **CAM**, cell adhesion molecule; **cAMP**, adenosine-3',5'-cyclic monophosphate; **Cdc42**, cell division cycle 42 protein; **ConA**, concanavalin A; **cPLA2**, cytoplasmatic phospholipase A2; **CREB**, cAMP response element binding protein; **CTAP-III**, connective tissue activating peptide-III; **DAG**, diacylglycerol; **E1A**, adenovirus type A early gene; **EAE**, experimental autoimmune encephalomyelitis; **ECM**, extracellular matrix; **EGF**, epidermal growth factor; **ELISA**, enzyme-linked immunosorbent assay; **ERB**, EGF receptor B; **ERK**, extracellular signal-regulated kinase; **ET-1**, endothelin-1; **Ets**, E26 avian erythroblastosis virus proto-oncogene; **FAK**, focal adhesion kinase; **fMLP**, formyl-methionyl-leucyl-phenylalanine; **GAS**, gamma interferon-activated sequence; **GCP-2**, granulocyte chemotactic protein-2; **Gp**, glycoprotein; **GRO-α**, growth-related oncogene-α; **GTPase**, guanosine triphosphatase; **HGF/SF**, hepatocyte growth factor/scatter factor; **HIV**, human immunodeficiency virus; **HTLV-1**, human T-cell lymphotropic virus-1; **ICAM-1**, intercellular cell adhesion molecule-1; **IFN**, interferon; **IFNAR**, IFN-α/β receptor; **ISRE**, IFN-stimulated regulatory element; **IKK**, IκB kinase; **IL-**, interleukin-; **JAK**, Janus-protein tyrosine kinase; **JNK/SAPK**, c-jun N-terminal kinase/stress-activated protein kinase; **LFA-1**, lymphocyte function-associated antigen-1; **LPS**, lipopolysaccharide; **LTB4**, leukotriene B4; **MAPK**, mitogen-activated protein kinase; **MAPKAPK**, MAPK-activated protein kinase; **MAPKK**, MAPK kinase; **MAPKKK**, MAPKK kinase; **MEK/MKK**, MAPK/ERK kinase; **MEKK**, MEK kinase; **MMP**, matrix metalloproteinase; **MPO**, myeloperoxidase; **MT-MMP**, membrane-type MMP; **NF-κB**, nuclear factor-kappa B; **NGAL**, neutrophil gelatinase B-associated lipocalin; **NIK**, NF-κB-inducing kinase; **PDGF**, platelet-derived growth factor; **PEA3**, polyomavirus enhancer A-binding protein-3; **PECAM**, platelet endothelial cell adhesion molecule; **PF-4**, platelet factor-4; **PGE2**, prostaglandin E_2; **PI-3K**, phophatidylinositol 3-kinase; **PKA**, protein kinase A; **PKC**, protein kinase C; **PMA**, phorbol 12-myristate 13-acetate; **PTK**, protein tyrosine kinase; **Ras**, rat sarcoma oncogene; **RBE**, retinoblastoma binding element; **RGD**, Arg-Gly-Asp; **RTK**, receptor tyrosine kinase; **SCC**, squamous cell carcinoma; **SDF-1**, stromal-cell derived factor-1; **SDS-PAGE**, sodium dodecyl sulfate polyacryla-

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mide gel elctrophoresis; **SH**, Src homology; **Smad**, similar to mothers against decapentaplegic homolog; **SNP**, single nucleotide polymorphism; **Sp1**, stimulating protein-1; **SPF**, specific pathogen-free; **Src**, Rous sarcoma protooncogene; **STAT**, signal transducer and activator of transcription; **TAK1**, TGF-β activated kinase-1; **TFPI**, tissue factor pathway inhibitor; **TGF-β**, transforming growth factor-β; **TIE**, TGF-β1 inhibitory element; **TIMP**, tissue inhibitor of metalloproteinases; **TNF-α**, tumor necrosis factor-α; **t-PA**, tissue-type plasminogen activator; **TPA**, 12-O-tetradecanoyl-phorbol-13-acetate; **TRE**, TPA response element; **u-PA**, urokinase; **v-**, viral; **VCAM-1**, vascular cell adhesion molecule-1; **VEGF**, vascular endothelial growth factor; **VLA-4**, very late antigen-4; YB, Y-box protein

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ABSTRACT: The matrix metalloproteinases (MMPs) form an enzyme family of which gelatinase B (MMP-9) represents the largest and most complex member. We focus here on the biochemical properties, regulation, and functions of gelatinase B. The tight regulation of gelatinase B activity is highly complex and is established at five different levels. The transcription of the gelatinase B-gene depends on various *cis*-elements in its gene promotor and is induced or repressed by a large variety of soluble factors, including cytokines, growth factors, and hormones and by cellular contacts acting through specific signaling pathways. The specific regulation of its secretion occurs in cells storing gelatinase B in granules. After secretion, progelatinase B must be activated through an activation network. The enzyme activity is further regulated by inhibition and by other mechanisms, such as fine-tuning and stabilization by glycosylation. The ability of gelatinase B to degrade components of the extracellular matrix and to regulate the activity of a number of soluble proteins confers an important role in various physiological and pathological processes. These include reproduction, growth, development, inflammation, and vascular and proliferative diseases.

1. BIOCHEMISTRY OF GELATINASE B

1.1. Structure of Gelatinase B

1.1.1. Primary Structure

Gelatinase B belongs to the family of matrix metalloproteinases (Nagase and Woessner, 1999; Yong *et al*., 2001; Egeblad and Werb, 2002). The MMP-family is characterized by the presence of conserved protein domains: a prodomain, an active domain and a Zn^{2+}-binding domain. All MMPs, except MMP-7 and MMP-26, contain an additional carboxyterminal hemopexin-domain. Membrane-type MMPs (MT-MMPs) are bound to membranes through a carboxyterminal hydrophobic anchor. In the cases of MT-MMP-1, –2, –3, and –5 this hydrophobic anchor is a transmembrane domain and these MT-MMPs contain also a short intracellular domain, whereas MT-MMP-4 and –6 are membrane-anchored through a glycosyl-phosphatidyl-inositol anchor (Itoh *et al*., 1999b; Kojima *et al*., 2000). Gelatinases have a gelatin-binding fibronectin domain, composed of three fibronectin-repeats, inserted between the active-site domain and the Zn^{2+}-binding domain, and gelatinase B contains an additional Ser/Thr/Pro-rich collagen type V domain in a suggested hinge region (Figure 1). In comparison with the other MMPs, gelatinase B is structurally one of the most complex members of the family (Cuzner and Opdenakker, 1999; Opdenakker *et al*., 2001a; Opdenakker *et al*., 2001b; Van den Steen *et al*., 2001).

The Zn^{2+}-binding domain of human gelatinase B contains the conserved sequence AHEXGHXXGXXH, in which the three histidines are responsible for the coordination of the catalytic Zn^{2+}-ion. Together with the active domain, the Zn^{2+}-binding domain forms the active site and is essential for the enzymatic activity. In the human proenzyme, the fourth ligand of the Zn^{2+} is $cysteine_{86}$ of the conserved sequence PRCGXPD in the prodomain. This prodomain is removed by various types of proteolysis or is distorded by substrate binding

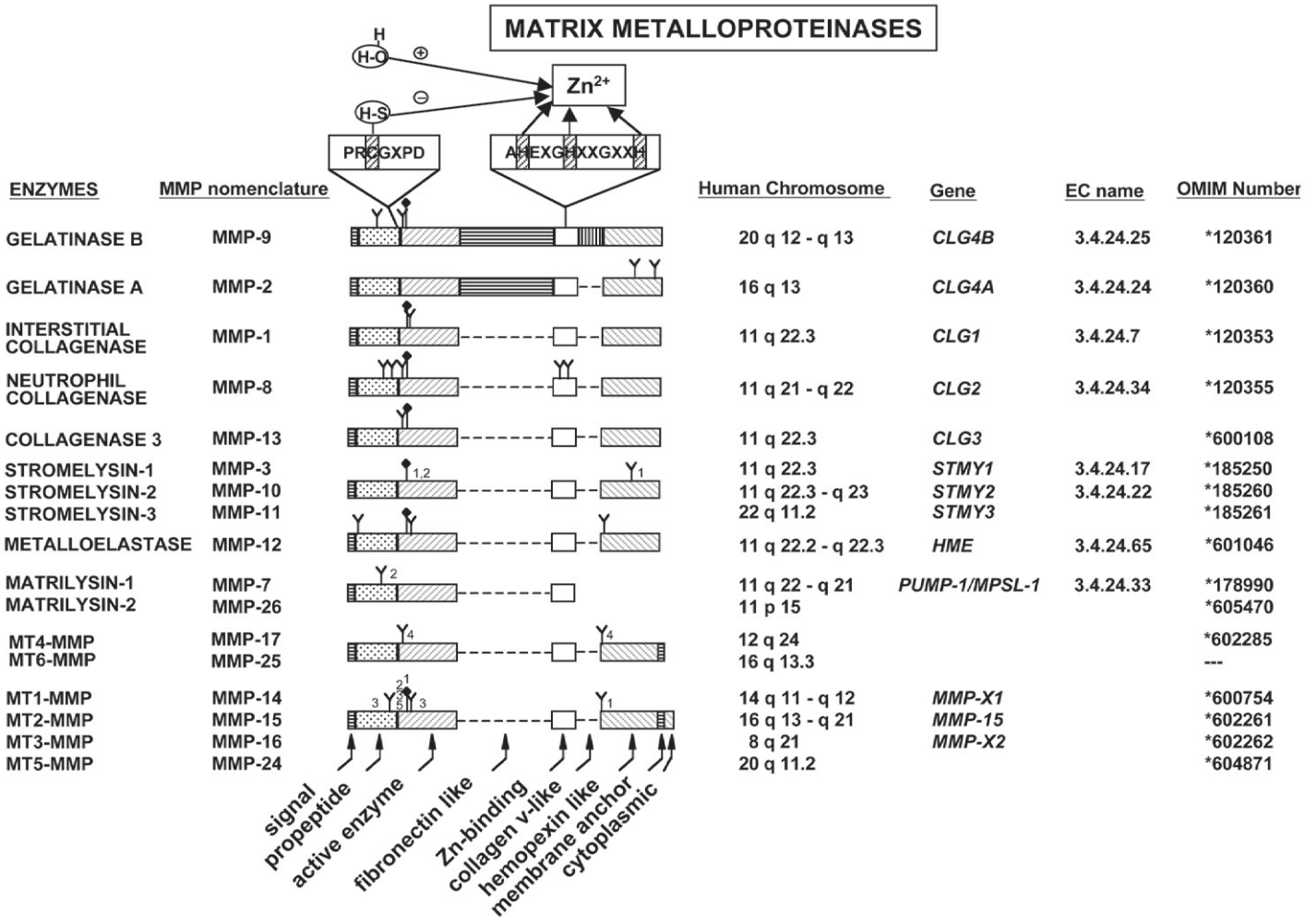

FIGURE 1. Domain structure of MMPs. MMPs are multidomain enzymes with a prodomain, an active domain, a Zn^{2+}-binding domain and a hemopexin domain (except MMP-7 and MMD-26). Additionally, membrane-bound MMPs contain a membrane anchor and a cytoplasmic domain at the carboxyterminus. Gelatinases contain a gelatin-binding fibronectin-like domain and gelatinase B contains also a serine-, threonine-, and proline-rich collagen type V-like domain, which is theoretically an attachment site for multiple O-linked glycans. N-glycosylation sites, one of which is conserved among most MMPs (↑), are indicated with a Y-symbol. The numbers aside the respective N-linked attachment sites indicate the type of stromelysin, matrilysin, or membrane-type MMP involved. The consensus sequence of the conserved sequon is indicated on top in one-letter code for amino acids.

(Bannikov *et al*., 2002) to yield the active enzyme through the cysteine-switch mechanism (Van Wart and Birkedal-Hansen, 1990) (see also further). The function of the hemopexin-domain is less clear. It was suggested to play a role in the substrate specificity of collagenases. However, for gelatinase B it was clearly shown that it is important for the binding of the tissue inhibitors of metalloproteinases (TIMPs) (see further). The fibronectin type II repeats in gelatinases are responsible for binding to gelatin, laminin, and collagens type I and IV. This is in contrast to collagenases, where the collagen-binding capacity is conferred by the hemopexin domain. The activation status of gelatinase B is also important, because pro-gelatinase B binds with higher affinity to collagen type I and to gelatin, and with lower affinity to collagen type IV compared with activated gelatinase B (Allan *et al*., 1995).

The primary structures of the human (Huhtala *et al*., 1991) and mouse gelatinase B genes (Masure *et al*., 1993) have been compared. The gene sequences and the distribution of 13 exons and introns are similar (Masure *et al*., 1993). By analysis of the relation of gelatinase B with other MMPs at the genomic and cytogenetic levels (Figure 1), it is evident that many human MMP genes colocalise on chromosome 11, subband q22. Remarkably, all human membrane-type MMPs are at different chromosomes and also gelatinase B occupies a unique site on human chromosome 20, subband q12-q13 (St. Jean *et al*., 1995). The mouse gelatinase B gene has been localized on mouse chromosome 2 (Leco *et al*., 1997).

1.1.2. Posttranslational Modifications

Human natural gelatinase B is a heavily glycosylated MMP (Rudd *et al*., 1999; Mattu *et al*., 2000; Van den Steen *et al*., 2001). It contains three possible attachment sites for N-linked glycans, one of which is situated in the prodomain. The two others are located in the active domain. One of the N-glycosylation sites in the active domain (N_{108}YS) is conserved in most of the MMPs (Figure 1), also in different species. Gelatinase B contains multiple O-linked sugars, most of which are probably located in the collagen type V-like domain. This domain contains repeats of T/SXXP, which constitute attachment sites for the multiple O-linked glycans. These O-linked glycans may serve to extend this protein domain into a "bottle-brush" structure, as was described for mucins. The structures of both N- and O-linked glycans were determined for a yeast-expressed recombinant mouse gelatinase B (Van den Steen *et al*., 1998b) and for natural gelatinase B from human neutrophils (Rudd *et al*., 1999; Mattu *et al*., 2000; Royle *et al*., 2002).

In many cell types, gelatinase B is produced as a mixture of monomers and homodimers. In addition, neutrophils produce a third form, a covalent complex of gelatinase B with neutrophil gelatinase B-associated lipocalin (NGAL) (Kjeldsen *et al*., 1993). These different forms of gelatinase B can be visualized on non-reducing SDS-PAGE or by gelatin zymography (Figure 2). After reduction only monomers are present, indicating that the homo- and heterodimerization occurs through disulfide-bonding (see also further). Recently, it was shown that

covalent dimerization has an influence on the activation by stromelysin-1 (MMP-3) (Olson *et al*., 2000). An analysis of the different forms by gel filtration chromatography indicates that noncovalent interactions between gelatinase B molecules may be present, possibly resulting in the formation of non-covalent trimers (unpublished results). Truncation of the proenzyme also occurs in neutrophils, resulting in the removal of the first 8 to 10 amino acid residues from the propeptide (Masure *et al*., 1991). The complete removal of the propeptide, resulting in enzyme activation, is discussed in a separate section.

1.1.3. Model of the Three-Dimensional Structure of Gelatinase B

The three-dimensional structure of recombinant forms of the catalytic domain of gelatinase B, without hemopexin and collagen type V domain, has been determined recently by X-ray crystallography (Rowsell *et al*., 2002; Elkins *et al*., 2002). However, the crystal structure of the complete enzyme has not yet been obtained, probably because its heterogeneity is an obstacle for crystallization. A number of other recombinant MMPs or MMP domains have already been crystallized and their structures analyzed. Particularly interesting is the structural determination of a recombinant full-length gelatinase A (MMP-2) variant (Morgunova *et al*., 1999). Gelatinase A is the MMP with the closest sequence similarity to gelatinase B, and the structure of its catalytic domain is very similar to that of gelatinase B (Rowsell *et al*., 2002). Therefore, it was possible to generate a model of gelatinase B starting from the crystallography data of gelatinase A, by adding the N-linked glycans and the Ser/Thr/Pro-rich domain with a number of O-linked glycans (Mattu *et al*., 2000) (Plate 1*). The prodomain is bound in the catalytic cleft by several hydrogen bonds and, as expected, the conserved Cys is coordinated with the catalytic Zn^{2+} ion. The fibronectin type II repeats each contain four Cys residues, of which the first forms a disulfide bridge with the third Cys and the second with the fourth Cys. The repeats were found to possess a hydrophobic pocket, which probably accounts for the binding to gelatin. The hemopexin domain consists of a four-bladed propeller-structure in which the first blade is linked to the fourth blade by a disulfide bridge. In gelatinase A, the first two blades are oriented to the catalytic domain and form hydrogen bonds with the fibronectin domain. In gelatinase B, the hemopexin domain is probably spaced from the other domains by the Ser/Thr/Pro-rich domain, because protein domains with clustered O-glycans often form rigid structures (see also further). The other two blades are turned away from the catalytic site and are probably the binding site for TIMP-2 in gelatinase A (or TIMP-1 in the case of gelatinase B). In gelatinase B, two additional cysteines are present (Cys_{449} in the collagen type V domain and Cys_{615} in the hemopexin domain) that may be free and that may be responsible for the covalent homodimerization or heterodimerization with NGAL. The latter was observed with the gelatinase B isolated from neutrophils (see further). In the partial crystal structures of most MMPs, a second Zn^{2+}-ion has been found and considered to play a structural role.

* Plate 1 appears following page 382.

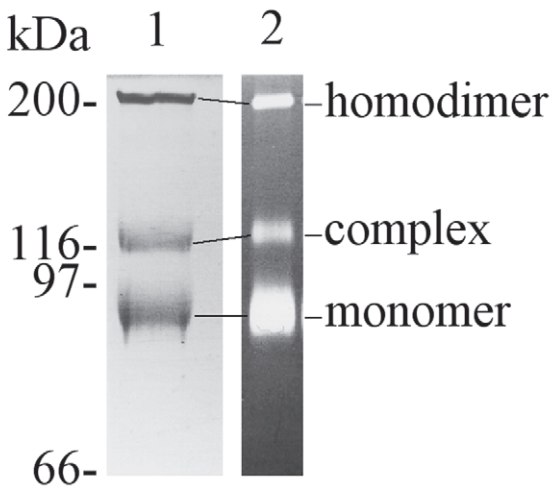

FIGURE 2. SDS-PAGE and zymography analysis of purified gelatinase B from granulocytes. Gelatinase B from human granulocytes occurs as monomers, disulfide-linked homodimers, and disulfide-linked complexes of gelatinase B with neutrophil gelatinase B-associated lipocalin (NGAL). After purification, the different forms can be visualized by nonreducing SDS-PAGE and protein staining (lane 1). Gelatinase B forms in unpurified crude samples can be visualized by gelatin substrate zymography followed by Coomassie Blue staining (lane 2).

However, by analysis of gelatinase B in solution it is now clear that only one Zn^{2+}-ion is present in the full-length enzyme (Kleifeld *et al*., 2000).

1.2. Methods and Reagents for the Detection and Purification of Gelatinase B

The most sensitive and widely used assay for the detection of gelatinase B is substrate zymography. This technique was developed in the late 1970s (Granelli-Piperno and Reich, 1978) for the study of plasminogen activators (Heussen and Dowdle, 1980; Roche *et al*., 1983) and later adapted for other proteases, including gelatinases (Nakagawa and Sakata, 1986; Masure *et al*., 1990). Polyacrylamide gels are copolymerized with gelatin and, after electrophoresis of the protein samples in the presence of sodium dodecylsulphate (SDS), the gels are washed to remove the SDS. Overnight incubation at 37°C allows the reactivated enzyme to degrade "in gel" the copolymerized substrate. Subsequently, the gels are stained with Coomassie Brilliant Blue and the areas where the gelatin substrate has been degraded by gelatinases develop into white spots on a blue background (Figure 2). The advantages of this method are its picogram sensitivity (Kleiner and Stetler-Stevenson, 1994) and the possibility of discriminating between various forms of gelatinases on the basis of different molecular weights. This allows the differentiation between gelatinases A and B, between proforms (which become activated by the action of detergents, see further) and active species, and between monomers and covalently linked homo-

or heterodimers. However, noncovalent complexes, such as complexes of gelatinase B with TIMP, are readily dissociated by the SDS and are separated during the electrophoresis. The quantification of zymolytic activity is possible by densitometry scanning and comparison with titration curves of standard preparations (Masure *et al*., 1990). Technically more difficult are the *in situ* zymographic techniques for semiquantitative detection of gelatinase B in tissue sections (Galis *et al*., 1995).

Polyclonal antisera and monoclonal antibodies are now also available, allowing the detection of human gelatinase B immunoreactivity in sensitive ELISA assays (Paemen *et al*., 1995) or by immunohistochemistry (Grillet *et al*., 1997; van den Oord *et al*., 1997). Inhibitory monoclonal antibodies recognize, by definition, the activated form of the enzyme and therefore make it possible to discriminate between pro- and activated forms of gelatinase B (Paemen *et al*., 1995).

Recently, similar observations were made after the generation of a panel of monoclonal antibodies against mouse gelatinase B by a combinatorial approach of hybridoma technology and immunization of gelatinase B-deficient mice with recombinant mouse enzyme (unpublished results).

For the analysis of gelatinase B activity in biological samples, a range of techniques was developed. Fluorescence assays are based on the use of quenched fluorogenic peptide substrates (Knight *et al*., 1992) or on the flow cytometric analysis of fluorescent-labeled gelatin coated on polystyrene microspheres (St-Pierre *et al*., 1996). A disadvantage of the fluorimetric method is low specificity; these assays do not allow the distinction between gelatinases A and B, enzyme complexes and other gelatinolytic activities. By replacement of radioactive gelatin as a substrate in earlier studies (Harris and Krane, 1972) with, for example, biotinylated gelatin, efficient nonisotopic methods became available. With the use of standard preparations of gelatinase B (with known activity), these assays have become also useful for the discovery and titration of gelatinase B inhibitors (Paemen *et al*., 1996).

Recently, an immunocapture assay for MMPs was described by Hanemaaijer *et al*. (Hanemaaijer *et al*., 1998). Specific MMPs (e.g., gelatinase B) are immobilized to solid phase on a plate coated with a specific (polyclonal or monoclonal) antibody. The activity of the captured enzyme is detected using a genetically engineered prourokinase containing a sequence recognized by MMPs. Cleavage of this sequence results in the activation of prourokinase. Urokinase activity is detected by the conversion of a chromogenic substrate. The specificity of this type of assay is based and depends on the antibody used, and its sensitivity is in the low nanogram range.

Gelatinase B was initially purified using classic multistep chromatographic procedures, using, for example, concanavalin-A-sepharose chromatography followed by ion-exchange chromatography and gel filtration (Rantala-Ryhanen *et al*., 1983). One of the disadvantages is the breakdown of the rather unstable enzyme during these time-consuming purification schemes. Stability can be increased by working at 4°C and by the addition of Ca^{2+}. However, long purification procedures need to be avoided. The enzyme is also highly unstable in acid conditions, rendering classic immuno-affinity chromatography of

little use. Therefore, gelatin-Sepharose affinity chromatography with the use of 10% dimethylsulfoxide (DMSO) in the elution buffer (Hibbs *et al*., 1987) is a method of choice, because it is a rapid single-step procedure and highly selective for gelatinases. Gelatinase A can be separated from gelatinase B, for example, by concanavalin-A-sepharose chromatography (Rantala-Ryhanen *et al*., 1983), and TIMPs can be removed using gel filtration in the presence of SDS (Van Ranst *et al*., 1991). To obtain pure gelatinase B, the enzyme source is of considerable importance. For instance, neutrophilic granulocytes do not produce gelatinase A or TIMP-1, in contrast to most other gelatinase B-producing cell types. Neutrophils are also particularly suited as a source, because they contain large amounts of gelatinase B in the tertiary granules (Masure *et al*., 1991). In addition, these granules are rapidly released (20 min) after a secretagogue stimulus, making long induction times unnecessary. This is also beneficial for the stability and integrity of the enzyme ([Van den Steen *et al*., 2000] and data not shown). Complexes of gelatinase B with NGAL can be removed by immuno-affinity chromatography using antibodies against NGAL (Van den Steen *et al*., 2000), and monomeric gelatinase B can be separated from dimers by gel filtration chromatography (unpublished results).

The DNAs of human and mouse gelatinase B were cloned in 1989 (Wilhelm *et al*., 1989) and in 1993 (Masure *et al*., 1993), respectively. This not only yielded information on the complete primary structure of gelatinase B, but also allowed recombinant expression. For instance, human gelatinase B was expressed in a baculovirus-based expression system (George *et al*., 1997) and recombinant mouse gelatinase B was produced in a yeast (*Pichia pastoris*) (Masure *et al*., 1997).

1.3. Substrate Specificity

The substrate-specificity of various MMPs has been reviewed recently (Imper and Van Wart, 1998). We discuss here only the major features of the substrate-specificity of gelatinase B. The specificity depends on the primary sequence of the substrate, because, in general, endoproteases possess a clear preference for peptide sequences that can bind in the groove of the catalytic site. However, the three-dimensional conformation and accessibility of the cleavage site in a substrate is important too. Finally, exosites on the enzyme may bind to distant sites on the substrate and also promote hydrolysis.

The primary sequence specificity has been analyzed using short synthetic peptide substrates derived from the sequence Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln (corresponding to sites P4-P3-P2-P1-P1'-P2'-P3'-P4') (Netzel-Arnett *et al*., 1993) (Table 1). It was found that shortening the peptide beyond P3-P3' strongly reduces the hydrolysis rate. This indicates that interaction of the peptide with the respective S3-S3' sites on the enzyme is required. The most clear amino acid preference was found at the P1 and P1' sites. Only small amino acids (Gly, Ala, Pro) are well tolerated at the P1 site. A possible explanation for this comes from the crystal structure of the catalytic site of MMP-8 (Bode *et al*., 1994), where the S1 site is rather undefined and unable to bind larger amino acids. At the P1' site, a clear preference is noticed for hydrophobic residues (Ile, Leu, Tyr,

Table 1. Primary sequence substrate specificity of different subsites of gelatinase B

Cleavage of a synthetic peptide							
P4	P3	P2	P1	P1'	P2'	P3'	P4'
					Phe_{385}		
		Leu_{294}			Trp_{238}		
		Met_{182}			Leu_{238}		Thr_{161}
		Tyr_{153}			Arg_{196}	Ala_{141}	His_{121}
		Val_{106}	Ala_{110}	Met_{179}	Gln_{127}	Ser_{131}	Ala_{111}
Gly_{100}	**Pro_{100}**	**Gln_{100}**	**Gly_{100}**	**Ile_{100}**	**Ala_{100}**	**Gly_{100}**	**Gln_{100}**
	$Ala_{9.4}$	Arg_{85}	Pro_{46}	Tyr_{95}	Hyp_{12}	Val_{49}	
	$Asn_{<5}$	Hyp_{15}	His_{44}	Leu_{79}		Arg_{45}	
		Asp_{9}	Tyr_{30}	Val_{25}		Met_{35}	
			Glu_{29}	Gln_{20}			
			Phe_{26}	$Ser_{<5}$			
			Gln_{13}	$Arg_{<5}$			
			Met_{12}	$Trp_{<5}$			
			Leu_{9}	$Pro_{<5}$			
			$Val_{<5}$	$Glu_{<5}$			

The used reference peptide is indicated in bold and the relative preferences of each subsite of the enzyme for the indicated single amino acid substitutions in this reference peptide are in subscript (Netzel-Arnett *et al.*, 1993).

Met), which is in accordance with the general observation that the S1' site of MMPs consists of a more or less deep hydrophobic pocket (Imper and Van Wart, 1998; Rowsell *et al.*, 2002). At the P2 subsite, hydrophobic residues are also preferred, and the preferences of the other sites are indicated in Table 1. In another study, using a phage display peptide library, the preference for hydrophobic residues at P1' and of Pro at P3 was confirmed, and a relative preference for Arg at P2 was documented (Kridel *et al.*, 2001).

A limited number of physiological substrates have been described, together with the respective cleavage sites (Table 2). The fibronectin domain probably plays a role as exosite by binding the collageneous substrates and thereby increasing the hydrolysis efficiency, as shown for gelatinase A (Murphy *et al.*, 1994). The best-known substrates for gelatinase B are denatured collagens (gelatins). The cleavage sites of gelatinase B in type II gelatin, a major substrate of neutrophil gelatinase B in rheumatoid arthritis, has been analyzed in detail only recently (Van den Steen *et al.*, 2002). This study confirmed the findings with synthetic peptide substrates, but also provided new important clues. The comparison of the cleavage sites in type II gelatin shows that gelatinase B cleaves collagen type II always after a Gly-residue (P1 position). At P1', a clear preference for hydrophobic residues exists and at P3 for Pro. Only 4% of the amino acids at P2' are found to be posttranslationally modified (mostly by hydroxylation of prolines) and 71% of the residues at

Table 2. Comparison of the cleavage sites by gelatinase B in natural substrates

Substrate	P6	P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'	P5'	P6'
Gelatin	A	P^{OH}	**G**	**P**	Q	**G**	F_{42}	Q	**G**	N	$\mathbf{P^{(OH)}}$	**G**
type II[a]	P	P^{OH}	**G**	**P**	Q	**G**	A_{93}	R	**G**	F	$\mathbf{P^{OH}}$	**G**
	P	M	**G**	**P**	R	**G**	L_{147}	P^{OH}	**G**	E	R	**G**
	R	T	**G**	**P**	A	**G**	A_{159}	A	**G**	A	R	**G**
	P	A	**G**	A	A	**G**	A_{162}	R	**G**	N	D	**G**
	A	R	**G**	**P**	E	**G**	A_{207}	Q	**G**	P	R	**G**
	S	P^{OH}	**G**	**P**	A	**G**	A_{225}	A	**G**	N	$\mathbf{P^{OH}}$	**G**
	P	L	**G**	**P**	K	**G**	Q_{273}	T	**G**	E	$\mathbf{P^{OH}}$	**G**
	E	R	**G**	**P**	S	**G**	L_{357}	A	**G**	P	K^{OHEX}	**G**
	L	A	**G**	**P**	K^{OHEX}	**G**	A_{363}	N	**G**	D	$\mathbf{P^{OH}}$	**G**
	L	P^{OH}	**G**	A	R	**G**	L_{381}	T	**G**	R	$\mathbf{P^{OH}}$	**G**
	P	P^{OH}	**G**	**P**	Q	**G**	A_{414}	R	**G**	Q	$\mathbf{P^{OH}}$	**G**
	L	P^{OH}	**G**	A	P^{OH}	**G**	L_{447}	R	**G**	L	$\mathbf{P^{OH}}$	**G**
	A	P^{OH}	**G**	**P**	S	**G**	F_{483}	Q	**G**	L	$\mathbf{P^{OH}}$	**G**
	L	V	**G**	**P**	R	**G**	E_{519}	R	**G**	F	$\mathbf{P^{OH}}$	**G**
	A	Q	**G**	**P**	P*	**G**	L_{567}	Q	**G**	M	$\mathbf{P^{OH}}$	**G**
	P	P*	**G**	**P**	A	**G**	A_{618}	N	**G**	E	K^{OHEX}	**G**
	P	P*	**G**	**P**	A	**G**	F_{654}	A	**G**	P	$\mathbf{P^{OH}}$	**G**
	P	Q	**G**	**P**	T	**G**	V_{699}	T	**G**	P	K^{OHEX}	**G**
	A	Q	**G**	**P**	P^{OH}	**G**	A_{714}	T	**G**	F	$\mathbf{P^{OH}}$	**G**
	P	P*	**G**	**P**	Q	**G**	L_{795}	A	**G**	Q	R	**G**
	L	A	**G**	Q	R	**G**	I_{801}	V	**G**	L	$\mathbf{P^{OH}}$	**G**
	P	V	**G**	**P**	P*	**G**	L_{846}	T	**G**	P	A	**G**
	L	K	**G**	H	R	**G**	F_{954}	T	**G**	L	Q	**G**
Procollagen	G	G	N	F	A	A	Q_{157}	M	A	G	G	F
type II	G	N	F	A	A	Q	M_{158}	A	G	G	F	D
Propeptide[b]	G	A	Q	L	G	V	M_{174}	Q	G	P	M	
	A	Q	L	G	V	M	Q_{175}	G	P	M		
Collagen α1(V) [c]	P	P	**G**	P	P	**G**	V_{440}	V	**G**	P	Q	**G**
Collagen α2(V) [c]	P	P	**G**	P	P	**G**	L_{446}	R	**G**	E	R	**G**
Collagen α1(XI) [c]	P	P	**G**	P	G	**G**	V_{440}	V	**G**	P	Q	**G**
MBP[d]	P	V	V	H	F	F	K_{91}	N	I	V	T	P
	K	G	R	G	L	S	L_{111}	S	R	F	S	W
	L	S	L	S	R	F	S_{115}	W	**G**	A	E	**G**
	G	G	R	A	S	D	Y_{134}	K	S	A	H	K
ET-1[e]	H	V	V	**P**	Y	**G**	L_{33}	G	S	P	R	S
Plg[f]	S	V	V	A	P	P	P_{466}	V	V	L	L	P
$Aβ_{1-40}$[g]	G	A	I	I	G	L	M_{35}	V	**G**	G	V	V
	E	V	H	H	Q	K	L_{17}	V	F	F	A	E
	G	S	N	K	G	A	I_{31}	I	**G**	L	M	V
	I	G	L	M	V	**G**	G_{38}	V	V	I	A	T
Link protein[h]	H	D	R	A	I	H	I_{17}	Q	A	E	N	**G**
SDF-1[i]	-	-	K	**P**	V	S	L_5	S	Y	N	C	P
IL-8[j]	A	V	L	**P**	R	S	A_7	K	E	L	R	C
GRO-α[j]	A	S	V	A	T	E	L_7	R	C	Q	C	L
	N	I	Q	S	V	N	V_{28}	K	S	P	G	P
CTAP-III[j]	E	S	L	D	S	D	L_{14}	Y	A	E	L	R
	S	D	L	Y	A	E	L_{18}	R	C	M	C	I
	Y	A	E	L	R	C	M_{21}	C	I	K	T	T
	G	I	H	**P**	K	N	I_{34}	Q	S	L	E	V

Table 2 (continued)

	N	I	Q	S	L	E	V_{39}	I	**G**	K	G	I
	V	E	V	I	A	T	L_{55}	K	D	G	R	K
	A	P	R	I	K	K	I_{73}	V	Q	K	K	L
Aggrecan[k]	V	D	I	D	E	N	F_{342}	F	**G**	V	G	**G**
TFPI[l]	E	L	P	**P**	L	K	L_{21}	M	H	S	F	C
MBL	S	Q	**G**	P	K	**G**	Q_{72}	K	**G**	D	R	**G**
variants[m]	E	V	K	L	A	N	M_{81}	E	A	E	I	N
	L	Q	**G**	P	P^{OH}	**G**	K_{46}	L	**G**	P	**P^{OH}**	**G**

Residues showing a large consensus are shown in bold. At P1', hydrophobic residues are indicated in italic and the position in the sequence is indicated in subscript. For the cleavage of collagen types V and XI, rabbit gelatinase B was used. P^{OH}, hydroxyproline; K^{OHex}, glycosylated hydroxylysine; P*, Pro with probable but uncertain hydroxylation. $A\beta_{1-40}$, amyloid peptide-β(1-40); CTAP-III, connective tissue activating peptide-III; ET-1, endothelin-1; GRO-α, growth related oncogene-α; IL-, interleukin-; MBL, mannose binding lectin; MBP, myelin basic protein; Plg, plasminogen; SDF-1, stromal cell-derived factor-1; TFPI, tissue factor pathway inhibitor. [a] Van den Steen *et al.*, 2002; [b] Fukui *et al.*, 2002; [c] Niyibizi *et al.*, 1994; [d] Proost *et al.*, 1993a; [e] Fernandez-Patron *et al.*, 2001; [f] Patterson and Sang, 1997; [g] Backstrom *et al.*,1996; [h] Nguyen *et al.*, 1993; [i] McQuibban *et al.*, 2001; [j] Van den Steen *et al.*, 2000; [k] Fosang *et al.*, 1992; [l] Belaaouaj *et al.*, 2000; [m] Butler *et al.*, 2002.

P5'. This is significantly different from the 40% modifications on residues in front of Gly, as was observed in collagen type II in general. Recently, the aminoterminal prodomain of procollagen type II was shown to be cleaved by gelatinase B (Fukui *et al.*, 2002). Collagen type V can also be cleaved by gelatinase B (Hibbs *et al.*, 1987); however, it is unclear whether gelatinase B can cleave native full-length type IV collagen (Wilhelm *et al.*, 1989; Mackay *et al.*, 1990; Okada *et al.*, 1992). Other extracellular matrix substrates include aggrecan (Fosang *et al.*, 1992), link protein (Nguyen *et al.*, 1993), and elastin (Senior *et al.*, 1991). In man, gelatinase B was also shown to degrade myelin basic protein, resulting in the release of encephalitogenic peptides (Proost *et al.*, 1993a). Another autoantigen, BP180 or type XVII collagen, which is important in bullous pemphigoid, is a membrane-bound hemidesmosome protein, containing an extracellular collagenous domain. This domain is a substrate for gelatinase B, and its cleavage may be at the basis of the observed blistering in bullous pemphigoid (Ståhle-Bäckdahl *et al.*, 1994) (see also further). In addition to these structural components, other gelatinase B substrates are functional proteins. These include the serine protease inhibitors α_1-proteinase inhibitor, α_1-antitrypsin, and α_1-antichymotrypsin (Desrochers *et al.*, 1992; Sires *et al.*, 1994), substance P (Backstrom and Tökés, 1995), galactoside binding proteins CBP30 and CBP35 (Mehul *et al.*, 1994; Ochieng *et al.*, 1994), interleukin(IL)-2 receptor-α (Sheu *et al.*, 2001), transforming growth factor-β (TGF-β) (Yu and Stamenkovic, 2000), and tissue factor pathway inhibitor (TFPI) (Belaaouaj *et al.*, 2000). It was also found that gelatinase B can degrade amyloid-β peptide (1-40) ($A\beta_{1-40}$), with possible implications for the pathogenesis of Alzheimer's disease (Backstrom *et al.*, 1996). The proinflammatory cytokine IL-1β is activated (Schönbeck *et al.*, 1998), and angiostatin is cleaved from plasminogen by gelatinase B (Cornelius *et al.*, 1998; Patterson and Sang, 1997). Also, pro-tumor necrosis factor-α (proTNF-α) can be processed by gelatinase B, although

with lower efficiency than by other MMPs (Gearing *et al*., 1994). Finally, recently we have shown that gelatinase B is able to process CXC-chemokines. In particular, gelatinase B cleaves the six aminoterminal amino acid residues from IL-8(1-77), generating the more active IL-8(7-77) and thereby providing a positive feedback loop, because IL-8 is able to induce the release of gelatinase B from neutrophils (Van den Steen *et al*., 2000). Other CXC chemokines, such as connective tissue activating peptide-III (CTAP-III), growth-related oncogene-α (GRO-α), platelet factor-4 (PF-4) (Van den Steen *et al*., 2000), and stromal-cell derived factor-1 (SDF-1) (McQuibban *et al*., 2001) are inactivated. A positive feedback loop was also demonstrated between neutrophil gelatinase B and endothelin-1 (ET-1), because ET-1 (1-32) induces the release of gelatinase B from neutrophils and gelatinase B cleaves big ET-1 into ET-1(1-32) (Fernandez-Patron *et al*., 2001).

2. REGULATION OF GELATINASE B ACTIVITY

Gelatinase B activity is regulated by five mechanisms: gene transcription, secretion, activation, inhibition, and glycosylation. Various studies and overviews have already dealt with the regulation of gelatinase B production and summarized the inducers that stimulate gelatinase B gene expression (Cuzner and Opdenakker, 1999; Opdenakker *et al*., 2001b; Yong *et al*., 2001; Egeblad and Werb, 2002). Here we have taken the approach to compare the gene promoters of gelatinase A and B to explain the differences in protein expression. It has been established by now that gelatinase B expression, that is, the production of proenzyme by cells, is not necessarily concomitant with gene transcription and mRNA synthesis. In most cell types, gene transcription of gelatinase B is inducible, and after translation the enzyme is immediatley secreted through the normal secretory pathway. It is secreted usually together with variable amounts of its inhibitor TIMP-1 and with the more constitutively produced gelatinase A. However, in the neutrophilic granulocyte, transcription of the gelatinase B gene occurs only in the latest stage of neutrophil development during which large amounts of the enzyme are stored in granules. Therefore, gelatinase B functions as a terminal differentiation marker of neutrophil development. The content of these granules is rapidly secreted after stimulation with specific secretagogues. In addition, neutrophils do not make gelatinase A or TIMPs, but are the only cell type to secrete a complex of gelatinase B with NGAL.

The literature on the regulation of gelatinase B activity is rather skewed toward gene transcription. Therefore, this aspect is covered first, by a broad comparison between gelatinases B and A. Alternatively to the general presentation, we describe the various steps influencing transcription, starting from the gene and the interacting transcription factors. Then the signal transduction pathways and the cytoplasmic adaptor mechanisms are summarized. The review of gene transcription closes with the cellular receptors and ligands that trigger transcription. Thereafter, the regulation by secretion, activation, inhibition, and glycosylation are discussed, also in proportion with the available literature.

2.1. Transcriptional Regulation of Gelatinase B and A

2.1.1. Functional cis-Elements in the Promoter/Enhancer Regions of the Gelatinase B and A Genes

The 2.2-kbp promoter sequence in the 5'-flanking region of the human gelatinase B gene (Figures 3 and 4), contains several consensus motifs for regulatory elements and resembles more the promoters of the interstitial collagenase (MMP-1) and stromelysin-1 (MMP-3) genes than that of the gelatinase A gene (Huhtala *et al*., 1990a; Huhtala *et al*., 1991). At position –29 a TATA motif-like sequence is located, but no CAAT motif is found in the gelatinase B gene promoter. A consensus sequence for the binding of nuclear stimulating protein-1 (Sp 1), also named GC box, is present at –563 bp relative to the transcriptional start site. More proximally, at position –54 bp, a retinoblastoma binding element (RBE) or GT box has been identified in human (Sato and Seiki, 1993; Himelstein *et al*., 1997) that is highly conserved throughout different species (Sato *et al*., 1993; Campbell *et al*., 2001) and is also recognized by Sp1. Three additional units of GGGG(T/A)GGGG sequence or GT boxes have been detected (Sato *et al*., 1993). A consensus sequence of a TGF-β1 inhibitory element (TIE) is located at –474 bp (Huhtala *et al*., 1991). Furthermore, the promoter contains (at least) four 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-responsive elements (TRE) or activator protein-1 (AP-1) binding sites that potentially can bind members of the c-Fos and c-Jun families of transcription factors. The AP-1 motif located at –79, and the more distal site at –209 bp, are also highly conserved in various mammalian species (Sato *et al*., 1993; Campbell *et al*., 2001). The former was shown to be essential and sufficient for basal and c-Jun/c-Fos-induced promoter activity in HT1080 cells and osteosarcoma OST cells (Sato and Seiki, 1993), and mutation of this site abolishes all promoter activity (Gum *et al*., 1997). Several sequences with homology to the polyomavirus enhancer A-binding protein-3 (PEA3), which are recognized by the products of the Ets-1 and Ets-2 proto-oncogenes (Sato and Seiki, 1993), can be found in the gelatinase B gene promoter (He, 1996). All three PEA3 sites, as shown in Figure 3, are situated in the region between positions –599 and –531, which has been reported to be required for basal activity of the gelatinase B gene promoter (Sato and Seiki, 1993). The PEA3 elements that are localized at –541 and –571 bp, are responsible for activation by the Ets-related protein E1AF (Higashino *et al*., 1995). The gelatinase B gene promoter region contains a nuclear factor-κB (NF-κB) motif at position –600 that matches the subtype p65 NF-κB binding site (Han *et al*., 2001) and is highly conserved throughout species (Sato *et al*., 1993; Campbell *et al*., 2001), and a second motif at –328 bp that matches the subtype p50 NF-κB binding site (Han *et al*., 2001). In addition, two AP-2 motifs and a microsatellite segment of alternating CA residues ($(CA)_n$) are conserved between the human and mouse promoters (Huhtala *et al*., 1991; Sato *et al*., 1993; Masure *et al*., 1993; He, 1996; Campbell *et al*., 2001). Conflicting studies debate the possible involvement of the $(CA)_n$ sequence in the regulation of gelatinase B

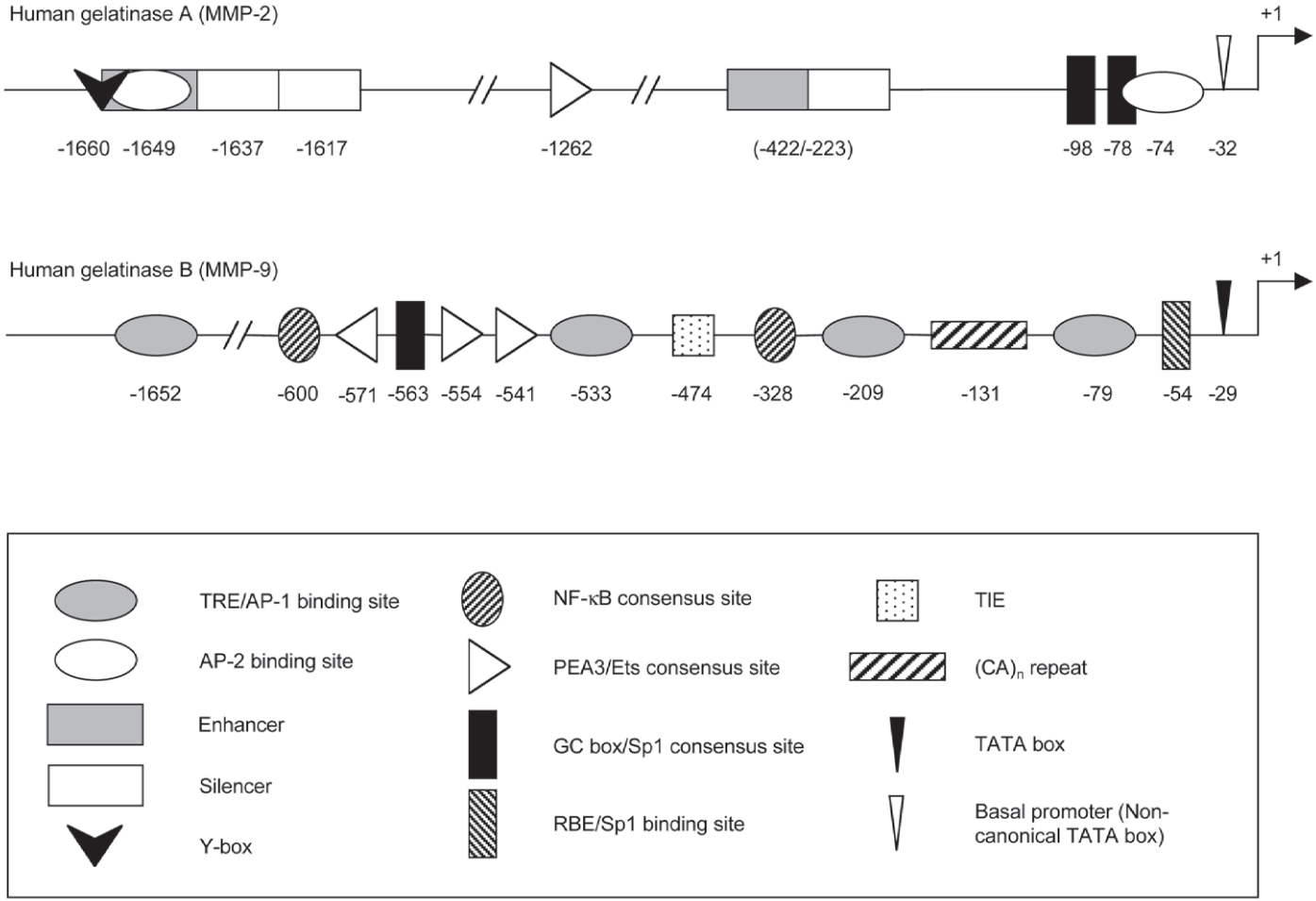

FIGURE 3. Major regulatory *cis*-elements in the promoter/enhancer regions of the gelatinase A and B genes. Although gelatinase A and gelatinase B are highly similar enzymes at the protein level, their transcriptional regulation is completely different. This is illustrated here by comparing the most important *cis*-regulatory elements in the gene promoters of both gelatinases, based on the sequence data and references in Figure 4 and Table 3. Only these elements with established functional importance in the response to polypeptide or hormone inducers are depicted, and their positions and sequences are printed in bold in Table 3. The 5'-end positions of the transcription factor binding sequences (5' → 3') are indicated as negative numbers.

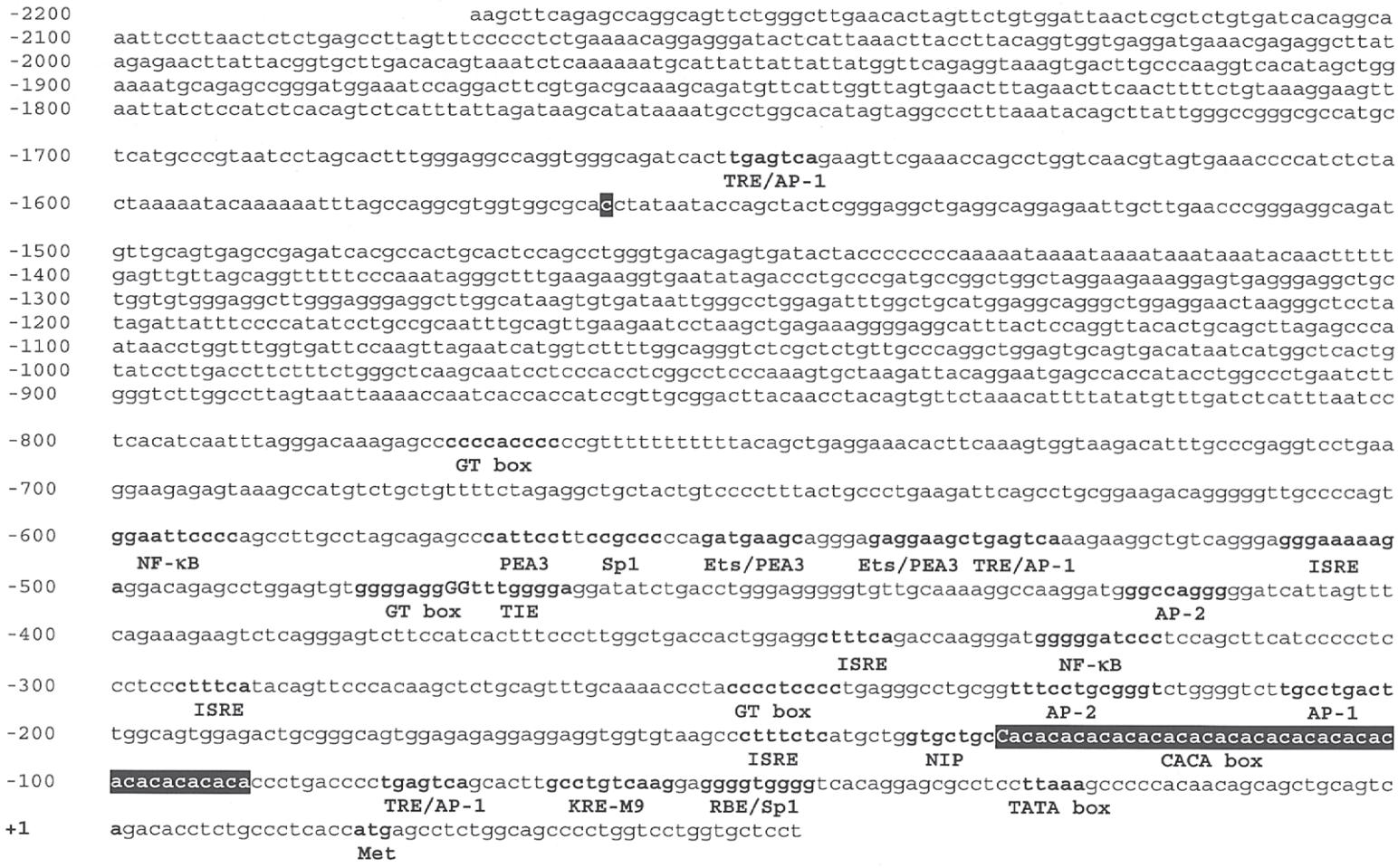

FIGURE 4. Nucleotide sequence of the gelatinase B gene promoter and transcription factor binding sites. The gelatinase B gene (Accession number D10051) determined by Huhtala *et al.* (Huhtala *et al.*, 1991) is used to locate the various consensus sites in the 5'-flanking region of the gelatinase B gene relative to the transcriptional initiation site. The latter is 19 bp upstream from the initiation codon (adg) for the amino acid methionine (Huhtala *et al.*, 1991; Sato and Seiki, 1993). Wherever two consensus sequences overlap, the overlapping bases are printed in uppercase letters. The single nucleotide polymorphism and the polymorphic $(CA)_n$ dinucleotide repeat region are shown as white letters in a black box. Abbreviations of the different elements are explained throughout the text.

gene expression (see below). In one study, it has been proven to be a transcription-activating domain in the gelatinase B gene (Himelstein *et al*., 1998), whereas in other studies no functional importance has been found. Furthermore, a KRE-M9 element (5'-GCCTGTCAAG-3') has been identified recently between positions –66 and –57 bp. This element differs in only one base from a potential AP-2 binding sequence in the human involucrin gene promoter, namely, the keratinocyte differentiation factor-1 regulatory element-4 (KRE-4). Together with the closely spaced AP-1 site, it is important for gelatinase B gene transcription upon stimulation with Ca^{2+} in human keratinocytes (Kobayashi *et al*., 2001).

Several *cis*-regulatory regions appear to act synergistically in basal and induced human gelatinase B gene transcriptional responses. The AP-1 element at –79 bp seems to be necessary but not sufficient for cytokine- and phorbol ester-enhanced gelatinase B gene transcription in fibrosarcoma cells and cooperates with NF-κB and Sp1 elements at positions –600 and –563, respectively (Sato and Seiki, 1993). Besides being absolutely required for upregulation of gelatinase B gene expression by TNF-α (Sato and Seiki, 1993; Hozumi *et al*., 2001), the NF-κB motif at position –600 has also been demonstrated to regulate the response to IL-1β (Yokoo and Kitamura, 1996), Bcl-2 (Ricca *et al*., 2000), human immunodeficiency virus (HIV)-1-Tat (Kumar *et al*., 1999), the metastasis suppressor KiSS-1 (Yan *et al*., 2001), or to synergistic combinations of cytokines and growth factors (Bond *et al*., 1998; Bond *et al*., 2001), and to act in concert with other motifs, in particular AP-1 sites (Sato and Seiki, 1993; Yokoo and Kitamura, 1996; Bond *et al*., 1998; Bond *et al*., 2001). The Ets-responsive element (PEA3) at position –541 has been implicated in gelatinase B gene promoter activation by c-H-*ras* (Gum *et al*., 1996; Himelstein *et al*., 1997), E1AF (Higashino *et al*., 1995), fibroblast cell contact (Himelstein *et al*., 1998), and epidermal growth factor (EGF) (Watabe *et al*., 1998), and may function in concert with the TRE/AP-1 element at position –533 bp (Gum *et al*., 1996; Himelstein *et al*., 1997; Watabe *et al*., 1998). These two *cis*-elements were identified previously in the collagenase promoter as a specialized inducible enhancer module, and defined as a 'TPA and oncogene-responsive unit' or TORU (Gutman and Wasylyk, 1990). The RBE (–54) and AP-1 (–79) elements are involved in gelatinase B gene promoter activation by v-*src* in fibrosarcoma cells (Sato *et al*., 1993), by c-H-*ras* in human adenocarcinoma cells (Gum *et al*., 1996), and by c-H-*ras*/v-*myc* in rat embryo cells (Himelstein *et al*., 1997). Upregulated gelatinase B expression by SK-N-SH neuroblastoma cells during spontaneous conversion from epithelial to neuroblast phenotype is regulated by the RBE and NF-κB (–600 bp) elements (Farina *et al*., 1999). Transient transfection experiments of deleted promoter constructs into human fibroblasts have indicated the presence of an enhancer sequence in a region between –600 and –500 bp, which includes Sp1, AP-1, NF-κB recognition elements and a PEA-3 site, and regulates responsiveness of the gelatinase B gene promoter to TNF-α (He, 1996). Finally, the proximal 670-bp promoter, containing also the downstream AP-1 (–79 bp) and RBE (–54 bp) *cis*-elements besides NF-κB, Sp1, and PEA3, has been implicated in basal transcriptional activity (Himelstein *et al*., 1997; Vegeto *et al*., 2001). In general, it is accepted that

full activation of the gelatinase B gene promoter depends on the concerted action of several *trans*-acting factors and that the AP-1 (–79) binding site is an indispensable *cis*-element. The signals to the NF-κB or Sp-1 sites, which are not present in interstitial collagenase and stromelysin-1 promoters, are the specific determinants for the inducibility of the gelatinase B gene.

By comparison, it is interesting to notice that the promoter structure of the gelatinase A gene is completely different from that of gelatinase B (Figure 3) and other MMP family members. Consequently, the transcriptional regulation of gelatinase A is quite different from that of gelatinase B. In the following sections the comparisons of the promotor structures and the inducers of transcription of both genes exemplify the highly controllable regulation of gelatinase B. In contrast to the gene promotor of gelatinase B, the gelatinase A gene promotor contains heterogeneous initiation sites for transcription (Huhtala *et al*., 1990a; Huhtala *et al*., 1990b; Levy *et al*., 1991; Bian and Sun, 1997) and does not contain a TATA motif-like sequence, a CAAT box, or regulatory binding sequences for AP-1, NF-κB, TIE, and other transcription factors. However, a TACATCT sequence, representing a noncanonical TATA box and located 32 bp upstream from the major start site of transcription (Huhtala *et al*., 1990b), has been shown to provide basal promoter activity (Templeton and Stetler-Stevenson, 1991). Common regulatory elements contained in the ~2.2-kbp gelatinase A gene promoter sequence (Table 3) are two GC boxes and an additional CCACC sequence at position –1307, which also can serve to bind Sp1, several AP-2 binding sequences, and a number of incomplete PEA3 sites. One potential AP-2 binding site, whose transcriptional regulatory role has not yet been investigated, is located in the 5' untranslated region behind the transcription initiation site (Huhtala *et al*., 1990a). A strong enhancer (r2) element is located at position –1655 bp and has been found to be essential for basal, high-level, and inducible transcription of the gelatinase A gene. In this region a Y-box element and an AP-2 binding sequence are contained that bind the synergistically interacting nuclear proteins YB-1 and AP-2, respectively. YB-1 has binding specificity for the consensus sequence CTGATTGGCTAA, which contains an inverted CCAAT box. Depending on the cell type, YB-1 acts as a positive or negative regulator of transcription driven by the gelatinase A gene promoter (Mertens *et al*., 1999). The AP-2 binding site has been shown to be a major target for transcriptional repression by the adenovirus type A E1A oncogene product (Frisch and Morisaki, 1990; Somasundaram *et al*., 1996). In addition, a perfect binding site of 20 bp for the common tumor suppressor and transcription factor p53 has later been colocalized with the r2 enhancer sequence (Bian and Sun, 1997), and encompasses the AP-2 site. The p53/r2 element, identified as critical for gelatinase A gene promoter activity in some cells, such as human sarcoma and fibrosarcoma (HT1080) cell lines (Frisch and Morisaki, 1990; Bian and Sun, 1997), has been shown to be nonfunctional in astroglioma cells (Qin *et al*., 1999), and thus contributes to cell-specific expression of gelatinase A. Within a 40-bp region immediately downstream of the r2 enhancer sequence, a transcriptional silencer sequence containing a modular array of functional elements and negatively affecting constitutive promoter

Table 3. Position and sequence of the regulatory elements in the promoter region of the human gelatinase A and B genes

Promoter element[a]	Position (bp)[b]	Sequence (5'→ 3')[b]	References
Gelatinase A (MMP-2)			
AP-2 binding site	+147 (+154)	CCCCAGGC	(Huhtala *et al.*, 1990a;Tryggvason *et al.*, 1992)
	-22 (-15)	GGCTGCCC	(Bian and Sun, 1997)
	-74 (-67)	**CCCCCAGCCC**	(Bian and Sun, 1997)
	-81 (-74)	CCCCCCG	(Bian and Sun, 1997)
	-1649 (-1642)	**GCCTGAACT**	(Frisch and Morisaki, 1990;Somasundaram *et al.*, 1996)
Basal promoter (non-canonical TATA)	**-32 (-25)**	**TACATCT**	(Templeton and Stetler-Stevenson, 1991)
GC box /Sp1 consensus site	**-78 (-71)**	**CCCGCCC**	(Huhtala *et al.*, 1990a)
	-98 (-91)	**GGGCGG**	(Huhtala *et al.*, 1990a)
	-1307 (-1300)	CCACC	(Price *et al.*, 2001)
ISRE	-138 (-131)	GAAAAGT	(Hujanen *et al.*, 1994)
	-156 (-149)	AGAAAGGAA	(Hujanen *et al.*, 1994)
	-168 (-161)	GAAAAGA	(Hujanen *et al.*, 1994)
PEA3/Ets consensus site	-152 (-145)	AGGAAA	(Bian and Sun, 1997)
	-547 (-540)	AGGAAG	(Bian and Sun, 1997)
	-559 (-552)	AGGAAA	(Bian and Sun, 1997)
	-1057 (-1050)	AGGAAA	(Bian and Sun, 1997)
	-1262 (-1255)	**CAGGAAGC (Ets-1)**	(Bian and Sun, 1997)
GCN4	-224 (-217)	TGACGA	(Bian and Sun, 1997)
CREB	-309 (-302)	GGACGTCA	(Bian and Sun, 1997;Hasan and Nakajima, 1999)
Enhancer/silencer	**-422/-223**	**ND**	(Templeton and Stetler-Stevenson, 1991)
GATA-1 (reversed)	-795 (-788)	CTATCT	(Price *et al.*, 2001)
c-myc/c-myb	-1169 (-1162)	CAACTG	(Bian and Sun, 1997)
AP-1 (reversed)	-1277 (-1270)	AGAAGTCA	(Bian and Sun, 1997)
Silencer	**-1617 (-1610)**	**AGCCGCAGAGACTTTTCTA (siΔ3)**	(Frisch and Morisaki, 1990)
	-1627 (-1620)	**CTGAGACCCA (x2/s2)**	(Frisch and Morisaki, 1990)
	-1637 (-1630)	**CTGAAGCCCA (x1/s1)**	

Enhancer r2/p53 binding site	**-1655 (-1648)**	**AGACAAGCCT GAACTTGTCT**	(Bian and Sun, 1997)
Y-box element	**-1660 (-1653)**	**CCACCAGACAAG**	(Mertens *et al.*, 1999)
Gelatinase B (MMP-9)			
TATA	**-29**	**TTAAA**	(Huhtala *et al.*, 1991;He, 1996)
GT box	**-54**	**GGGGTGGGG (RBE/Sp1 binding)**	(Sato *et al.*, 1993;Himelstein *et al.*, 1997;Campbell *et al.*, 2001)
	-252	CCCCTCCCC	(Sato *et al.*, 1993)
	-481	GGGGAGGGG	(Sato *et al.*, 1993)
	-774	CCCCACCCC	(Sato *et al.*, 1993)
KRE-M9	-66	GCCTGTCAAG	(Kobayashi *et al.*, 2001)
TRE/AP-1 binding site	**-79**	**TGAGTCA**	(Huhtala *et al.*, 1991;He, 1996)
	-209	**TGCCTGACT**	(Campbell *et al.*, 2001)
	-533	**TGAGTCA**	(Huhtala *et al.*, 1991;Sato and Seiki, 1993;He, 1996)
	-1652	**TGAGTCA**	(Sato and Seiki, 1993)
CACA box/microsatellite	-131	$(CA)_n$	(Sato *et al.*, 1993;He, 1996)
NIP	-138	GTGCTGCC	(Sato and Seiki, 1993)
ISRE	-151	CTTTCTC	(Hujanen *et al.*, 1994)
	-295	CTTTCA	(Hujanen *et al.*, 1994)
	-345	CTTTCA	(Hujanen *et al.*, 1994)
	-509	GGGAAAAAGA	(Hujanen *et al.*, 1994)
AP-2 binding site	-230	TTTCCTGCGGGT	(Campbell *et al.*, 2001)
	-421	GGCCAGGG	(Campbell *et al.*, 2001)
NF-κB consensus site	**-328**	**GGGGGATCCC (p50)**	(Han *et al.*, 2001)
	-600	**GGAATTCCCC (p65)**	(Sato and Seiki, 1993;He, 1996;Campbell *et al.*, 2001)
TIE	**-474**	**GGTTTGGGGA**	(Huhtala *et al.*, 1991;Sato and Seiki, 1993;He, 1996)
PEA3/Ets consensus site	**-541**	**GAGGAAGC (Ets/E1AF)**	(Sato and Seiki, 1993;Higashino *et al.*, 1995;He, 1996;Gum *et al.*, 1996)
	-554	**GATGAAGC (Ets)**	(Sato and Seiki, 1993)
	-571	**CATTCCT (E1AF)**	(Higashino *et al.*, 1995)

Table 3 (continued)

GC box/Sp1 consensus site	**-563**	**CCGCCC**	(Huhtala *et al.*, 1991;He, 1996)

[a]All regulatory promoter elements listed in the table are also marked in Figure 3 and 4. Abbreviations used are: AP-1/2, activating protein-1/2; *c-myc/c-myb*, proto-oncogene found in avian myelocytomatosis virus; CREB, cAMP response element binding protein; Ets, product of *ets* proto-oncogene found in E26 avian erythroblastosis virus; GCN4, yeast transcriptional activator; ISRE, interferon-stimulated regulatory element; KRE-M9, keratinocyte differentiation factor-1 regulatory element; MMP, matrix metalloproteinase; NF-κB, nuclear factor-kappa B; NIP, nuclear inhibition protein; p53, tumor suppressor; PEA3, polyomavirus enhancer A-binding protein-3; RBE, retinoblastoma binding element; Sp1, stimulating protein 1; TIE, transforming growth factor-beta inhibitory element; TRE, 12-O-tetradecanoylphorbol-13-acetate response element. [b]The 5'-end position of each binding sequence (5' → 3') relative to the start site of transcription is given in basepairs (bp) (Huhtala *et al.*, 1990a;Huhtala *et al.*, 1990b;Huhtala *et al.*, 1991;Levy *et al.*, 1991;Bian and Sun, 1997). For the gelatinase A promoter elements, the first positions mentioned are calculated relative to the major transcription initiation site (Huhtala *et al.*, 1990a;Huhtala *et al.*, 1990b), whereas the positions relative to the secondary initiation site are shown between brackets (Levy *et al.*, 1991;Bian and Sun, 1997). The positions and sequences of promoter sites that are shown in Figure 3, are depicted in bold. ND, the exact sequence has not been determined.

activity is located. Cell-type-specific expression of the gelatinase A gene was suggested to be partly determined by this silencer (Frisch and Morisaki, 1990; Qin *et al*., 1999). Besides this well-established enhancer/silencer module, another putative enhancer that increased gelatinase A activity in highly metastatic A2058 melanoma cells has been observed somewhere within the positions –223 and –422 bp, upstream of the basal promoter. Within the same region, a putative silencer sequence that causes decreased activity of the basal promoter was found in the nonmetastatic melanoma HT144 cell line. Apparently, the regulation of the gelatinase A gene is cell-type specific and is able to contribute to the production of the metastatic phenotype (Templeton and Stetler-Stevenson, 1991).

The cooperative action between promoter elements was also found to be necessary for the constitutive activity of gelatinase A, as observed in astroglioma cells (Qin *et al*., 1999). Simultaneous binding of both Sp1 and Sp3 transcription factors to the Sp1 site at –98 bp, and of AP-2 to the AP-2 consensus sequence at –74 bp, resulted in the synergistic enhancement of gelatinase A gene promoter activation.

2.1.2. Functional Polymorphisms in the Promoter/Enhancer Regions of Human Gelatinases

Polymorphisms in the gene promoter sequences of human gelatinase A and gelatinase B have been implicated in the regulation of gene expression and susceptibility to various diseases. Out of five identified sequence variants (Zhang *et al*., 1999a), the gelatinase B gene promoter region on human chromosome 20 contains two polymorphic sequences with functional importance, namely, a single nucleotide substitution at –1562 bp and a $(CA)_n$ dinucleotide repeat at position –131 bp. The single nucleotide polymorphism (SNP) at –1562 bp is due to a C to T substitution (–1562 C→T). This base transition results in the loss of binding of a nuclear protein to this region and an increase in transcriptional activity in macrophages. In these cells, the C/C genotype leads to low promoter activity, whereas the C/T and T/T genotypes result in high transcriptional activity (Zhang *et al*., 1999b). In contrast, promoter activity did not differ significantly between –1562C and –1562T alleles when evaluated in primary amnion epithelial cells, WISH amnion-derived, or THP-1 cells (Ferrand *et al*., 2002).

Similarly, the $(CA)_n$ microsatellite polymorphism may influence transcriptional activity of the gelatinase B gene promoter due to its localization close to the transcriptional start site and several important transcription factor binding sites, including the TRE, Sp1, and NF-κB consensus sequences (Figure 3). Alternatively, the polymorphic sequence can alter DNA conformation and thus modulate transcriptional activity (Himelstein *et al*., 1998). Variation in the length of the repetitive element indeed modulates promoter activity in human HT1080 fibroblasts in *in vitro* reporter assays (Peters *et al*., 1999), in human esophageal squamous cell carcinoma (SCC) cell lines (Shimajiri *et al*., 1999), in 293 cells (Maeda *et al*., 2001), and in human amnion epithelial and WISH amnion-derived cells (Ferrand *et al*., 2002). The highest promoter activity has been observed in reporter constructs containing the $(CA)_{21}$ (Shimajiri *et al*., 1999; Maeda

et al., 2001) or $(CA)_{23}$ (Peters *et al*., 1999) alleles, having 21 or 23 tandem repeats, respectively. The polymorphic promoter element has been shown to serve as a binding site for a sequence-specific DNA-binding protein, with the strength of nuclear protein binding being dependent on the number of CA repeats present (Peters *et al*., 1999).

The multiallelic $(CA)_n$ microsatellite in the gelatinase B gene promoter region exhibits a bimodal distribution of the allele frequencies in the American white (St Jean *et al*., 1995; Peters *et al*., 1999), Finnish (Yoon *et al*., 1999), Swedish, Belgian, Sardinian (Nelissen *et al*., 2000; 2002a), African-American (Ferrand *et al*., 2002), and southern English population (Zhang *et al*., 2001), with the highest incidence of the $(CA)_{14}$ allele and a second peak at the $(CA)_{21}$, $(CA)_{22}$, and $(CA)_{23}$ alleles (Table 4). This is in sharp contrast to Japanese people, in whom the allele containing 21 repeats is most prevalent, followed by the alleles $(CA)_{22}$ and $(CA)_{20}$ in one study (Shimajiri *et al*., 1999), or $(CA)_{23}$ and $(CA)_{22}$ in another (Maeda *et al*., 2001). In the two last studies, none (Maeda *et al*., 2001) or only few individuals (Shimajiri *et al*., 1999) had one or two alleles, respectively, with 14, 18, or 19 $(CA)_n$ repeats. Another striking observation from this comparison is the fact that the $(CA)_{16}$ and $(CA)_{17}$ alleles are not tolerated in all American white, Asian, and Caucasian populations.

Genetic studies were performed to detect possible association of both functional and polymorphic markers in the human gelatinase B gene promoter with several pathological conditions. The $(CA)_n$ microsatellite polymorphism was studied in relation to abdominal aortic aneurysm and intracranial aneurysm in case-control studies in Caucasian populations. An association of this genetic polymorphism with intracranial aneurysm has been found in only one study, but no association with abdominal aneurysm has been detected (St Jean *et al*., 1995; Yoon *et al*., 1999; Peters *et al*., 1999; Zhang *et al*., 2001). The functional –1562 C→T SNP has also been found not to be associated with intracranial aneurysm in England (Zhang *et al*., 2001). Likewise, both $(CA)_n$ microsatellite and –1562 C→T polymorphisms were analyzed in case-control samples and simplex families of multiple sclerosis, but no association has been found (Nelissen *et al*., 2000 and 2002a). However, the microsatellite $(CA)_{21}$ allele has been found to be protective in the development and progression of overt nephropathy in Japanese subjects with type 2 diabetes (Maeda *et al*., 2001), whereas the $(CA)_{14}$ allele has been associated with significantly increased risk for preterm premature rupture of fetal membranes in African-American women (Ferrand *et al*., 2002). Furthermore, an association of the C→T single nucleotide polymorphisms at position –1562 with severity of coronary atherosclerosis (Zhang *et al*., 1999b) and with the area of complicated coronary lesions (Pollanen *et al*., 2001) has been observed in Caucasian subjects. Only in the latter study, positive association of the promoter activity genotype with the risk of myocardial infarction has been detected.

In the gene promoter of gelatinase A on human chromosome 16, six single base substitutions have been identified, of which three variants map onto consensus sequences for Sp1 (–1306 bp), a cell cycle-dependent element (CDE; CGCGG, at +220 bp), and an inverted GATA-1 site (–795 bp). The majority of promoter variants are nonfunctional neutral SNPs. Only the common –1306

Table 4. Allele frequencies[a] of the $(CA)_n$ microsatellite polymorphism in the human gelatinase B promoter in different ethnic populations

Ethnicity and geographic origin of populations	Number of alleles	$(CA)_n$ microsatellite alleles[k]													
		$(CA)_{14}$	$(CA)_{15}$	$(CA)_{16}$	$(CA)_{17}$	$(CA)_{18}$	$(CA)_{19}$	$(CA)_{20}$	$(CA)_{21}$*	$(CA)_{22}$	$(CA)_{23}$*	$(CA)_{24}$	$(CA)_{25}$	$(CA)_{26}$	$(CA)_{27}$
Caucasian Western populations															
Western Pennsylvania, USA[b]	188	**0.511**	0.021	-	-	-	0.005	0.021	**0.154**	**0.191**	0.085	0.011	-	-	-
Kuopio, Finland[c]	342	**0.526**	0.003	-	-	-	0.029	0.032	**0.187**	**0.158**	0.050	0.012	-	-	0.003
Stockholm, Sweden[d]	290	**0.541**	0.021	-	-	-	0.010	0.021	**0.197**	**0.138**	0.069	-	-	0.003	-
Cagliari, Sardinia[e]	250	**0.672**	0.036	-	-	-	0.024	0.016	**0.116**	0.092	0.024	0.020	-	-	-
Wessex, England[f]	316	**0.569**	0.037	-	-	-	-	0.005	0.021	**0.160**	**0.170**	0.032	0.005	-	-
Leuven, Belgium[g]	284	**0.563**	0.018	-	-	-	0.007	0.028	**0.194**	**0.106**	0.063	0.021	-	-	-
African American population															
Pennsylvania/Detroit, USA[h]	430	**0.190**	0.048	0.007	0.016	0.030	0.083	**0.167**	**0.198**	**0.149**	0.095	0.014	-	-	-
Eastern populations															
Kitakyushu, Japan[i]	223	0.009	-	-	-	0.013	0.004	**0.112**	**0.713**	**0.117**	0.031	-	-	-	-
Otsu, Japan[j]	144	-	-	-	-	-	0.021	0.028	**0.424**	**0.139**	**0.354**	0.021	0.014	-	-

[a]Allele frequencies exceeding the 10% level are depicted in bold. [b](St Jean *et al.*, 1995), [b](Peters *et al.*, 1999), [c](Yoon *et al.*, 1999), [d,e](Nelissen *et al.*, 2000), [f](Zhang *et al.*, 2001), [g](Nelissen *et al.*, unpublished results, 2002a), [h](Ferrand *et al.*, 2002), [i](Shimajiri *et al.*, 1999), [j](Maeda *et al.*, 2001). [k]$(CA)_n$ denotes a microsatellite allele with n CA-repeats. The alleles with highest promoter activity (Shimajiri *et al.*, 1999; Peters *et al.*, 1999; Maeda *et al.*, 2001) are indicated with '*'.

C→T transition influences gelatinase A gene promoter activity in an allele-specific manner. The presence of a T at the –1306 site abolishes the Sp1 site. The Sp1 transcription factor binds only to the –1306C allele containing sequence. The –1306C allele provides a twofold higher promoter activity compared with the –1306T allele, as assessed by a functional study of reporter gene activity (Price *et al*., 2001).

2.1.3. Transcriptional Regulation of Human Gelatinase B and A Gene Expression

2.1.3.1. Cellular Sources and Inducibility

Gelatinase A is widely expressed in most human tissue cell types, circulating leukocytes, and tumor cells, whereas gelatinase B is produced by selected cell types, including keratinocytes, monocytes, tissue macrophages, and polymorphonuclear leukocytes, and by a variety of malignant cells. The production of both gelatinases is stimulated in response to a variety of inducers, such as tumor promoters, growth factors, cytokines, oncogene products, and physiological substances, such as metal ions, reactive oxygen species, or hormones. Again, this regulation is quite different between gelatinase A and B. It should be noted that neutrophils, in contrast to other cell types, show a rather exceptional gelatinase expression pattern, in that the synthesis of gelatinase A is completely absent, whereas gelatinase B is expressed during maturation and subsequently stored within secondary or tertiary granules (Cowland and Borregaard, 1999). Therefore, stimulation of mature neutrophils does not result in upregulation of gelatinase B synthesis, but may induce release of the enzyme by degranulation. To allow a comparison of the extensive literature, the different producer cell types of both gelatinases and their inducers, including growth factors, cytokines, secondary messengers, and phorbol esters, are summarized in Tables 5 and 6.

Expression of most MMPs is normally low in tissues and is induced when remodeling of the extracellular matrix (ECM) is required. The differential response of the gelatinase A and gelatinase B genes to inducers is related to differences in the promoter sequences of the genes. The location and number of consensus sites, recognized by available transcription factors, determines gene transcription. As discussed above, the promoter region of the gelatinase B gene contains a set of such regulatory elements. Accordingly, basal expression of gelatinase B, which is low in most cell types, is highly responsive to most, if not all growth factors and cytokines. Induced levels can be more than a 100-fold of the basal expression levels. This is in sharp contrast with the gelatinase A gene, of which the promoter region contains only few conserved *cis*-elements. Gelatinase A is expressed constitutively by most cells, at least *in vitro*, and appears to be only moderately induced or repressed (two- to fourfold) (Birkedal-Hansen *et al*., 1993), or the gene is not responsive at all. Transcription is also regulated in a tissue-specific manner (Frisch and Morisaki, 1990; Marti *et al*., 1993; Harendza *et al*., 1995). Therefore, the gelatinase A gene may be considered as a housekeeping gene, involved in the maintenance of normal extracellular matrix turnover.

Table 5. Cellular sources and regulators of human gelatinase B expression

Producer cell type[a]	Effect[b]	Inducer/repressor[c]	References
Normal cells			
Articular cartilage	+	IL-1α	(Mohtai *et al.*, 1993)
Astrocytes	+	IL-1β; PMA	(Apodaca *et al.*, 1990; Korzus *et al.*, 1997)
	+/-	(PMA,TNF-α)/(IFN-β, IFN-γ)	(Ma *et al.*, 2001)
Chondrocytes	+	IL-1α; IL-1β; TNF-α	(Lefebvre *et al.*, 1991; Mohtai *et al.*, 1993)
Dendritic cells	+/-	(IL-1β, TNF-α)/IFN-β	(Bartholomé *et al.*, 2001)
	-	IFN-β	(Bartholomé *et al.*, 2001)
Endothelial cells	+	PMA; PMA+(IL-1α, TNF-α); TNF-α	(Mackay *et al.*, 1992; Hanemaaijer *et al.*, 1993; Fisher *et al.*, 1994; Cornelius *et al.*, 1995; Foda *et al.*, 1996; Genersch *et al.*, 2000; Hummel *et al.*, 2001; Nelissen *et al.*, 2002 b)
	NE	IFN-β; IL-1; TNF-α; VEGF	(Mackay *et al.*, 1992; Lamoreaux *et al.*, 1998; Nelissen *et al.*, 2002b)
Epithelial cells	+	IL-1β; PMA; TNF-α	(Yao *et al.*, 1997; Hofmann *et al.*, 1998; Yao *et al.*, 1998; Hozumi *et al.*, 2001)
	NE	IL-1β	(Hozumi *et al.*, 2001)
Fibroblasts	+	(bFGF, PDGF)+(IL-1α,TNF-α); IL-1α; IL-1α+PMA; IL-1β; Oncostatin M; PDGF; PMA; TGF-β+(IL-1β, TNF-α); TNF-α; TNF-β	(Masure *et al.*, 1990; Fridman *et al.*, 1990; Moll *et al.*, 1990; Unemori *et al.*, 1991; Mackay *et al.*, 1992; Zeng and Millis, 1994; Unemori *et al.*, 1994; He, 1996; Sato *et al.*, 1996; Korzus *et al.*, 1997; Bond *et al.*, 1998; Hofmann *et al.*, 1998; Singer *et al.*, 1999; Bond *et al.*, 2001; Han *et al.*, 2001)
	+/-	TNF-α/IL-1α	(Sato *et al.*, 1996b)
	NE	bFGF; CSF-1; IFN-α; IFN-β; IFN-γ; IL-1α; IL-1β; IL-6; IL-10; PDGF; PMA; TGF-α; TGF-β; TGF-β+(EGF, IL-6, IL-8, PDGF); TNF-α	(Wilhelm *et al.*, 1989; Unemori *et al.*, 1991; Salo *et al.*, 1991; Mackay *et al.*, 1992; Gohji *et al.*, 1994b; Unemori *et al.*, 1994; Lacraz *et al.*, 1995; Sato *et al.*, 1996b; Korzus *et al.*, 1997; Wassenaar *et al.*, 1999; Han *et al.*, 2001)
Glial cells	NE	TGF-β	(Giraudon *et al.*, 1997)
Keratinocytes	+	EGF; HGF/SF; PMA; TGF-α; TGF-β; TGF-β+TNF-α; TNF-α	(Wilhelm *et al.*, 1989; Salo *et al.*, 1991; McCawley *et al.*, 1998; Makela *et al.*, 1998; Han *et al.*, 2001)
	+/-	TNF-α/IFN-γ	(Makela *et al.*, 1998)
	NE	HGF/SF; IL-1β	(Dunsmore *et al.*, 1996; Kobayashi *et al.*, 1998a)
B lymphocytes	+	IL-1β; IL-8; IL-13; PMA	(Trocme *et al.*, 1998)
	-	TGF-β	(Trocme *et al.*, 1998)
T lymphocytes	+	IL-1; IL-2; MIP-1α; MIP-1β; PMA; RANTES; TNF-α	(Montgomery *et al.*, 1993; Weeks *et al.*, 1993b; Leppert *et al.*, 1996; Johnatty *et al.*, 1997)
	+/-	IL-2/IFN-β	(Leppert *et al.*, 1996)
	-	IFN-β; IFN-γ; IL-1; IL-2; TNF-α	(Leppert *et al.*, 1996; Stuve *et al.*, 1996; Johnatty *et al.*, 1997)
Macrophages	+	GM-CSF; PMA	(Welgus *et al.*, 1990; Lacraz *et al.*, 1992; Mautino *et al.*, 1997)
	+/-	(IL-1β, LPS, TNF-α)/IFN-γ; *S. Aureus*/IL-4; (LPS, *S. aureus*)/IL-10	(Lacraz *et al.*, 1992; Lacraz *et al.*, 1995; Saren *et al.*, 1996)
	-	IFN-γ; IL-4; IL-4+IFN-γ; IL-10	(Shapiro *et al.*, 1990; Lacraz *et al.*, 1992; Lacraz *et al.*, 1995; Mautino *et al.*, 1997)
	NE	IL-2; IL-6	(Lacraz *et al.*, 1992; Lacraz *et al.*, 1995)
Mesangial cells	+	IL-1β; PMA	(Martin *et al.*, 1994; Kitahara *et al.*, 2001)
Mesothelial cells	+	IL-1β; IL-1β+TNF-α; PMA; TNF-α	(Marshall *et al.*, 1993)
	NE	IFN-γ	(Marshall *et al.*, 1993)
Monocytes	+	GM-CSF; GM-CSF+(IL-1β, TNF-α); IL-1β; MCP-1; M-CSF; PMA; SPARC; TGF-β; TNF-α	(Wahl *et al.*, 1993; Kitagawa *et al.*, 1996; Shankavaram *et al.*, 1997; Zhang *et al.*, 1998; Xie *et al.*, 1998; Klier and Nelson, 1999; Vos *et al.*, 2000)
	+/-	ConA/(IFN-γ, IL-4); (GM-CSF+TNF-	(Corcoran *et al.*, 1992; Wahl and Corcoran, 1993;

Table 5 (continued)

		α)/IL-4	Zhang *et al.*, 1998)
	-	IL-10	(Mertz *et al.*, 1994; Lacraz *et al.*, 1995)
	NE	IL-4	(Corcoran *et al.*, 1992; Wahl *et al.*, 1993)
Mononuclear cells	+	IL-1β; IL-17; PMA	(Opdenakker *et al.*, 1991b; Jovanovic *et al.*, 2000; Nelissen *et al.*, 2002b)
	+/-	IL-17/(IL-4, IL-10, IL-13); MCP-1/IFN-β; (PHA+PMA)/IFN-β	(Stüve *et al.*, 1997; Lou *et al.*, 1999; Jovanovic *et al.*, 2000)
	-	IL-4; IL-13; IFN-β	(Ozenci *et al.*, 2000; Jovanovic *et al.*, 2000; Galboiz *et al.*, 2001; Nelissen *et al.*, 2002)
	NE	IFN-β	(Galboiz *et al.*, 2001)
Neurons	+	IL-1β	(Vecil *et al.*, 2000)
	-	TGF-β	(Vecil *et al.*, 2000)
Skin	+	TGF-β+TNF-α	(Han *et al.*, 2001)
Smooth muscle cells	+	IL-1α; IL-1β; PMA; TNF-α	(Kenagy *et al.*, 1994; Galis *et al.*, 1994a; Gurjar *et al.*, 2001)
	+/-	rCD40L/IFN-γ	(Schönbeck *et al.*, 1997)
	NE	CTGF; TGF-β	(Galis *et al.*, 1994a; Fan and Karnovsky, 2002)
Stem cells, $CD34^+$	+	G-CSF; GM-CSF; IL-3; IL-6; IL-8; M-CSF; MIP-1α; SCF; SDF-1; TNF-α	(Janowska-Wieczorek *et al.*, 1999; Janowska-Wieczorek *et al.*, 2000)
Tumor cells			
Adenocarcinoma	+	Amphiregulin; EGF; Heregulin-β1; TGF-β; TNF-α	(Price *et al.*, 1996; Kondapaka *et al.*, 1997; Greene *et al.*, 1997; Watabe *et al.*, 1998; Duivenvoorden *et al.*, 1999; Sehgal and Thompson, 1999; Reddy *et al.*, 1999; Yao *et al.*, 2001; Dong *et al.*, 2001)
	NE	Amphiregulin; EGF; Heregulin; IGF-1; IL-1; KGF; PMA; TNF-α	(Mackay *et al.*, 1992; Kondapaka *et al.*, 1997; Dong *et al.*, 2001)
Astroglioma	+/-	(PMA,TNF-α)/(IFN-β, IFN-γ)	(Ma *et al.*, 2001)
	NE	IFN-γ; TNF-α	(Qin *et al.*, 1998)
Carcinoma	+	bFGF; EGF; HGF/SF; IL-1; PMA; TGF-α; TGF-β; TGF-β+PMA; TNF-α	(Moll *et al.*, 1990; Mackay *et al.*, 1992; Shima *et al.*, 1993; Juarez *et al.*, 1993; Xie *et al.*, 1994a; Miyake *et al.*, 1997; Moore *et al.*, 1997; Simon *et al.*, 1998; Hofmann *et al.*, 1998; McCawley *et al.*, 1998; Ikebe *et al.*, 1998; Huang *et al.*, 1999; Simon *et al.*, 2001; Ellerbroek *et al.*, 2001a; Beppu *et al.*, 2002)
	+/-	TNF-α/(IL-4)	(Beppu *et al.*, 2002)
	-	IFN-α	(Slaton *et al.*, 2001)
Cervical cell line	+	TGF-β	(Agarwal *et al.*, 1994)
	-	EGF	(Agarwal *et al.*, 1994)
Endothelial cell line	+	PMA; TNF-α	(Nelimarkka *et al.*, 1998;Genersch *et al.*, 2000)
Fibrosarcoma	+	PMA; TGF-β; TNF-α	(Wilhelm *et al.*, 1989; Okada *et al.*, 1990b; Kerr *et al.*, 1990; Moll *et al.*, 1990; Brown *et al.*, 1990; Tryggvason *et al.*, 1990; Huhtala *et al.*, 1991; Kubota *et al.*, 1991; Okada *et al.*, 1992; Mackay *et al.*, 1992; Morodomi *et al.*, 1992; Lauricella-Lefebvre *et al.*, 1993; Sato and Seiki, 1993; He, 1996; Moore *et al.*, 1997)
	+/-	PMA/TGF-β; (PMA,TNF-α)/(IFN-β, IFN-γ)	(Tryggvason *et al.*, 1990; Ma *et al.*, 2001)
	NE	IL-1α; TGF-β; TGF-β+PMA	(Kerr *et al.*, 1990; Huhtala *et al.*, 1991; Mackay *et al.*, 1992; Lauricella-Lefebvre *et al.*, 1993)
Glioma	+	EGF; IL-1β; PMA; TNF-α	(Apodaca *et al.*, 1990; Nakano *et al.*, 1995; Chintala *et al.*, 1998; Estève *et al.*, 1998)
	NE	IL-6	(Nakano *et al.*, 1995)
Hepatoma	+	PMA; TNF-α	(Masure *et al.*, 1990; Sato and Seiki, 1993)

Table 5 (continued)

T lymphoblastoma	+	IL-2; IL-4; MIP-1α; RANTES	(Xia *et al.*, 1996)
Lymphoid cells	+	PMA	(Weeks *et al.*, 1993b)
T lymphoma	+	PMA; TGF-β	(Zhou *et al.*, 1993)
Melanoma	+	IFN-α; IFN-γ; IL-1β; PMA; TGF-β; TNF-α	(Masure *et al.*, 1990; Mackay *et al.*, 1992; Houde *et al.*, 1993; Lauricella-Lefebvre *et al.*, 1993; Hujanen *et al.*, 1994; MacDougall *et al.*, 1995; Janji *et al.*, 1999)
	-	IFN-α; IFN-γ	(Hujanen *et al.*, 1994)
	NE	IL-1α; IL-1β; PMA; TGF-β	(Lauricella-Lefebvre *et al.*, 1993; MacDougall *et al.*, 1995)
Mesothelioma	+	HGF/SF	(Harvey *et al.*, 2000)
Monocytic leukemia	+	bFGF; IL-1β; PMA; PMA+(IL-1α, TNF-α); TNF-α	(Wilhelm *et al.*, 1989; Welgus *et al.*, 1990; Moll *et al.*, 1990;Van Ranst *et al.*, 1991; Morodomi *et al.*, 1992; Saarialho-Kere *et al.*, 1993; Watanabe *et al.*, 1993; McMillan *et al.*, 1996b; Weston and Weeks, 1996; Chong *et al.*, 2001; Nelissen *et al.*, 2002b)
	+/-	TNF-α/IL-4	(Chizzolini *et al.*, 2000)
	-	IFN-β	(Nelissen *et al.*, 2002b)
	NE	IL-1α	(Watanabe *et al.*, 1993)
Myeloblastic leukemia	+	TNF-α; TNF-β	(Kubota *et al.*, 1996)
Myeloma	NE	IL-1β; IL-6; IL-10; TGF-β; TNF-α	(Barille *et al.*, 1997)
Neuroblastoma	+	IL-1; PMA; TNF-α	(Mackay *et al.*, 1992; Chambaut-Guerin *et al.*, 2000)
Osteosarcoma	+	PMA; TNF-α	(Masure *et al.*, 1990; Okada *et al.*, 1990b; Sato and Seiki, 1993; Kawashima *et al.*, 1994)
	-	TGF-β	(Duivenvoorden *et al.*, 1999)
	NE	bFGF; EGF; IL-1α; PDGF; TGF-β	(Okada *et al.*, 1990b)
Promyelocytic leukemia	+	PMA; TNF-α	(Moll *et al.*, 1990; Davis and Martin, 1990; Ries *et al.*, 1994; Xie *et al.*, 1998; Ismair *et al.*, 1998)
	+/-	PMA/α-TNF-α; α-TNF-α	(Ries *et al.*, 1994)
Salivary gland cell line	+	IFN-γ; IFN-γ+TNF-α	(Wu *et al.*, 1997)
Stromal cells	+	TNF-α (MNCs, giant cell tumor of bone)	(Rao *et al.*, 1999)

[a]Producer cell types are listed in alphabetical order. [b]+, inducing effect; -, repressive effect; NE, no effect. [c]Whenever combined interacting agents are used, '+' indicates a synergistic action, whereas '/' separates the activating substance (in front) and the modulating compound (in the back). The prefix 'r' indicates a recombinant protein.

'α' –indicates a neutralizing antibody. Abbreviations used are: bFGF, basic fibroblast growth factor; CD40L, CD40 ligand (gp39); ConA, concanavalin A; CSF, colony-stimulating factor; CFGF, connective tissue growth factor; EGF, epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; HGF/SF, hepatocyte growth factor/scatter factor; IFN, interferon; IGF-I, insulin-like growth factor I; IL, interleukin; KGF, keratinocyte growth factor; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; M-CSF, macrophage colony-stimulating factor; MIP-1, macrophage inflammatory protein-1; MNC, mononuclear cell; PDGF, platelet-derived growth factor; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; RANTES, regulated upon activation and normally T cell expressed and secreted; SCF, stem cell factor; SPARC, secreted protein, acidic and rich in cysteine or osteonectin; SDF-1, stromal cell-derived factor-1; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor

Another general comment on cellular sources and gelatinase inducibility is that in the literature apparently conflicting results were published. An inducer of gelatinase B in one cell line may be downregulating this enzyme in another cell type or in the same cells when co-induced with other factors. Therefore, the complete cellular context needs to be evaluated, and here we attempt to give a general picture by comparing and complementing the published data. The cellular context *in vivo* includes both soluble factors and cellular interactions with extracellular matrix and tissue cells. These interactions are discussed separately after a summary of the signal transduction pathways.

2.1.3.2. Regulatory MAPK Pathways

Most of the regulatory mechanisms mediated by soluble inducers, such as growth factors and cytokines, occur primarily at the transcriptional level and are initiated by the binding of the stimulating factor to its cell surface receptor. Signals exerted by extracellular stimuli are transmitted to the nucleus. A major mechanism for this signal transduction involves activation of serine-threonine kinases related to the mitogen-activated protein kinase (MAPK) superfamily (Robinson and Cobb, 1997; Chang and Karin, 2001). In mammals, the involve-

Table 6. Cellular sources and regulators of human gelatinase A expression

Producer cell type[a]	Effect[b]	Inducer/repressor[c]	References
Normal cells			
Astrocytes	NE	IL-1β; oncostatin M; PMA	(Apodaca *et al.*, 1990; Korzus *et al.*, 1997)
Chondrocytes	NE	IL-1β; TNF-α	(Lefebvre *et al.*, 1991)
Endothelial cells	+	HGF/SF; VEGF	(Lamoreaux *et al.*, 1998; Wang and Keiser, 2000; Toschi *et al.*, 2001)
	NE	IFN-β; IL-1; PMA; PMA+(IL-1α, TNF-α); TNF-α	(Mackay *et al.*, 1992; Hanemaaijer *et al.*, 1993; Cornelius *et al.*, 1995; Hummel *et al.*, 2001; Nelissen *et al.*, 2002b)
Epithelial cells	NE	IL-1; PMA; TNF-α	(Hofmann *et al.*, 1998)
Fibroblasts	+	IFN-γ; IL-1α; IL-1β; TGF-β; TNF-α;	(Overall *et al.*, 1989; Unemori *et al.*, 1991; Overall *et al.*, 1991; Zeng and Millis, 1994; Unemori *et al.*, 1994; Gohji *et al.*, 1994a)
	-	IL-1α; IL-1β; PMA	(Brown *et al.*, 1990; Hecker-Kia *et al.*, 1997)
	NE	EGF; IFN-α; IFN-β; IFN-γ; IGF-II; IL-1α; IL-1α+(PMA, TNF-α); IL-1β; IL-1β+TGF-α; IL-6; oncostatin M; PDGF; PMA; TGF-α; TGF-β; TNF-α	(Salo *et al.*, 1989; Masure *et al.*, 1990; Moll *et al.*, 1990; Brown *et al.*, 1990; Okada *et al.*, 1990a; Salo *et al.*, 1991; Mackay *et al.*, 1992; Unemori *et al.*, 1994; Gohji *et al.*, 1994a; Sato *et al.*, 1996b; Korzus *et al.*, 1997; Hofmann *et al.*, 1998; Singer *et al.*, 1999; Wassenaar *et al.*, 1999)
Glial cells	+	TGF-β	(Giraudon *et al.*, 1997)
Keratinocytes	+	TGF-β	(Salo *et al.*, 1991)
	NE	IL-1β; PMA; TNF-α	(Salo *et al.*, 1991; Makela *et al.*, 1998; Kobayashi *et al.*, 1998a)
T lymphocytes	+	IL-2	(Leppert *et al.*, 1996)
	NE	IFN-β	(Leppert *et al.*, 1996)
Mesangial cells	+	TGF-β	(Marti *et al.*, 1994)
	NE	IL-1β; PMA	(Martin *et al.*, 1994; Kitahara *et al.*, 2001)
Mesothelial cells	NE	IFN-γ; PMA	(Marshall *et al.*, 1993)
Monocytes	+	MCP-1; TGF-β	(Wahl *et al.*, 1993; Klier and Nelson, 1999)
Mononuclear cells	+	IFN-β	(Galboiz *et al.*, 2001)
	-	IFN-β	(Galboiz *et al.*, 2001)
	NE	PMA	(Welgus *et al.*, 1990)
Smooth muscle cells	+	CTGF; IL-1α; TNF-α	(Galis *et al.*, 1994a; Fan and Karnovsky, 2002)
	NE	PMA; TGF-β	(Kenagy *et al.*, 1994; Galis *et al.*, 1994a)
Stem cells, $CD34^+$	+	G-CSF; GM-CSF; IL-3; IL-6; IL-8; M-CSF; MIP-1α; SCF; SDF-1; TNF-α	(Janowska-Wieczorek *et al.*, 1999; Janowska-Wieczorek *et al.*, 2000)
Tumor cells			
Adenocarcinoma	+	TGF-β	(Greene *et al.*, 1997; Duivenvoorden *et al.*, 1999; Sehgal and Thompson, 1999)
	NE	Amphiregulin; EGF; Heregulin; IL-1; PMA; TNF-α	(Mackay *et al.*, 1992; Price *et al.*, 1996; Kondapaka *et al.*, 1997)
Astroglioma	-	IFN-γ; IFN-γ+TNF-α; TNF-α;	(Qin *et al.*, 1998)
	NE	IL-4; IL-10	(Qin *et al.*, 1998)
Carcinoma	+	bFGF; EGF; HGF/SF; TGF-β	(Tienari *et al.*, 1994; Gohji *et al.*, 1994b; Miyake *et al.*, 1997; Huang *et al.*, 1999)
	-	EGF; HGF/SF; IFN-β; IFN-γ; TGF-α	(Fabra *et al.*, 1992; Gohji *et al.*, 1994a; Kato *et al.*, 1995; McCawley *et al.*, 1998)
	NE	EGF; IL-1; PMA; TGF-β; TGF-β+PMA; TNF-α	(Moll *et al.*, 1990; Mackay *et al.*, 1992; Shima *et al.*, 1993; Xie *et al.*, 1994a; Hofmann *et al.*, 1998; Ikebe *et al.*, 1998; Ellerbroek *et al.*, 2001; Beppu *et al.*, 2002)

Table 6 (continued)

Cervical cell line	+	TGF-β	(Agarwal *et al.*, 1994)
	-	EGF	(Agarwal *et al.*, 1994)
Endothelial cell line	-	TNF-α	(Nelimarkka *et al.*, 1998)
Fibrosarcoma	+	TGF-β	(Salo *et al.*, 1989; Brown *et al.*, 1990; Tryggvason *et al.*, 1990; Huhtala *et al.*, 1991; Kubota *et al.*, 1991)
	-	IL-1α; PMA	(Brown *et al.*, 1990; Huhtala *et al.*, 1991)
	NE	IL-1α; PMA; TNF-α	(Wilhelm *et al.*, 1989; Salo *et al.*, 1989; Moll *et al.*, 1990; Brown *et al.*, 1990; Tryggvason *et al.*, 1990; Mackay *et al.*, 1992; Lauricella-Lefebvre *et al.*, 1993)
Glioma	+	PMA; TGF-β	(Nakano *et al.*, 1995; Uhm *et al.*, 1996)
	NE	IL-6; PMA	(Apodaca *et al.*, 1990; Nakano *et al.*, 1995)
Hepatoma	NE	PMA	(Masure *et al.*, 1990)
Maxillary tumor	NE	EGF	(Mizoguchi *et al.*, 1991)
Melanoma	+	IFN-α; IFN-γ; TGF-β	(Brown *et al.*, 1990; Hujanen *et al.*, 1994)
	-	EGF; IFN-α; IFN-γ; IL-1α;PMA;	(Turpeenniemi-Hujanen *et al.*, 1986; Brown *et al.*, 1990; Hujanen *et al.*, 1994)
	NE	IL-1α; IL-1β; PMA; TGF-β; TNF-α	(Masure *et al.*, 1990; Brown *et al.*, 1990; Mackay *et al.*, 1992; Houde *et al.*, 1993; Lauricella-Lefebvre *et al.*, 1993; MacDougall *et al.*, 1995; Janji *et al.*, 1999)
Mesothelioma	NE	HGF/SF	(Harvey *et al.*, 2000)
Monocytic leukemia	+	PMA	(Moll *et al.*, 1990)
	NE	PMA	(Wilhelm *et al.*, 1989)
Myeloblastic leukemia	NE	TNF-α; TNF-β	(Kubota *et al.*, 1996)
Neuroblastoma	NE	IL-1; PMA; TNF-α	(Mackay *et al.*, 1992; Chambaut-Guerin *et al.*, 2000)
Osteosarcoma	NE	bFGF; EGF; IL-1α; PDGF; PMA; TGF-β; TNF-α	(Masure *et al.*, 1990; Okada *et al.*, 1990b; Kawashima *et al.*, 1994; Duivenvoorden *et al.*, 1999)
Promyelocytic leukemia	+	PMA	(Moll *et al.*, 1990)
Salivary gland cell line	+	IFN-γ; IFN-γ+TNF-α	(Wu *et al.*, 1997)
Stromal cells	NE	IL-1β; IL-6; TGF-β1; TNF-α	(Barille *et al.*, 1997; Rao *et al.*, 1999)

[a]Producer cell types are listed in alphabetical order. [b]+, inducing effect; -, repressive effect; NE, no effect. [c]Whenever combined interacting agents are used, '+' indicates a synergistic action. Abbreviations used are: bFGF, basic fibroblast growth factor; CTGF, connective tissue growth factor; EGF, epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; HGF/SF, hepatocyte growth factor/scatter factor; IFN, interferon; IGF-II, insulin-like growth factor II; IL, interleukin; MCP-1, monocyte chemotactic protein-1; M-CSF, macrophage colony-stimulating factor; MIP-1α, macrophage inflammatory protein-1α; PDGF, platelet-derived growth factor; PMA, phorbol 12-myristate 13-acetate; SCF, stem cell factor; SDF-1, stromal cell-derived factor-1; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor

ment of at least three well-characterized groups of MAPK family members have been identified, namely, the extracellular signal-regulated kinases (ERK)1/2, the c-jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK)1/2, and the p38 proteins (p38α/β/γ/δ) (Figure 5). These MAPKs are activated through sequential phosphorylation of their upstream MAPKK kinases (MAPKKK) and MAPK kinases (MAPKK): respectively, c-Raf and MAPK/ERK kinase 1/2 (MEK1/2, also known as MAPK kinase, MKK1/2) for ERK1/2, MEK kinase (MEKK)1/2/3 and MKK4/7 (also called JNK kinase 1/2, JNKK1/2, respectively) for the JNK/SAPKs, and apoptosis signal regulating kinase 1 (ASK1 or MEKK5) or TGF-β-activated kinase 1 (TAK1) and MKK3/6 for the p38MAPKs. Each of these modules may be regulated by upstream small guanosine triphosphatases (GTPases), including Ras and the members of the Ras-related Rho family of small GTP-binding proteins/GTPases, Rac and Cdc42. The activated forms of Rac and Cdc42 typically are efficient activators of the MAPK cascades leading to JNK and p38 activation, whereas stimulation of Ras leads to activation of the Raf-MEK-ERK module. In general, considerable crosstalk exists between distinct MAPK cascades. Many MAPKs activate specific effector kinases, the so-called MAPK-activated protein kinases (MAPKAPKs), and are inactivated by MAPK phosphatases (MKPs) that physiologically interact with MAPKKs. Only part of the MAPK pool that is recruited

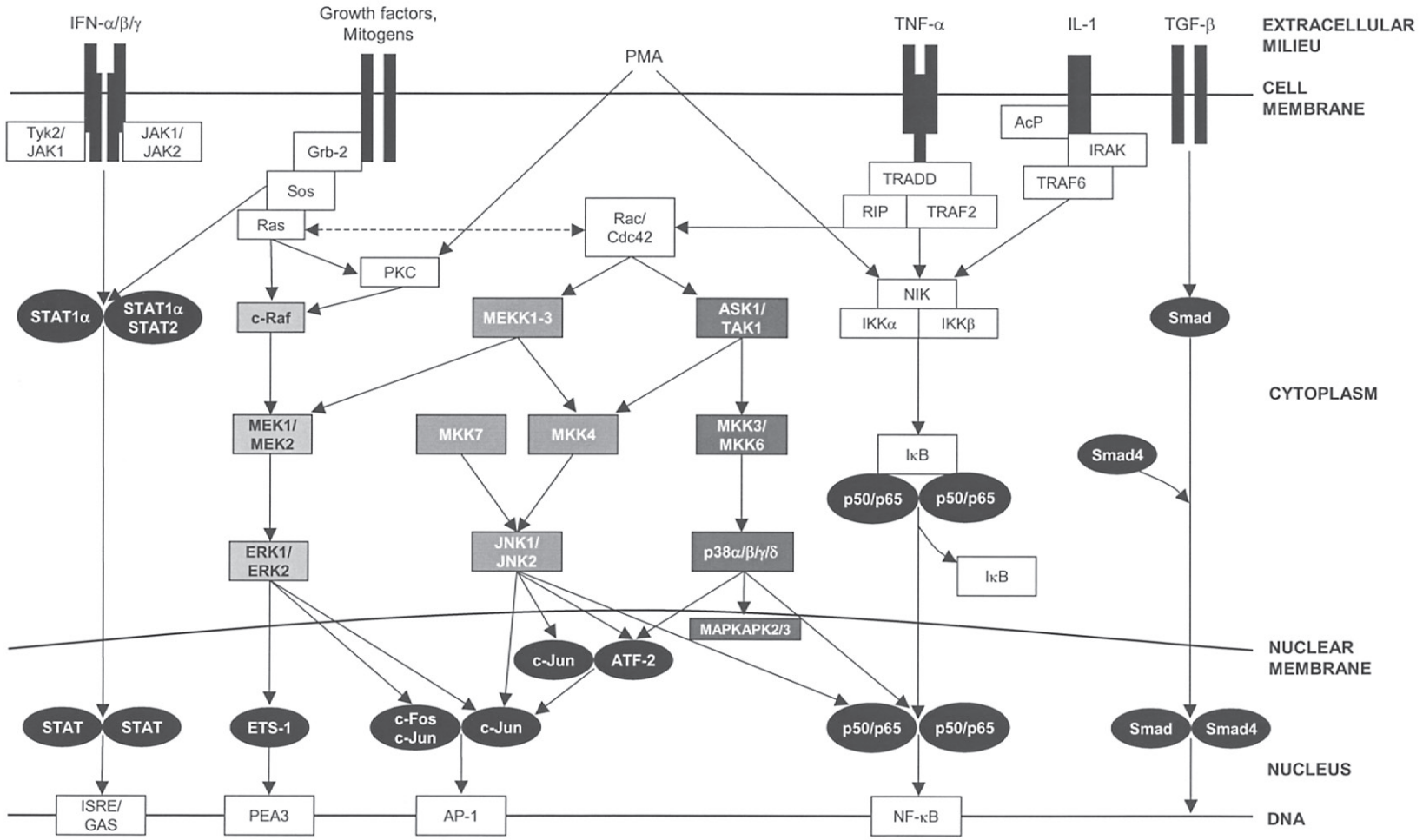

FIGURE 5. Intracellular signaling pathways that contribute to gelatinase (A and) B gene transcription. A basic scheme of the signaling cascades that are elicited by the most important stimulatory or inhibitory cytokines, growth factors, and phorbol esters is shown. The three different MAPK modules are depicted in gray boxes. Signal molecules that function in the cytoplasm are shown in a white box, whereas these that translocate to the nucleus to form complexes with co-activators or co-suppressors, or to act as transcription factors directly, are denoted in white letters within black ellipses. The arrows within and in between transduction pathways are based on publications about gelatinase B regulation and represent only part of the complexity that may exist in other regulatory systems.For clarification see list of abbreviations.

and activated on stimulation by extracellular inducers translocates to the nucleus and targets transcription factors that are prebound to DNA. Specific substrate recognition by the MAPKs is determined by the interactions involving distinct phosphoacceptor sites, composed of serine or threonine followed by a proline, and docking sites on the substrate. Once activated, JNKs phosphorylate activating transcription factor (ATF)-2 and c-Jun proteins, and thereby enhance their ability to activate c-*jun* transcription without affecting DNA binding. Most MAPKs, especially the ERKs, phosphorylate Ets transcription factors that are involved in early induction of c-*fos* genes, whose products heterodimerize with Jun proteins to form AP-1 complexes. Furthermore, ERKs act on c-Fos, and cAMP response element binding protein (CREB). The p38 proteins phosphorylate and enhance the activity of ATF-2, which can further mediate c-*jun* transcription through complexing with Jun proteins, and thus can affect AP-1 activity. Figure 5 illustrates this stepwise control of the transcription factor cascades. For further details on MAPK signaling, we refer to some excellent reviews (Cano and Mahadevan, 1995; Robinson and Cobb, 1997; Chang and Karin, 2001). Due to the occurrence of cytokine mixtures in complex organisms, the so-called cytokine soups, many feedback control interactions exist for these cascades. In general, enzyme cascades amplify signals, and therefore it seems unnecessary for all substrates and enzymes to become activated. In addition, to balance such chain reactions, compensation of kinases by phosphatases is provided as a shut-off mechanism.

The classic ERK mitogenic cascade is strongly activated after stimulation of cells with growth factors, serum, and phorbol esters. Additionally, this family of kinases has been implicated in diverse cellular responses, such as chemical or osmotic stress, cell differentiation, and migration. The JNK/SAPK and p38MAPK signaling pathways are only weakly activated by mitogens, but are highly stimulated on exposure to inflammatory cytokines, such as TNF-α and IL-1, and a wide variety of environmental stress inducers.

Different signaling cascades are involved in MMP regulation, depending on the stimulus, cell type, and the MMP. All three MAPK signaling pathways have been demonstrated to be involved in human gelatinase B gene regulation. Constitutive upregulation of gelatinase B through oncogenic transformation of human ovarian adenocarcinoma OVCAR-3 cells by v-Ras was reported to be mediated through a MEK1-independent signaling pathway (Gum *et al*., 1996). MAPK activity was essential for gelatinase B expression in oncogenic transformed rat embryo cells and in tumorigenic SCC cells, which display constitutive activation of both ERK and/or JNK/SAPK (Gum *et al*., 1997; Himelstein *et al*., 1997; Simon *et al*., 1999). In the SCC cells, phorbol ester-induced gelatinase B secretion required stimulation of the p38 MAPK pathway (Simon *et al*., 1998; Simon *et al*., 2001). Basal gelatinase B protein expression and promoter activity seem to be driven by the three MAPK signaling cascades

and rely on the activation of the proximal AP-1 motif (–79 bp) (Gum *et al*., 1997; Crowe *et al*., 2001; Simon *et al*., 2001).

Due to the lack of well-characterized regulatory elements in the gelatinase A gene promoter/enhancer sequence, historically the gelatinase A gene has been considered refractory to modulation, either inhibition or enhancement. Nevertheless, many inducing agents promote the conversion of specific MMP proenzyme forms to the active enzyme, or exert their effect by altering translated intracellular protein levels or the stability of secreted protein. These types of regulations may complement the regulation of gelatinase A activity. However, only a few reports describe regulatory pathways that induce gelatinase A gene transcription, and most of these are based on studies with rat cells. In these studies, constitutive activation of the Ras-MEK1 MAPK pathway, but not the JNK pathway, was shown to be critical and sufficient for the augmented activation and secretion of gelatinase A in Concanavalin A (ConA)-activated rat 3Y1 fibroblasts, and in v-*src*- (Thant *et al*., 1997; Kurata *et al*., 2000) and v-*crk*-transformed cells (Liu *et al*., 2000a). Signaling by MEK1 was not found to be active in gelatinase A secretion by human rheumatoid synovial fibroblasts, which underlines the variety of signaling modules involved in gelatinase A induction in different cell types (Smolian *et al*., 2001). A role for p38 activity in basal gelatinase A production was demonstrated in human ovarian cancer cells (Ellerbroek *et al*., 2001a).

The main stimulatory effects on gelatinase A gene transcription were demonstrated to be governed by the potent enhancer region r2 at position –1655 bp in the human (Frisch and Morisaki, 1990) and the functional analogous RE-1 region at –1322 bp in the rat promoter (Harendza *et al*., 1995). Both sequences were shown to bind the nuclear proteins YB-1 and AP-2. It was suggested that initial binding of AP-2 to double-stranded r2/RE-1 components, recruits the lower affinity YB-1 protein, which causes the unwinding of the DNA double-helix, and subsequently potentiates heteromer formation with additional single strand-specific transcription factors (Mertens *et al*., 1998; Mertens *et al*., 1999). The transcription factor AP-2 appears to direct transcriptional activation in response to two different signal transduction pathways, one involving the phorbol ester- and diacylglycerol (DAG)-activated protein kinase C (PKC), the other involving adenosine-3',5'-cyclic monophosphate (cAMP)-dependent protein kinase A (PKA) (Imagawa *et al*., 1987). Bottles *et al*. recently described a second promoter region between –1435 and –1375 bp in the rat gelatinase A gene, termed RE-2, that contributed substantially to the gelatinase A gene promoter activity in heart-derived endothelial cells from spontaneously hypertensive rats, but not in these of normotensive rats. It is not yet clear what factors associate with RE-2 (Bottles *et al*., 1999).

2.1.3.3. Modulation of mRNA Half-Life and Translational Efficiency

Modulation of mRNA half-life at the posttranscriptional level has been observed to be involved in the regulation of

gelatinase gene expression in response to inducers. For example, treatment of monocytic precursor U937 cells with phorbol 12-myristate 13-acetate (PMA) stimulated gelatinase B expression at the transcriptional level, and subsequent exposure to bacterial lipopolysaccharide (LPS) increased the half-life of gelatinase B mRNA (Saarialho-Kere *et al*., 1993). TGF-β1 upregulated gelatinase A in human gingival fibroblasts (Overall *et al*., 1991) and gelatinase B in human prostate cancer cell lines (Sehgal and Thompson, 1999) through increased mRNA stability. In the latter study, the TGF-β1-mediated induction of gelatinase B mRNA levels was found to require *de novo* synthesis of mRNA-stabilizing proteins, rather than decreased levels of destabilizing binding peptides. This posttranscriptional mechanism of MMP gene regulation may be mediated by activated MAPKs that remain in the cytoplasm and interact with cytoplasmatic target proteins (Chang and Karin, 2001).

In a recent study, in murine prostate carcinoma cells, translational efficiency of the gelatinase B mRNA in polysomes was found to be cell line dependent (Jiang and Muschel, 2002).

2.1.3.4. Transcriptional Control by Cytokines, Growth Factors, and Phorbol Ester

In specific cell types, human gelatinase B is induced by multiple polypeptide factors, including EGF, platelet-derived growth factor (PDGF), hepatocyte growth factor/scatter factor (HGF/SF), basic fibroblast growth factor (bFGF), TGF-α, amphiregulin, TNF-α, IL-1α, IL-1β, interferon (IFN)-α, IFN-γ, and TGF-β, as well as by phorbol ester stimulation. Phorbol esters mimic the activating signaling cascade of some of these cytokines (*vide infra* and see Table 5 for additional references). Downregulation of gelatinase B production is generally observed with IFN-β, IFN-γ, IL-4, and IL-10, and in some cell types with IFN-α, IL-1α, IL-13, EGF, PMA, and TGF-β. Modulation of human gelatinase A expression is executed by only a small subset of factors that act to increase or decrease gelatinase B production (Table 6). Gelatinase A is induced by TGF-β in mesangial cells, keratinocytes, glial cells, melanoma, adenocarcinoma, glioma, fibrosarcoma, and fibroblast cell lines, and by IL-1α and TNF-α in fibroblasts and smooth muscle cells, by IFN-α and IFN-γ in melanoma cells after short-term treatment, and by IL-1β in some fibroblasts. Its constitutive expression was also observed to be repressed by IL-1α, IL-1β, IFN-α, IFN-γ, and TNF-α in independent studies, and furthermore by IFN-β, TPA, EGF, HGF/SF, and TGF-α. In general, the main inducers that highly enhance gelatinase B production and only slightly alter gelatinase A levels are phorbol esters, growth factors, such as EGF, and the proinflammatory cytokines IL-1β and TNF-α. In addition, TGF-β, which downregulates most other MMPs, enhances the expression of both gelatinase A and B. It should be noted that LPS is also a very potent inducer of gelatinase A and B *in vitro*, which may act either directly or indirectly via cytokine stimulation on the regulation of gene expression. The effects of this bacterial component on gelatinase gene transcription are further discussed in Section 2.1.3.7. It is obvi-

ous that the view that we have about transcriptional control, and that stems from data of *in vitro* experiments, is rather a simplified version of the general picture *in vivo*. In complex organisms cytokines always occur as a mixture, and these soluble factors are complemented by solid-phase regulation through contact of cells with extracellular matrices and/or other cells. The different inducing and repressing signals are then integrated in the cell at the levels of signal transduction and transcriptional regulation.

2.1.3.4.1. Inducing Cytokines

Both IL-1 and TNF-α have been found to stimulate the production of most MMPs, including gelatinase B (Okada *et al*., 1990b; Lefebvre *et al*., 1991; Saren *et al*., 1996). Gelatinase A expression was found to be upregulated only in fibroblasts, smooth muscle cells, and $CD34^+$ stem cells, but remained unaffected in most cell types after treatment with the individual cytokines (Tables 5 and 6).

Signaling by inflammatory cytokines, such as IL-1β and TNF-α, at the nuclear level is accepted to be mainly mediated through activation of the transcription factors NF-κB and AP-1 via JNK/SAPK or p38 MAPK pathways (Baud and Karin, 2001). Following receptor-proximal events, various IL-1 and TNF-α receptor-associated factors are recruited, which are efficient activators of JNKs and p38 MAPKs (Figure 5). Phosphorylation by these MAPKs activates c-Jun, c-Fos, and ATF subunits, which may form a heterogeneous collection of dimeric AP-1 transcription factors. The pathway that leads to activation of NF-κB in response to inflammatory agonists involves more upstream components of the JNK signaling cascade, namely, MEKK1, MEKK2, and MEKK3, as well as the related MAPKKK, named NF-κB-inducing kinase (NIK). These MAPKKKs can activate the IκB kinase (IKK) complex, which consists of the IKKα and IKKβ subunits, and site-specifically phosphorylates IκB (Zhao and Lee, 1999). The latter is an inhibitor protein that retains the transcription factor NF-κB in its latent form as a complex of p50 (NF-κB1) and p65 (RelA) in the cytoplasm of nonstimulated cells. Once phosphorylated, IκBs are dissociated from the complex and proteolytically degraded by a cytosolic adenosine 5'-triphosphate-dependent protease complex, the 26S proteasome. After release, NF-κB translocates to the nucleus and is modulated further through phosphorylation by protein kinases, such as p38 MAPK, that are responsive to the stimulating mediator. At this point, a crosstalk with MAPK signaling pathways is possible (Baud and Karin, 2001).

NF-κB and/or AP-1 have been shown to regulate gelatinase B gene expression following TNF-α treatment of osteosarcoma cells, fibrosarcoma cells (Sato and Seiki, 1993), SCC cells (Ikebe *et al*., 1998; Beppu *et al*., 2002), dermal fibroblasts (Bond *et al*., 1998), vascular smooth muscle cells (Bond *et al*., 2001), salivary gland cells (Azuma *et al*., 2000), bronchial epithelial cells (Hozumi *et al*., 2001), and human skin (Han *et al*., 2001), and following IL-1 exposure in human dermal fibroblasts (Bond *et al*., 1998),

vascular smooth muscle cells (Bond *et al*., 2001), and rat glomerular mesangial cells (Yokoo and Kitamura, 1996; Eberhardt *et al*., 2000b). The influence of NF-κB on TNF-α-induced gelatinase B expression was substantiated further by the observation that pretreatment with synthetic proteasome inhibitors suppressed TNF-α-mediated NF-κB activation, as well as gelatinase B expression and cell migration of oral SCC cells (Ikebe *et al*., 1998).

The JNKs are particularly relevant in the TNF-α and IL-1-mediated induction of AP-1 activity, although the p38 MAPKs also affect AP-1 activity. Recent studies implicate the activation of the ERK pathway in TNF-α- and IL-1-stimulated expression of gelatinase B. For instance, Genersch *et al*. reported that, besides activation of the p38 MAPK pathway, TNF-α induction of gelatinase B expression in endothelial cells is also transmitted through sustained activity of ERK, independent of PKC and Ras signaling (Genersch *et al*., 2000). In another study, enhancement by TNF-α of gelatinase B production by *ras*-transformed keratinocytes was found to be dependent on p38 and ERK1/2 activity, with a role for JunB and c-Fos-containing AP-1 transcription factors (Johansson *et al*., 2000). Sustained ERK activation was also found to play a role in the induction by IL-1β in vascular smooth muscle cells and was dependent on IL-1β-stimulated superoxide generation (Gurjar *et al*., 2001b). Dual regulation by the c-Jun/AP-1 and tyrosine kinase-NF-κB pathways was demonstrated to be essential, but not sufficient, for the induction of gelatinase B by IL-1β in cultured rat mesangial cells (Yokoo and Kitamura, 1996), and a functional role for the ERK, JNK, and p38MAPKs was substantiated in these cells (Eberhardt *et al*., 2000b).

Besides IL-1β and TNF-α, other cytokines have also been shown to exert their upregulatory effect on gelatinase expression via MAPK signaling. In human monocytes/macrophages, for instance, IL-17 was found to induce gelatinase B production, which was related in part to autocrine stimulation by TNF-α, but was independent of IL-1β. The IL-17-stimulated macrophage signal transduction was mediated by both ERK1/2 and p38 MAPK, and IL-17-induced expression of AP-1 and NF-κB contributed to transactivation of the gelatinase B gene promoter (Jovanovic *et al*., 2000). Signaling via p38 MAPK was also observed to be required to upregulate gelatinase B secretion by both rat glioma cells and human T cells after binding of the chemokines stromal cell-derived factor 1α (SDF-1α) and macrophage inflammatory protein 1β (MIP-1β) to their receptors (Misse *et al*., 2001).

2.1.3.4.2. Inhibitory Cytokines

A limited number of cytokines are consistently reported to exert an inhibitory effect on basal or stimulated gelatinase B expression in various cell types, namely, IL-4, IL-10, IFN-γ, and IFN-β (Table 5). Gelatinase A expression was found to be reduced by the interferons only in tumor cells, including astroglioma (Qin *et al*., 1998), renal carcinoma (Gohji *et al*., 1994a), and metastatic melanoma cells (Hujanen *et al*., 1994). These findings may explain an inhibitory role for the interferons in tumor cell progression, because elevated levels of gelatinase A are known to be associated with invasive behavior. Unlike the generally observed inhibitory effect of IFN-β and IFN-γ on gelatinase

B expression, short-term treatment of highly metastatic human melanoma cells with (IFN-α and) IFN-γ (Hujanen *et al*., 1994), or treatment of human salivary gland cells with IFN-γ alone or combined with TNF-α (Wu *et al*., 1997), resulted in the upregulation of both gelatinases A and B.

The Th2 cytokines, IL-4 and IL-10, were shown to inhibit monocyte and macrophage production of gelatinase B at the pretranslational level (Corcoran *et al*., 1992; Lacraz *et al*., 1992; Wahl and Corcoran, 1993; Mertz *et al*., 1994; Lacraz *et al*., 1995; Mertz *et al*., 1996). This effect was observed to be cell type-specific (Lacraz *et al*., 1995) and resulted, in large part, from the inhibition of prostaglandin E_2 (PGE_2) synthesis, due to the suppression of membrane-bound prostaglandin H synthase (PGHS)-2. The underlying mechanism has been demonstrated by experiments in which monocyte MMP production was inhibited by the cyclooxygenase inhibitor indomethacin, and this suppression was reversed by exogenous agents, such as PGE_2 and dibutyryl cAMP (Bt_2cAMP), which elevate the intracellular levels of cAMP. These findings further substantiated the requirement of PGE_2 and cAMP stimulation for the induction of macrophage MMP synthesis. The initial steps in the PGE_2-cAMP pathway involve the phosphorylation and activation of cytoplasmatic phospholipase A_2 ($cPLA_2$) by MAP kinases. This $cPLA_2$ mediates liberation of arachidonic acid from membrane phospholipids, which is subsequently metabolized into prostaglandins, including PGE_2, by PG synthase. PGE_2 then activates adenylate cyclase resulting in increased levels of cAMP. cAMP in turn elevates the intracellular levels of ornithine decarboxylase, resulting in the generation of the polyamine putrescine, which can interact with DNA. Additional events are cAMP-mediated activation of PKA and transactivation of the production of c-Fos, c-Jun, and CREB proteins (Wahl and Corcoran, 1993). Other soluble stimulatory agents, reported to exert their promoting or inhibitory effect on monocyte/macrophage gelatinase B expression through the PGE_2/cAMP-dependent mechanism are IFN-γ (Wahl *et al*., 1990), Con A (Wahl and Corcoran, 1993), *Vibrio cholerae* and *Bordetella pertussis* toxin (Corcoran *et al*., 1994), SPARC (secreted protein, acidic and rich in cystein) (Shankavaram *et al*., 1997), LPS (Pentland *et al*., 1995; Shankavaram *et al*., 1998), and IL-17 (Jovanovic *et al*., 2000).

Besides the direct inhibition of PGE_2 synthesis, both IL-4 and IL-10 suppress many other monocyte/macrophage functions, such as the production of IL-1α, IL-1β, TNF-α, IL-6, IL-8, reactive nitrogen and oxygen intermediates, and class II MHC expression (Lacraz *et al*., 1992; de-Waal *et al*., 1993; Mertz *et al*., 1994). Because most of these products, for example, IL-1 and TNF-α (Smith *et al*., 1992), are capable of inducing PGE_2 formation and MMP production, they may indirectly be responsible for the suppressive effect of IL-4 and IL-10 on gelatinase B production in monocytes/macrophages. In cells other than monocytes/macrophages, it is likely that IL-4 and/or IL-10 inhibit gelatinase expression by a PGE_2/cAMP-independent mechanism. Recently, IL-4 was demonstrated to suppress gelatinase B gene expression, as well as protein production and activation in TNF-α-stimulated SCC cells. Because the cytokine also partially blocked NF-κB activation in the TNF-α-stimulated cells, it was suggested that IL-4 may suppress gelatinase B expression in tumor cells, and hence block tumor progression, by targeting

NF-κB signaling. Interleukin-10 did not show a similar effect (Beppu *et al.*, 2002).

In parallel with IL-4 and IL-10, the inhibition of monocyte/macrophage gelatinase B production by IFN-γ was also shown to result primarily from the suppression of eicosanoid synthesis (Wahl and Corcoran, 1993). While IFN-γ is generally considered to be an activator of monocyte/macrophage function, this cytokine also suppresses $cPLA_2$ activity, thus reducing the release of arachidonic acid needed for the endogenous synthesis of PGE_2. Decreased PGE_2 levels may then result in the observed IFN-γ-mediated downregulation of c-*fos* mRNA, whose product, in complexing with c-Jun, is an essential *trans*-activating factor mediating gelatinase B gene transcription. Another mechanism by which both IFN-γ and IFN-β may exert their effects on gelatinase transcription is based on specific IFN signaling. Type I (IFN-α/β) and type II IFNs (IFN-γ), synthesized by virus-infected cells, and by activated T cells and NK cells, respectively, signal through distinct but related pathways. Both types of interferon implicate Janus-protein tyrosine kinases (JAKs) and signal transducers and activators of transcription (STATs; see Figure 5) (Stark *et al.*, 1998). In the unliganded IFN-α/β receptor, subunit IFNAR1 associates with the Janus kinase Tyk2, whereas IFNAR2 associates with JAK1, STAT1α, and STAT2. Ligand-induced dimerization of the receptor at the cell surface results in a tyrosine phosphorylation cascade inside the cell that promotes the formation of STAT1α/STAT2 heterodimers. After the release of the activated heterodimers from the receptor, they are transported to the nucleus for DNA binding and stimulation of transcription. Together with p48, a member of the interferon regulatory factor (IRF) family, STAT1α and STAT2 can also form a heterotrimeric complex, known as latent cytosolic IFN-stimulated gene factor 3 (ISGF3). After translocation to the nucleus, ISGF3 binds to IFN-stimulated regulatory elements (ISRE, consensus sequence A/GGGAAANNGAAACT) in the promoter of target genes. A similar mechanism regulates the cellular response to IFN-γ. In unstimulated cells, the subunits of the IFN-γ receptor, IFNGR1 and IFNGR2, associate with JAK1 and JAK2, respectively. Binding of IFN-γ to the receptor subsequently induces oligomerization of the receptor subunits, which leads to the *trans*-phosphorylation and activation of the JAKs. Through phosphorylation, the activated JAKs recruit and bind two latent STAT1α proteins that are activated and dissociate from the receptor as a homodimer. The active STAT1α homodimers translocate to the nucleus, bind to specific gamma-activated sequence (GAS) elements of IFN-γ-inducible genes, and stimulate their transcription.

The presence of a putative GAS element in any of the gelatinase promoter/enhancers has not been reported, while the presence of ISRE elements in the gelatinase B gene is not clear. The complete ISRE consensus sequence is not present, but incomplete ISRE sequences (between positions –168 and –132 in the gene promotor of gelatinase A and at positions –509, –345, –295, and –151 in the gelatinase B gene promotor; see Table 3 and Figure 4) could possibly bind STAT-dimers and mediate the stimulatory effects of (IFN-α and) IFN-γ in melanoma cells (Hujanen *et al.*, 1994).

Alternatively, STAT can also bind transcriptional co-activators in IFN-γ- or IFN-β-regulated gene transcription (Ramana *et al*., 2000). The latter mechanism was suggested to contibute to transcriptional suppression of PMA- and TNF-α-mediated gelatinase B mRNA induction and protein synthesis by IFN-γ and IFN-β in a variety of cells, including human astroglioma cells, fibrosarcoma cells, and primary astrocytes. Possibly, the co-activators CREB-binding protein (CBP)/p300, which interact with AP-1 and NF-κB, and are important for optimal gelatinase B transcription, were recruited away from the transcription complex by activated STAT-1α (Ma *et al*., 2001).

2.1.3.4.3. Growth Factors

Most growth factors fail to regulate gelatinase A production in any cell type, whereas both normal and malignant cells respond with increased gelatinase B production (Tables 5 and 6). This is frequently observed in parallel with altered cellular function, including cell growth, differentiation, migration, and morphogenesis. These responses are regulated by receptor tyrosine kinase (RTK) activation and are ligand dependent (McCawley *et al*., 1999). For example, the activation of only a small subset of receptors, namely, of the EGF receptor and the HGF/SF receptor, by EGF, TGF-α, and HGF/SF, was able to induce gelatinase B expression in keratinocytes and promote cell motility, although multiple ligands are mitogenic for these cells (McCawley *et al*., 1998).

The EGF receptor (EGFr or ErbB1) is a transmembrane protein with intrinsic RTK activity that is activated after binding of ligand (e.g., EGF, TGF-α, amphiregulin). The activation of ErbB1 involves homo- and heterodimerization with other EGFr family members, such as ErbB2, and *trans*-phosphorylation of receptors at tyrosine autophosphorylation sites. These sites serve as binding sites for Src homology 2 (SH2) domains of a variety of small adaptor proteins that link different proteins involved in signal transduction, such as Grb-2 (Figure 5). Grb-2 itself forms a complex with the guanine nucleotide-releasing factor Sos via its SH3 domains. Thereupon, the Grb-2/Sos complex is recruited to an activated RTK, thus translocating Sos to the plasma membrane, where it is close to its target protein Ras and can stimulate exchange of guanosine 5'-diphosphate (GDP) for guanosine 5'-triphosphate (GTP). Once in the active GTP-bound state, Ras interacts with several effector proteins, such as Raf and phosphatidylinositol 3-kinase (PI-3K) (Figure 5). The Grb-2/Sos complex thus links a variety of surface receptors to a number of downstream Ras/MAP kinase signaling cascades. Within the MAPK family, the ERK and JNK pathways are typically activated by RTK-dependent signaling, and thus commonly stimulated by growth factors (Schlessinger, 2000). In several studies, growth factors induced gelatinase B expression via concerted and sustained ras-dependent (Chen *et al*., 1993) JNK and ERK signaling (McCawley *et al*., 1999; Zeigler *et al*., 1999; Hauck *et al*., 2001). For example, c-Jun was found to be activated exclusively by a distinct JNKK-JNK module, independent of ERK, whereas c-Fos expression was solely ERK-dependent in HGF- or EGF-stimulated keratinocytes. Their coordinated and prolonged activation was necessary to generate an increase in production of gelatinase B (Zeigler *et al*., 1999). By analogy with growth factor-induced sig-

naling, invasive behavior of tumor cells and coincident increase of gelatinase B have also been observed to be regulated by the ERK- and JNK-dependent signaling modules (Lakka *et al*., 2000). In recent studies, a role for the p38 MAPK signaling in growth factor-induced gelatinase B expression has also been demonstrated. Indeed, all three MAPKs were found to contribute to both basal and EGF-upregulated gelatinase B expression in human ovarian carcinoma (Ellerbroek *et al*., 2001a). Furthermore, augmented ERK and p38 kinase activities were involved in the upregulation of gelatinase B protein and mRNA levels by PDGF in rat arterial smooth muscle cells (Cho *et al*., 2000), and by heregulin-β1 in human breast cancer cells (Yao *et al*., 2001).

Regarding the involved promoter *cis*-elements, the closely spaced PEA3 (–541 bp) and AP-1 binding (–533 bp) sites were shown to potentiate activation of the gelatinase B gene promoter in human breast tumor cell lines in response to EGF. The transcription factors Ets-1 and Ets-2 were shown to mediate this response (Watabe *et al*., 1998). Fan and Karnovsky recently found increased gelatinase A activity, mediated at the mRNA levels, after treatment of vascular smooth muscle cells by connective tissue growth factor (CTGF). The AP-2 transcription factor was shown to be responsible for most of this transcription (Fan and Karnovsky, 2002).

2.1.3.4.4. Transforming Growth Factor-β

Transforming growth factor-β is a multifunctional cytokine belonging to the TGF-β superfamily, of which the synthesis, activation, and signaling are tightly regulated (Zhu and Burgess, 2001). TGF-β is synthesized as a biologically inactive precursor protein, which is a dimer containing the mature TGF-β and the pro-domain, called TGF-β latency associated protein (LAP). Its efficient secretion, correct folding, and deposition onto the ECM is mediated by a latent TGF-β-binding protein (LTBP) that associates with the latent TGF-β complexes and co-secretes with it. The activation of latent TGF-β complexes may occur after association with thrombospondin or $\alpha_v\beta_6$ integrin, or by the proteolytical action of enzymes, including plasmin, gelatinase A, and gelatinase B (Yu and Stamenkovic, 2000). Active TGF-β then binds to a preformed heteromeric receptor complex of two distinct type I and type II serine/threonine kinase receptors, TβRI and TβRII, and forces a reorientation between the two receptor chains. Reorientation results in productive interactions between the kinase domains and formation of an active ligand-receptor complex. The TβRII subunit of the complex actually binds TGF-β, while TβRI serves to directly bind and phosphorylate downstream intracellular substrates, called Smads (Smad1/2/3/5/8), at the C-terminus. Smad6 and 7 may inhibit this activating process. Once phosphorylated, Smad2 and 3 dissociate from the complex, each forming a new complex with common Smad4 that subsequently moves into the nucleus (Figure 5). Smad activation and nuclear translocation can be prevented by several signaling systems, such as ras-MAPK. In the nucleus, Smad complexes associate with DNA-binding transcription factors (e.g., AP-1, ATF-2), co-activators (e.g., CBP/p300) or co-suppressors (e.g., Ski, SnoN) to regulate target gene expression (Zhu and Burgess, 2001).

Gene expression of most MMPs is inhibited by TGF-β, which can be medi-

ated by binding of a c-Fos-containing protein complex to the TIE, a *cis*-acting element found in the promoter region of the MMP genes, with the exception of the gelatinase A gene (Kerr *et al*., 1990). AP-1 sites reportedly cooperate with the TIE element in this TGF-β-mediated repression (Benbow and Brinckerhoff, 1997). Another mechanism involved in TGF-β-induced downregulation of MMP expression is the destabilization of mRNA (Rydziel *et al*., 1997).

In contrast to the repressive effect of TGF-β on most other MMPs, TGF-β increases both gelatinase A and gelatinase B expression in human peripheral blood monocytes (Wahl *et al*., 1993), keratinocytes (Salo *et al*., 1991; Johansson *et al*., 2000), bone-metastatic tumor cells (Duivenvoorden *et al*., 1999), prostate cancer cells (Sehgal and Thompson, 1999), cervical epithelial cells (Agarwal *et al*., 1994), and in various other nontumorigenic and tumorigenic cell lines. Transcriptional upregulation of the gelatinase genes by TGF-β has also been reported to occur by altering transcription levels or mRNA stability (Brown *et al*., 1990; Overall *et al*., 1991; Greene *et al*., 1997; Sehgal and Thompson, 1999; Han *et al*., 2001). Gelatinase B appears to be affected only by the combination of TGF-β with other cytokines in fibroblasts, and this finding was consistent throughout several studies. Furthermore, both gelatinases seem unresponsive to the cytokine in smooth muscle cells and gelatinase B expression can even be inhibited in some cell types, including B lymphocytes, fetal neurons, fibrosarcoma, and osteosarcoma (Tables 5 and 6).

Upregulation of the gelatinases after treatment with TGF-β was dependent on MAPK signaling, as recently reported in Ras-transformed human epidermal keratinocytes. In these cells, activation of MMP-9 expression, as well as slightly increased MMP-2 production, were observed with TGF-β, and both effects were dependent on the TGF-β-mediated activation of p38 MAPK and ERK1/2. By analogy with TNF-α stimulatory effects in these cells, TGF-β also upregulated the mRNAs for c-*jun*, *jun*B, and c-*fos*, and evidence was found for involvement of JunB and c-Fos-containing AP-1 dimers in induced gelatinase B expression. It was suggested that the effect of p38 MAPK on gelatinase B expression involved stabilization of the transcripts (Johansson *et al*., 2000).

2.1.3.4.5. Phorbol Esters

Constitutive gelatinase A expression is not altered by phorbol esters, whereas gelatinase B expression levels are highly upregulated in many cell types (Masure *et al*., 1990 and 1991; Opdenakker *et al*., 1991a and b; Houde *et al*., 1993). Phorbol esters, in particular phorbol-myristate-acetate (PMA) that is an analogue of the second messenger diacylglycerol (DAG), directly activate the serine/threonine kinase PKC (Nishizuka, 1984). Thereupon, most PKC isoforms are translocated from the cytosol to the plasma membrane. Here, PKC can phosphorylate various substrates, leading to enhanced, transient transcription of the early immediate protooncogenes c-*fos* and/or c-*jun* (Houde *et al*., 1993) through the classic mitogenic Raf-MEK-ERK cascade. By subsequent formation of the protein complex AP-1, the expression of genes containing AP-1 binding sites, such as *gelatinase B*, is activated (Masure *et al*., 1990; Mackay *et al*., 1992). Analysis of the upstream mediators of Raf in PMA signaling in endothelial cells revealed that PKC, but not Ras, activates Raf. Therefore, it was hypothesized that

PMA triggers two independent signaling pathways that may lead to gelatinase B expression, the PKC-Raf-MEK-ERK cascade, and another pathway involving Ras, but not ending in ERK activation (Genersch *et al*., 2000). Although PMA has been regarded in the past as a specific ERK activator, it was demonstrated recently that this tumor promoter activates all three MAPK subfamilies, in particular p38 MAPK. In an SCC cell line, PMA could induce gelatinase B secretion by stimulation of the p38 MAPK pathway and its downstream MAPKAPK-2 (Simon *et al*., 1998), and a role for p38α in the regulation of PMA-induced *gelatinase B* promoter activity was shown (Simon *et al*., 2001).

Although the transcription factor AP-1 is believed to be a major mediator of the effects of activated PKC (Angel *et al*., 1987), evidence exist that NF-κB, Ets, and Sp1 are also involved in the regulation of *gelatinase B* promoter activity by phorbol esters. Both the TRE (–79 bp) and the upstream binding element for Sp1 (–563 bp) contributed to PMA inducibility of gelatinase B in fibrosarcoma, hepatoma, and osteosarcoma cells (Sato and Seiki, 1993). In endothelial cells, PMA was found to require the synergistic action of *trans*-acting transcription factors binding upstream of the AP-1 motif at position –533 bp, including NF-κB, Sp1, Ets, and AP-1 (Genersch *et al*., 2000).

The absence of response elements for AP-1 and NF-κB in the gelatinase A gene promoter region may attribute to its lack of responsiveness to phorbol esters in most cells. Unlike the absence of a regulatory effect on gelatinase A production, the enzyme activity is often augmented in several cell types (Mackay *et al*., 1992; Nelissen *et al*., 2002b), and this effect has been shown to be regulated by PKC in highly invasive gliomas (Uhm *et al*., 1996). However, despite the missing functional *cis*-elements in both the human and rat gelatinase A genes, exposure of rat glomerular mesangial cells to phorbol ester, as well as $2PGE_2$ and cAMP analogues resulted in enhanced gelatinase A gene transcription and synthesis (Marti *et al*., 1993; Zahner *et al*., 1997). In the latter study, it was suggested that a functional AP-2 element may regulate the responses to these stimulatory factors. PGE_2-induced effects were substantiated to be mediated, at least in part, through the positive transcriptional action of the rat RE-1 enhancer element (Mertens *et al*., 1998).

2.1.3.4.6. Synergistic Induction

Compared with single inducers, combinations of cytokines and/or growth factors are generally more efficient in stimulating or inhibiting gene expression, because these lead to additive or synergistic modulating effects. The cooperation between two agents that function through different intracellular signal transduction modules, such as growth factors and cytokines, are generally most efficient. Gelatinase B gene expression has been observed to be modulated by several positive and negative synergisms between cytokines, growth factors, and/or PMA (Lacraz *et al*., 1992; Marshall *et al*., 1993; Hanemaaijer *et al*., 1993; Unemori *et al*., 1994; Sato *et al*., 1996b; Zhang *et al*., 1998; Jovanovic *et al*., 2000; Han *et al*., 2001). For example, both TNF-α and PDGF enhanced gelatinase B mRNA and protein expression in rat arterial smooth muscle cells and had a synergistic stimulatory effect when combined. The individual inducers acted via activation of p38 MAPK and ERKs, and the same MAPKs were

shown to contribute to the synergistic effect (Cho *et al*., 2000). IL-1α or TNF-α interacted synergistically with either PDGF or bFGF to stimulate gelatinase B secretion in rabbit and human dermal fibroblasts. PDGF and bFGF typically activated the ERK1/2 MAPK pathway, leading ultimately to activation of AP-1, whereas IL-1α and TNF-α activated RTK-independent pathways, leading to the rapid activation of NF-κB. When acting together, AP-1 and NF-κB synergistically upregulated gelatinase B, whereas the expression was rather unaffected by the individual transcription factors (Bond *et al*., 2001). In contrast to gelatinase B and in line with the limited means to alter gelatinase A expression by single stimulating factors, only additive effects on expression of gelatinase A by combined inducers are found in the literature (Unemori *et al*., 1994).

2.1.3.5. Transcriptional Control by Cell Adhesion

2.1.3.5.1. Basic Mechanisms of Cell-Cell and Cell-Matrix Interactions

Cell adhesion molecules (CAMs) are localized at the surface of the interacting cellular partners and belong to distinct protein families, namely, selectins, mucins, integrins, cell adhesion molecules of the immunoglobulin (Ig) superfamily, and cadherins. Their synthesis and cell surface expression can be upregulated under the influence of local stimuli, including cytokines. After stimulation, cell adhesion molecules on one cell bind to their respective complementary ligands presented on the other cell, facilitating the contact between both cells.

The selectins (E, P, and L selectins) are composed of an extracellular N-terminal lectin-like domain, an EGF-like domain, and variable numbers of short consensus repeats that exhibit homology with complement regulatory proteins. The extracellular parts are followed by transmembrane and short intracellular sequences. The lectin-like domains are mainly responsible for Ca^{2+}-dependent specific binding of glycosylated mucin-like ligands (e.g., P selectin glycoprotein ligand-1 via sialyl Lewis^x). Integrins are transmembrane heterodimeric glycoproteins comprised of α and β subunits. Most integrins bind ligands that are components of the ECM, but they can also bind to soluble ligands, such as fibrinogen, or to counterreceptors on other cells, such as intercellular adhesion molecules (ICAMs). The cytoplasmatic domains of the β chains are necessary and sufficient to target integrins to focal adhesion sites in a ligand-dependent manner, where they link to intracellular cytoskeletal complexes and bundles of actin filaments. The α cytoplasmatic domains regulate the specificity of the ligand-dependent interactions. Cell surface proteins of the Ig superfamily consist of a variable number of related Ig-like domains and bind mainly to integrins via heterotypic mechanisms, or to identical Ig superfamily members via homotypic interactions (e.g., platelet endothelial cell adhesion molecule [PECAM]-1 and neural cell adhesion molecule [NCAM]). Very late antigen (VLA)-4/vascular cell adhesion molecule (VCAM)-1 adhesion is predominantly responsible for prolonged cell adhesion at inflammatory sites, whereas the ICAMs mediate various critical intercellular adhesion events by engagement to their β_2 integrin (CD18) receptors, in-

cluding lymphocyte function-associated antigen (LFA)-1 (CD11a/CD18), Mac-1 (CD11b/CD18), and p150,95 (CD11c/CD18). The cadherin family is composed of transmembrane proteins that share an extracellular domain consisting of repeats of a cadherin-specific module. The classic cadherin subfamily, including N, P, R, B, and E cadherins, contain five such modules and are primarily calcium-dependent homotypic cell-cell adhesion molecules. After cell-cell adhesion, they localize in specialized sites, termed adherence junctions, where they establish linkages with the actin-containing cytoskeleton via intracellular proteins, called catenins, that bind to their cytoplasmatic domains (Juliano, 2002).

Cell-cell contact plays a fundamental role in the multistep progression of tumor cell invasion (Egeblad and Werb, 2002), as well as in the extravasation processes of leukocytes through the vascular endothelial basement membrane at sites of inflammation and subsequent invasion of the tissue (Springer, 1994). In leukocyte extravasation, which may be initiated after exposure to a local inflammatory trigger, the first cell-cell contact occurs with low affinity and is mediated by selectins and their mucin ligands. These multiple and reversible protein-sugar interactions result in the rolling of leukocytes onto the endothelial monolayer. Subsequently, leukocytes cease to roll and start to adhere more firmly through the engagement of rapid agonist-activated integrins, including VLA-4 ($\alpha_4\beta_1$), $\alpha_4\beta_7$, LFA-1 ($\alpha_L\beta_2$) and Mac-1, with their counterpartners. After strong protein-protein adhesion to the endothelium, an irreversible process starts whereby leukocytes migrate through the endothelial membrane. This involves integrins and cell adhesion molecules of the Ig superfamily, such as ICAM-1, VCAM-1, and PECAM-1.

Cellular invasion depends on the cooperation between adhesive and proteolytic mechanisms. To move through the ECM, cells must first adhere to it via cell-ECM contacts. The first, so-called primordial, contacts are highly labile, present at the cell front heading in the direction of migration, and involve anchoring onto the basement membrane collagen type IV constituent. These contents are rapidly remodeled through the specific degradation of collagen type IV by the proteolytic action of enzymes, such as the gelatinases. Migrating cells subsequently establish new contacts with fibronectin of the ECM in the central part of the cells. The latter are called focal contacts and possess a higher affinity than the primordial contacts. Both gelatinases A and B are actively involved in this cell migratory process (Legrand *et al.*, 1999), and co-localize with β_1 integrin that are incorporated into focal contacts (Partridge *et al.*, 1997). Numerous integrin-associated protein partners also exist, such as the receptor for uPA and the tetraspanins, that appear to be important in tethering ECM-degrading gelatinase activity to the adhesion sites (Chapman, 1997; Sugiura and Berditchevski, 1999).

2.1.3.5.2. Modulation of Gelatinase A and B Expression

It seems that gelatinases A and B, which contribute to ECM remodeling and have similar substrate specificity, can both be induced in particular cell types following firm adhesion to endothelium and to other cell types or matrix components. Indeed, the expression of gelatinase B is modulated by various cell-cell contact settings *in vitro*, involving human, murine, bovine, and rat cells (Table 7). Endothelial cells directly

Table 7. Regulation of gelatinase A and B expression by cell-cell contact

Species and producer cell type[a]	Cell line and/or cell origin[b]	Species and type of interacting cells[b]	Effect on gelatinase A[b]	Effect on gelatinase B[b]	Involved CAM Interaction[b]	References
Bovine endothelial cells	Retinal	Human glioblastoma (SNB19)	+	+	ND	(Nirmala *et al.*, 2000)
Human endothelial cells	HUVEC	Human polymorphonuclear leukocytes	NE (Activ)	NE	ND	(Schwartz *et al.*, 1998)
Human fibroblasts	Bone marrow	Human adenocarcinoma (breast, MDA-MB-231)	+	NE	ND	(Saad *et al.*, 2000)
	Dermal/ovarian tumor-derived	Human carcinoma (ovarian, PEO1, PEO14, SKOV3)	+	ND	ND	(Boyd and Balkwill, 1999)
	Skin	Human fibrosarcoma (HT1080)	+	+	ND	(Munaut *et al.*, 1995)
	HFL-1 (fetal lung)	Human carcinoma (colon, ALT-1)	NE	+	β_1	(Segain *et al.*, 1996)
Human monocytes	Mono Mac6	Human endothelial cells (HUVEC)	NE	+	ND	(Mostafa *et al.*, 2001)
	PB	Human T lymphocytes (BMS-2)	NE	+	CD40/CD40L	(Malik *et al.*, 1996)
		Human smooth muscle cells (airway)	+	+	ND	(Zhu *et al.*, 2001)
		Human chondrocytes (articular)	ND	-	ND	(Dreier *et al.*, 2001)
Human monocytic leukaemia	THP-1	Human endothelial cells (HUVEC)	NE	+	ND	(Amorino and Hoover, 1998)
		Human T lymphocytes; T lymphoblastoma (Jurkat)	ND	+	ND	(Lacraz *et al.*, 1994b)
		Human activated Th1, Th2 cells	ND	+	ND	(Chizzolini *et al.*, 2000)
		Human endothelial cells (HUVEC)	NE	NE	α-CD31/CD31	(Nelissen *et al.*, 2002b)
Human mononuclear cells	PB	Human endothelial cells (HUVEC)	NE	NE	α-CD31/CD31	(Nelissen *et al.*, 2002b)
		Human endothelial cells (HB-MVEC)	ND	+	LFA-1/ICAM-1	(Lou *et al.*, 1999)
Human myeloma cells	NCI-H929; XG-2; XG-6; SBN-1	Human mononuclear cells (bone marrow, stromal)	NE (Activ)	+	ND	(Barille *et al.*, 1997)
Human osteosarcoma cells	OST	Human fibroblasts (CCD18, S3, S4, B7)	NE	+	ND	(Kurogi *et al.*, 1996)
Human smooth muscle cells	Saphenous vein	Human T lymphocytes (PB)	NE (Activ)	+	CD40/CD40L	(Schonbeck *et al.*, 1997)
Human T lymphocytes	PB	Human glioblastoma (T98G)	+	ND	VLA-4/VCAM-1	(Kambara *et al.*, 1999)
Murine T lymphoma cells	164T2	Murine endothelioma (b-end.3)	NE	+	LFA-1/ICAM-1	(Aoudjit *et al.*, 1998)
Rat endothelial cells	RFC (microvascular)	Murine Th1 lymphocytes (C19)	+	NE	VLA-4/VCAM-1	(Romanic and Madri, 1994; Madri *et al.*, 1996; Graesser *et al.*, 1998)
Rat fibroblasts	REF (embryonal)	Rat embryo cell lines (H-*ras*, v-*myc* transformed, metastatic 2.8, 2.3, 2.10.1)	ND	+	ND	(Himelstein *et al.*, 1994; Himelstein *et al.*, 1998)
		Rat embryo cell lines (H-*ras*, E1A transformed, non-metastatic, RA1, 3, 4)	ND	-		
Human mast cell leukemia, $CD34^+$ mast cells	HMC-1	Human T lymphocytes; T lymphoblastoid cells (Jurkat)	NE	+ (exocytosis)	ND	(Baram *et al.*, 2001)

[a]Species and producer cell types are listed in alphabetical order. [b]Abbreviations used are : α-CD31, anti-CD31 monoclonal antibody; Activ, proteolytic activation of gelatinase to lower molecular weight forms; CAM, cell adhesion molecule; CD40L, CD40 ligand (gp39); ICAM-1, intercellular cell adhesion molecule-1; LFA-1, lymphocyte function-associated antigen-1; ND, not determined; NE, no effect; PB, peripheral blood; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4; +, inducing effect; -, repressive effect.

upregulate the expression of gelatinase B in monocytes (Amorino and Hoover, 1998; Mostafa *et al*., 2001) and in T cells (Aoudjit *et al*., 1998) via ICAM-1/LFA-1 interactions (Aoudjit *et al*., 1998; Lou *et al*., 1999). Activated T cells were capable of inducing gelatinase B expression in monocytes (Lacraz *et al*., 1994b; Malik *et al*., 1996) and mast cells (Baram *et al*., 2001) through direct intercellular contact. In addition, the interaction of CD40 on monocytes or smooth muscle cells with CD40 ligand (gp39) on T cells was shown to stimulate monocytic (Malik *et al*., 1996) and smooth muscle cell (Schönbeck *et al*., 1997) gelatinase B production. E-cadherin-mediated cell-cell contacts were involved in the downregulation of gelatinase B mRNA and protein levels in mouse SCC cells (Llorens *et al*., 1998). A relationship between gelatinase A production and intercellular adhesion events has also been observed. Transient upregulation of gelatinase A mRNA expression, protein, and activity in circulating murine $CD4^+$ Th1 cells or in T lymphocytes from patients with myelopathy was dependent on adhesion to VCAM-1-expressing endothelial or glioblastoma cells, respectively (Romanic and Madri, 1994; Kambara *et al*., 1999). Adhesion to rICAM-1 was not sufficient to elicit induction of gelatinase A (Romanic and Madri, 1994). Apparently, a functional relationship exists between LFA-1/ICAM-1 and gelatinase B, and between VLA-4/VCAM-1 and gelatinase A (Romanic and Madri, 1994; Madri *et al*., 1996; Lafrenie *et al*., 1996; Aoudjit *et al*., 1998; Graesser *et al*., 1998; Kambara *et al*., 1999; Yakubenko *et al*., 2000).

Besides *in vitro* experimental settings employing direct intercellular contact to investigate the influence of ligation of cell adhesion proteins on the expression of gelatinase A or gelatinase B, functional blockage or stimulation of cell surface adhesion receptors by their soluble or solid-phase purified antagonists have also been used (Table 8). Neutralizing antibodies are generally employed to identify the participating adhesive proteins by competition in an existing stimulatory interaction, whereas recombinant ligands and stimulatory antibodies may serve to mimic specific cell-cell encounters. The effect of these *in vitro* ligation events on gelatinase production is generally in good agreement with intercellular interaction studies, and it appears that immobilization of recombinant protein ligands, leading to effective adhesion and clustering of adhesion receptors, is often a critical step in promoting gelatinase A gene expression (Romanic and Madri, 1994; Yakubenko *et al*., 2000). The opposite has been observed in the induction of integrin receptor aggregation with antibodies. Antibodies seem to be more effective in gelatinase A upregulation when presented in a soluble form (Seftor *et al*., 1992; Seftor *et al*., 1993; Larjava *et al*., 1993; Chintala *et al*., 1996; Ellerbroek *et al*., 1999). Exposure to immobilized anti-integrin antibodies seems to result rather in the activation of latent progelatinase A (Stanton *et al*., 1998; Ellerbroek *et al*., 1999; Ellerbroek *et al*., 2001b). Both soluble and immobilized ligands and antibodies were able to induce gelatinase B expression (Larjava *et al*., 1993; Huhtala *et al*., 1995; Nelissen *et al*., 2002b). In general, multivalent ligand-receptor interaction, rather than simple ligand occupancy, seems to be required for induction of MMPs (Bafetti *et al*., 1998). However, cellular responses to engagement of cell adhesion proteins depend not only

Table 8. Regulation of gelatinase A and B expression after specific stimulation or neutralization of cell surface adhesion receptors

Species and producer cell type[a]	Cell line and/or cell origin[b]	CAM inducer or repressor[b]	Effect on gelatinase A[b]	Effect on gelatinase B[b]	Interacting CAM or ECM ligand[b]	References
Recombinant/transfected CAMs						
Human carcinoma	MCF-7 (breast)	N-cadherin (transfection)+bFGF	NE	+	ND	(Hazan *et al.*, 2000)
	COKFu (colon)	E-cadherin (transfection)	-	ND	ND	(Miyaki *et al.*, 1995)
	SW480 (colon)	$\alpha_v\beta_6$ (transfection)	NE	+	Col I	(Niu *et al.*, 1998; Agrez *et al.*, 1999)
Human fibroblasts	Gingival	sCD40L	NE	ND	CD40	(Wassenaar *et al.*, 1999)
Human fibrosarcoma	HT1080	VLA-4 (transfection)	+	ND	CS-1; VCAM-1	(Kawaguchi *et al.*, 1992)
Human mesenchymal cells	Fetal	sVCAM-IgG	NE (Activ)	NE	$\alpha_4\beta_1$	(Pender *et al.*, 2000)
Human monocytic leukaemia	THP-1	srCD31	NE	+	CD31	(Nelissen *et al.*, 2002b)
		irCD31	NE	NE		
Human squamous cell carcinoma	HN5	E-cadherin (transfection)	NE (Desactiv)	NE	ND	(Ara *et al.*, 2000)
Human smooth muscle cells	Aortic	Osteopontin+PDGF	NE	+	ND	(Bendeck *et al.*, 2000)
Human T lymphoblastoid cells	Jurkat	irVCAM-1	+	NE	$\alpha_4\beta_1$	(Yakubenko *et al.*, 2000)
		srVCAM-1	NE	NE		
Human T lymphocytes	PB	irVCAM-1	+	NE	$\alpha_4\beta_1$	(Yakubenko *et al.*, 2000)
		srVCAM-1	NE	NE		
Murine squamous cell carcinoma	HaCa4	E-cadherin anti-sense cDNA	NE	+	ND	(Llorens *et al.*, 1998)
Murine Th1 lymphocytes	Antigenic to MBP	irVCAM-1	+	NE	ND	(Romanic and Madri, 1994)
		irICAM-1	NE	NE		
Rat adenocarcinoma	R3327	E-cadherin (transfection)	-	ND	ND	(Luo *et al.*, 1999)
Rat glioma	BTC4; BT4Cn	NCAM-B (transfection)	ND	-	ND	(Edvardsen *et al.*, 1993)
Anti-CAM antibodies						
Canine epithelial cells	MDCK	sα-E-cadherin	ND	+	ND	(Fiorino and Zvibel, 1996)
Human carcinoma	LoVo C5 (colon)	sα-β_4	+	NE	LN	(Daemi *et al.*, 2000)
		sα-α_6; sα-β_1	NE	NE	ND	
	Hepatocellular	sα-$\alpha_3\beta_1$	NE (Desactiv)	NE	ND	(Giannelli *et al.*, 2001)
	DOV13 (ovarian)	sα-β_1 integrin	+	ND	Col I	(Ellerbroek *et al.*, 1999)
		iα-β_1	NE (Activ)	ND		
		iα-α_3; iα-β_1; iα-$\alpha_3\beta_1$	NE (Activ)	ND	Col I; FN	(Ellerbroek *et al.*, 2001b)

		iα-α_2; sα-α_2; sα-α_3; sα-β_1; sα-$\alpha_3\beta_1$	NE	ND		
Human endothelial cells	Capillary	iα-$\alpha_v\beta_3$; iα-β_1	NE (Desactiv)	ND	LN; VN	(Yan *et al.*, 2000)
Human epithelial cells	MDA-MB-231 (breast)	sα-α_3; sα-tetraspanin; iα-tetraspanin; iα-α_3	+	NE	LN	(Sugiura and Berditchevski, 1999)
Human fibrosarcoma	HT1080	iα-α_5; iα-β_1	NE (Activ)	ND	FN	(Stanton *et al.*, 1998)
		iα-α_6	NE	ND		
Human glioblastoma	SNB19; U251	sα-$\alpha_5\beta_1$	+	ND	FN	(Chintala *et al.*, 1996)
		sα-$\alpha_3\beta_1$	+	ND	Col; LN; FN	
Human keratinocytes	Mucosal	sα-α_3; sα-β_1	NE	+	FN	(Larjava *et al.*, 1993)
		sα-$\alpha_2\beta_1$	NE	NE	Col	
Human melanoma	A375M	sα-$\alpha_5\beta_1$	NE	ND	FN	(Seftor *et al.*, 1992)
		sα-$\alpha_v\beta_3$	+	ND	VN	
	C8161	sα-$\alpha_5\beta_1$	+	NE	FN	(Seftor *et al.*, 1993)
		sα-$\alpha_v\beta_3$	NE	NE	VN	
	LOX	sα-α_6; sα-β_1	NE	ND	LN	(Nakahara *et al.*, 1996)
	A375SM; M151; MC44H	sα-CD44	+	ND	(Col I; HN; FN; LN)	(Takahashi *et al.*, 1999)
Human mesenchymal cells	Fetal	sα-VLA-4	NE (Activ)	NE	$\alpha_4\beta_1$	(Pender *et al.*, 2000)
Human monocytic leukaemia	THP-1	iα-CD31	NE	+	CD31	(Nelissen *et al.*, 2002b)
		sα-CD31	NE	NE		
Human rhabdomyosarcoma	ND	sα-α_2; sα-α_3	+	ND	LN	(Kubota *et al.*, 1997)
Murine macrophages	RAW264.7	α-α_6 (= α-CD_w49f)	ND	NE	LN	(Khan and Falcone, 1997)
Rabbit fibroblasts	Synovial	iα-$\alpha_5\beta_1$	ND	+	FN	(Huhtala *et al.*, 1995)

[a]Species and producer cell types are listed in alphabetical order. [b]Abbreviations used are: Activ, proteolytic activation of gelatinase to lower molecular weight forms; bFGF, basic fibroblast growth factor; CAM, cell adhesion molecule; CD40L, CD40 ligand (gp39); Col, collagen; CS-1, connecting segment-1; Desactiv, disappearance of lower molecular weight activated forms; ECM, extracellular matrix; FN, fibronectin; HN, hyaluronate; ICAM-1, intercellular cell adhesion molecule-1; IgG, immunoglobulin G; LN, laminin; NCAM, neural cell adhesion molecule; ND, not determined; NE, no effect; PB, peripheral blood; PDGF, platelet-derived growth factor; VCAM, vascular cell adhesion molecule; VLA-4, very late antigen-4; VN, vitronectin; +, inducing effect; -, repressive effect. '+' between interacting substrates indicates a synergistic action. The prefices 'i' and 's' indicate either immobilized or soluble interacting agents, respectively, whereas 'r' stands for recombinant. Stimulating antibodies are indicated with 'α-'.

on ligand presentation, but also on the phenotypic characteristics of the cells involved, that is, the state of receptor activation, the density of receptors on the cell surface, and/or the presence of specific signaling pathways coupled to the adhesion receptor.

Because the gelatinases play a major role in ECM turnover by degrading gelatin, collagen types IV, V, VII, X, and XI, elastin, laminin, fibronectin, and proteoglycan core protein, the main adhesive event that regulates their expression is the contact of cells with ECM components (Table 9). These interactions are mediated by αβ integrin receptors on the cell surface that recognize specific sequences in the matrix proteins, such as the Arg-Gly-Asp (RGD) motif in fibronectin (Werb *et al*., 1989). For example, expression of both gelatinases A and B was induced by intact fibronectin in lymphoid tumor cells (Esparza *et al*., 1999; Vacca *et al*., 2001) and in human SCC cells (Thomas *et al*., 2001a) through mediation by the α_v integrin subunit. Additionally, both MMPs were induced after contact of various tissue cells with different intact ECM components (Table 9). In a number of studies, peptide fragments of ECM components that were shown to act as a ligand for specific integrin receptors were employed for stimulation of integrin clustering, such as the RGD-containing or connecting segment (CS)-1-containing region of fibronectin, or peptides derived from the α chain of laminin. These peptides resulted in different effects on gelatinase A or B expression (Turpeenniemi-Hujanen *et al*., 1986; Werb *et al*., 1989; Kanemoto *et al*., 1990; Sang *et al*., 1991; Huhtala *et al*., 1995; Corcoran *et al*., 1995; Kapila *et al*., 1996; Esparza *et al*., 1999). Finally, culture of a variety of cells in a three-dimensional collagen gel stimulates the cellular activation of progelatinase A rather than its *de novo* expression, and induces cell surface expression of membrane type-1 matrix metalloproteinase (MT1-MMP) in a coordinate way (*vide infra*) (Fishman *et al*., 1998; Haas *et al*., 1998). This effect can be mimicked by clustering of β_1 integrin receptors with immobilized antibodies (Ellerbroek *et al*., 1999). It was also observed that the activation state of gelatinase A is directly influenced by the characteristics of cell-ECM binding, such as the density of the ECM component (Yan *et al*., 2000) and the nature of the ECM protein (Stanton *et al*., 1998).

Various integrin-mediated pathways for the production of gelatinases A or B are activated during tumor development to facilitate cell invasion. For instance, a role for $\alpha_3\beta_1$ integrin in maintaining gelatinase B production by transformed epithelial cells (DiPersio *et al*., 2000) and mammary carcinoma cells (Morini *et al*., 2000) was found and was absent in normal primary keratinocytes (Larjava *et al*., 1993; DiPersio *et al*., 2000). The induction of gelatinase A and increased invasiveness of human tumor cells resulted from the ligation of the vitronectin $\alpha_5\beta_1$ receptor (Seftor *et al*., 1993; Chintala *et al*., 1996). Increased expression of the β_6 subunit by cells derived from colon carcinoma, SCC, ovarian carcinoma, or normal keratinocyte cultures was found to be associated with higher secretion of both gelatinases. This resulted in a more invasive and/or migratory phenotype, compared with cells expressing other α_v integrin partners (Agrez *et al*., 1999; Thomas *et al*., 2001a; Thomas *et al*., 2001b; Ahmed *et al*., 2002a; Ahmed *et al*., 2002b). The moderately invasive ability of human melanoma cells *in vitro* was increased by blocking the fibronectin $\alpha_v\beta_3$ receptor,

Table 9. Regulation of gelatinase A and B expression by cell-matrix interactions

Species and producer cell type[a]	Cell line and/or cell origin[b]	ECM substrate[b]	Effect on gelatinase A[b]	Effect on gelatinase B[b]	Interacting CAM ligand[b]	References
Hamster fibrosarcoma	HSV-2-induced	Col IV; FN	NE	ND	ND	(Teale *et al.*, 1988)
		LN	+	ND		
Human carcinoma	8701-BC (breast)	Col III	+	-	ND	(Minafra *et al.*, 1995)
		Col V	+	+		
		OF/LB Col	+	NE		
	NOM1 (ovarian)	FN	NE	+	ND	(Shibata *et al.*, 1998)
	Epithelial (ovarian)	Col I	NE	ND	$\alpha_2\beta_1$	(Fishman *et al.*, 1998)
	DOV13 (ovarian)	Col I	NE	ND	$\alpha_3\beta_1$	(Ellerbroek *et al.*, 2001b)
		Gel I	NE	ND	ND	
	32PC (prostate)	Col I	NE	+	$\alpha_2\beta_1$	(Dong *et al.*, 2001)
Human cytotrophoblasts	ND	FN; LN; VN	NE	+	ND	(Xu *et al.*, 2001b)
		Col IV	NE	-		
	MDA-MB-231 (breast)	LN-5	+	NE	$\alpha_3\beta_1$-tetraspanin	(Sugiura and Berditchevski, 1999)
		Col I	NE	NE	ND	
	Bronchial	Col I and III	NE	+	ND	(Yao *et al.*, 1997)
		Col IV	NE	+	ND	(Yao *et al.*, 1998)
Human endothelial cells	Capillary	Low-density FN	NE	ND	$\alpha_v\beta_3$; β_1	(Yan *et al.*, 2000)
Human fibroblasts	Ovarian tumor-derived	Col I	NE	ND	β_1	(Boyd and Balkwill, 1999)
	Skin	Col I	+	+	ND	(Munaut *et al.*, 1995)
Human fibrosarcoma	HT1080	Col I	+	+	ND	(Munaut *et al.*, 1995)
		FN	NE	ND	$\alpha_5\beta_1$	(Stanton *et al.*, 1998)
		FN	NE	ND	ND	(Turpeenniemi-Hujanen *et al.*, 1986)
		LN	+	ND	ND	(Reich *et al.*, 1995)
Human keratinocytes	ND	Col I	NE	+	ND	(Sarret *et al.*, 1992)
		Col IV	NE	-		
	Oral	FN	NE	+	$\alpha_v\beta_6$	(Thomas *et al.*, 2001b)
Human lymphoid tumor cells	Namalwa; U266; CEM	VN; FN	+	+	$\alpha_v\beta_3$	(Vacca *et al.*, 2001)
Human macrophages	Alveolar	Col I, III, and IV; ES; FN; LN	ND	NE	ND	(Shapiro *et al.*, 1993)
Human melanoma	LOX	LN peptides (AG-10, AG-32)	NE	ND	$\alpha_6\beta_1$	(Nakahara *et al.*, 1996)
	A2058	LN; LN peptide (P1)	+	ND	ND	(Turpeenniemi-Hujanen *et al.*, 1986)
	A375SM	FN	NE	NE	ND	(Takahashi *et al.*, 1999)
Human monocytes	PB	LN peptide (SIKVAV); LN peptide (SIKVAV) + ConA	ND	+	ND	(Corcoran *et al.*, 1995)
		Col I + platelets	ND	+	$\alpha_2\beta_1$; PSGL-1	(Galt *et al.*, 2001)
Human monocytic leukemia	THP-1	Col IV; FN; LN; TN	ND	+	$\alpha_6\beta_1$	(Khan and Falcone, 1997)
Human myeloid leukemia	HL-60	FN	ND	+	$\alpha_5\beta_1$/FN	(Xie *et al.*, 1998a; Xie *et al.*, 1998b)
Human neuroblastoma	SK-N-SH	Col IV	+	+	$\alpha_3\beta_1$; $\alpha_v\beta_3$	(Tzinia *et al.*, 2002)

Table 9 (continued)

Human osteosarcoma	KHOS-240	Col I	+	ND	ND	(Broberg *et al.*, 2001)
Human periodontal ligament cells	ND	FN	NE	+	ND	(Kapila *et al.*, 1996)
		RGD-containing FN peptide (120FN)	+	NE		
Human rhabdomyosarcoma	ND	LN; Matrigel	+	ND	α_2; α_3	(Kubota *et al.*, 1997)
Human squamous cell carcinoma	VB6	FN	+	+	$\alpha_v\beta_6$	(Thomas *et al.*, 2001b)
	SCC4	Col I	ND	+	$\alpha_2\beta_1$	(Vo *et al.*, 1998)
Human T lymphoblastoid cells	CCRF-CEM; Jurkat	FN; LDV- or RGD- containing FN peptide	+	+	α_4; α_5; α_v	(Esparza *et al.*, 1999)
	Jurkat	iCS-1-containing FN peptide	+	+	$\alpha_4\beta_1$	(Yakubenko *et al.*, 2000)
		sCS-1-containing FN peptide	NE	NE		
Human T lymphocytes	PB	iCS-1-containing FN peptide	+	+	$\alpha_4\beta_1$	(Yakubenko *et al.*, 2000)
		sCS-1-containing FN peptide	NE	NE		
Murine macrophages	RAW264.7	Col IV; FN; LN; TN	ND	+	ND	(Khan and Falcone, 1997)
Murine melanoma	B16F10; B16F1 (lung)	VN	+	ND	ND	(Bafetti *et al.*, 1998)
		RGD-containing VN peptide	NE	ND		
	B16F10 (lung)	FN	NE	ND	ND	(Turpeenniemi-Hujanen *et al.*, 1986)
		LN; LN peptide (PA22-2)	+	ND	ND	(Kanemoto *et al.*, 1990)
	B16F10; B16B16 (lung)	LN	+	ND	ND	(Reich *et al.*, 1995)
Rabbit bone cells	Neonatal	Col I + (hGH, hIGF-1)	+	NE	$\alpha_v\beta_3$	(Rousselle *et al.*, 2001)
		VN + (hGH, hIGF-1)	NE	NE	ND	
Rabbit fibroblasts	Synovial	FN; RGD-containing FN peptide (120FN)	NE	+	$\alpha_5\beta_1$	(Werb *et al.*, 1989; Huhtala *et al.*, 1995)
		CS-1-containing FN peptide	NE	-	$\alpha_4\beta_1$	(Huhtala *et al.*, 1995)
Rat endothelial cells	Capillary	Col I (3D)	+	NE	ND	(Haas *et al.*, 1998)
Rat peritubular cells	ND	LN	+	ND	ND	(Sang *et al.*, 1991)
		LN peptides (PA22, RGD, D6)	NE	ND		
Rat sertoli cells	ND	LN	+	ND	ND	(Sang *et al.*, 1991)
		LN peptides (PA22, RGD, D6)	NE	ND		
Rat smooth muscle cells	Vascular intima	Col VIII	+	+	$\alpha_2\beta_1$; $\alpha_1\beta_1$	(Hou *et al.*, 2000)

[a]Species and producer cell types are listed in alphabetical order. [b]Abbreviations used are: CAM, cell adhesion molecule; Col, collagen; ConA, concanavalin A; CS-1, connecting segment-1; ECM, extracellular matrix; ES, elastin; FN, fibronectin; Gel, gelatin; hGH, human growth hormone; hIGF-I, human insulin-like growth factor-I; LDV, Leu-Asp-Val; LN, laminin; ND, not determined; NE, No effect; OF/LB Col, onco-fetal/laminin-binding collagen; PB, peripheral blood; PSGL-1, P-selectin glycoprotein ligand-1; RGD, Arg-Gly-Asp; SIKVAV, Ser-Ile-Lys-Val-Ala-Val; TN, tenascin; VN, vitronectin; +, inducing effect; -, repressive effect; '+' between interacting substrates indicates a synergistic action. The prefices 'i' and 's' indicate either immobilized or soluble interacting agents, respectively.

and coincided with increased expression and secretion of gelatinase A (Seftor *et al*., 1992). Finally, binding of melanoma cell CD44 by a monoclonal antibody induced gelatinase A mRNA and protein expression, which was associated with enhanced cell migration and invasion (Takahashi *et al*., 1999).

2.1.3.5.3. Signaling Cascades

Upon adherence of circulating cells to other cells or matrix proteins, for instance, at sites of vascular injury, engagement of specific surface tethering molecules mediates outside-in signaling and synthesis of gene products by the communicating cells. A number of considerations can be made about this. First, the potential requirement of convergent signaling pathways for cells to synthesize MMPs in response to adhesion has been demonstrated. For instance, the coordinated interaction of monocytes with activated platelets and collagen synergistically induced the protein expression of gelatinase B. Multiple transcripts were generated when monocytes adhered to ECM proteins via β_1 integrins and a second signal was required for these mRNAs to be translated into the corresponding proteins (Galt *et al*., 2001). Second, it seems that signals transduced through the same integrin receptor after engagement of different ligands can lead to differential intracellular signals and MMP gene expression (Yakubenko *et al*., 2000). Furthermore, specific fragments of ECM constituents may differentially induce the expression of MMPs, by activating different integrin receptors (Kapila *et al*., 1996). Third, in contrast to *in vitro* adhesion systems, adhesion of leukocytes to corresponding extracellular ligands *in vivo* is not a stationary process, but initiates a migratory response and involves signaling that is produced by the binding of various receptor/ligand pairs. Finally, the expression of numerous genes is modulated after cell adhesion, including those of cytokines, growth factors, etc. Because MMP expression is regulated by cytokines, chemokines, and growth factors (see previous sections), transcriptional regulation of gelatinases A and B in response to adhesion may occur indirectly via these stimulatory factors and may involve the downstream signaling elicited by these factors. For instance, the gelatinase A level in the culture supernatants of human renal carcinoma was increased by their cultivation with mouse kidney or lung fibroblasts, which was demonstrated to be attributable partially to fibroblast TGF-β (Gohji *et al*., 1994b). Mast cell-T cell heterotypic adhesion upregulated mast cell gelatinase B expression, as well as expression and release of TNF-α, which was shown to regulate induction of gelatinase B expression (Baram *et al*., 2001). Thus, the overall mRNA production and protein output is a concerted cellular response that results from a crosstalk between all components of the migratory process.

Adhesion molecules that play a role in cell-cell contact, including Ig CAMs, selectins, and cadherins, have all been linked to various signaling processes. The same signaling pathways that are activated by growth factor receptors seem to be used by the cell-cell adhesion molecules (Figure 5). For example, exocytosis of latent progelatinase B from human neutrophils, induced by cross-linking of L selectin and Mac-1, was partially dependent on tyrosine phosphorylation (Wize *et al*., 1998). However, a considerable number of distinct mechanisms exist and remain to be explored. A common observation is the

crosstalk between the members of the different adhesion receptor families and integrin family members. Because transcriptional regulation of the gelatinase A and B genes by cell-cell interactions has not been studied much in detail yet, we refer to an excellent review for the general signaling picture (Juliano, 2002).

In contrast, significant insight into integrin signaling has accumulated during the last decade. After binding of integrins to arrays of ECM components, cells are anchored to specialized focal contact sites. At these sites, clustering of the integrin receptors is observed and triggers multiple signaling cascades that directly lead to MAPK activation and regulate target gene expression. In one of these cascades, cross-linking of integrins may result in tyrosine autophosphorylation of a non-receptor protein tyrosine kinase (PTK), named focal adhesion kinase (FAK or $pp125^{FAK}$). FAK is associated with the cytoplasmatic tail of various integrin β subunits via specialized cytoplasmatic proteins, including talin, vinculin, and paxillin. The major tyrosine autophosphorylation site pTyr-397 of FAK serves as a binding site for the SH2 domain of Src family PTKs, which phosphorylate additional tyrosines. This creates binding sites for other SH2 domain-containing adapter proteins, such as PI-3K and the Grb-2/Sos complex. Binding of Grb-2/Sos at pTyr-925 may lead to activation of the Ras cascade and subsequently to activation of the ERK1/2 pathway (Juliano, 2002). Besides FAK-dependent ERK activation, the existence of a distinct FAK-independent integrin signaling pathway has also been demonstrated (Lin *et al*., 1997). Another pathway of ERK activation involves the association of certain integrin α subunits with the Src-family kinase Fyn via the transmembrane protein caveolin-1. Fyn becomes activated after binding and subsequently causes tyrosine phosphorylation of the small adapter protein Shc, which contains a phosphotyrosine binding (PTB) domain. By association of the Grb-2/Sos complex with Shc, the complex is recruited to the cell membrane. This then triggers Ras and the downstream ERK cascade (Juliano, 2002). PI-3K activation, downstream of Ras, as well as Ras-independent mechanisms that signal via PKC upstream of Shc, may also activate ERKs (Clark and Brugge, 1995).

Besides activation of the ERKs, the JNK and p38 MAPK cascades have also been reported to be directly activated by integrin engagement. Therefore, integrins activate small GTP-binding proteins of the Rho family that regulate focal adhesion formation and actin skeleton organization (Clark and Brugge, 1995). Rho, in turn, activates PI-5K and the JNK MAPKs, whereas other members of the Rho subfamily, Rac-1 and Cdc42, activate PI-3K (Lin *et al*., 1997). The activation of p38 MAPK can also be triggered through independent pathways, but information on this is limited (Juliano, 2002).

Besides directly activating MAPKs, integrin signaling pathways may synergize with other pathways, especially those of growth factor receptors, receptors coupled to G-proteins, and cytokine receptors, to enhance or dampen signals elicited by each receptor. For instance, cell adhesion via integrin receptors leads to activation of several RTKs, via co-clustering with the receptors for insulin, EGF, PDGF, and FGF (Juliano, 2002). Stimulation of MAPKs ultimately leads to activation of several transcription factors, such as Ets-1, AP-1, and NF-κB, which may subsequently be involved in the transcriptional regulation of a number of ECM-degrading enzymes, including MMPs and serine proteases (see Figure 5). Signaling pathways that link activation of integrin receptors with gelatinase A and gelatinase B

production are still poorly understood, sometimes contradictory, and need further clarification. For instance, contact between lymphoid tumor cells and fibronectin, mediated by $\alpha_v\beta_3$, was found to rapidly recruit and activate cytoskeletal proteins, the tyrosine kinases FAK and pp60src, the adapter protein Grb-2, and the MAP kinase ERK2, which resulted in increased release of gelatinase A and gelatinase B activity (Vacca *et al*., 2001). In contrast, T lymphocytes in contact with the same ECM substrate transduced inhibitory signals for the expression of gelatinase A and gelatinase B through the Ras/Raf/ERK cascade, as well as through p38 MAPK, whereas only Src-type tyrosine kinases were observed to play a role in enhancing expression (Esparza *et al*., 1999). Furthermore, signaling via PI-3K was involved in the interaction of fibronectin with ovarian cancer cells (Thant *et al*., 2000), but not with T lymphocytes (Esparza *et al*., 1999). Gelatinase B secretion was observed to be induced in a PKC-dependent manner by enhanced expression of the growth-promoting integrin $\alpha_v\beta_3$ in colon cancer cells (Niu *et al*., 1998; Niu *et al*., 2001) or after contact of fibroblasts with primary tumor cells (Segain *et al*., 1996), but not after cell-fibronectin contact (Esparza *et al*., 1999; Thant *et al*., 2000). The activation of ERK1/2 appears to be a constant requirement for enhanced expression of gelatinase B in ovarian cancer cells in contact with ECM components via specific integrin ligation (Shibata *et al*., 1998; Thant *et al*., 2000; Ahmed *et al*., 2002a), but not in SCC cells (Vo *et al*., 1998; Tsang and Crowe, 2001). Finally, phospholipase D and its product phosphatidic acid, which results from the hydrolysis of phosphatidylcholine and other phospholipids (Liscovitch *et al*., 2000), were shown to be elicited by laminin in metastatic tumor cells, leading to induction of gelatinase A and enhanced invasiveness (Reich *et al*., 1995).

In addition to clustered integrins, FAK, and MAP kinases, focal contacts also contain specialized cytoplasmatic proteins (e.g., talin, vinculin, paxicillin) that help to bridge the gap between integrins and actin filaments of the cytoskeleton. These proteins attribute to integrin-induced remodeling of the actin cytoskeleton, which has been proven to affect both upstream and downstream events in the RTK/Ras/MAPK pathway (Juliano, 2002). Alterations in actin cytoskeleton organization may be directly linked to the activation and regulation of *de novo* gelatinase A and gelatinase B production. This production was found to be controlled at least by signal transduction through PKC, PI-3K, and FAK, tyrosine kinases of the Src family, and p38 MAP kinases (MacDougall and Kerbel, 1995; Tomasek *et al*., 1997; Chintala *et al*., 1998; Sugiura and Berditchevski, 1999; Lambert *et al*., 2001). Upregulated gelatinase A and B gene transcription after disruption of actin stress fibers was independent of Rho kinase and ERK1/2 activity (Lambert *et al*., 2001).

The preferential use of consensus sites in the gene promoters for gelatinase A and gelatinase B after adhesive interactions has not yet been investigated thouroughly. As intracellular signaling elicited by cell-cell or cell-ECM contact ends in similar MAPK pathways as the ones used by cytokines and growth factors (see previous section), the same transcription factor binding sites are expected to be involved. A few studies that are related to cell-cell contact confirm this hypothesis. For example, PKC-dependent induction of gelatinase B secretion after contact of fibroblasts with primary tumor cells was observed to require an

organized actin cytoskeleton and to implicate the AP-1 transcription complex (Segain *et al*., 1996). In addition, Ets (–541 bp) and Sp1 (–563 bp) recognition sequences in the upstream promoter region of the gelatinase B gene has been found to be required for the full activition of gelatinase B expression in rat fibroblasts responding to tumor cell contact (Himelstein *et al*., 1998).

2.1.3.6. Transcriptional Control by Hormonal Factors

As discussed in Section 3.1, highly regulated MMPs control several physiologic processes, such as embryonic development, uterine involution, and wound healing, all which rely on turnover of matrix components. The latter processes are also modulated by hormones, including retinoids, thyroid hormone, glucocorticoids, progesterone, and androgens. These kinds of agonists penetrate directly through cellular membranes, bind to specific members of the nuclear receptor superfamily, and up- or downregulate transcriptional activity of specific MMP genes, in various cell types (Schroen and Brinckerhoff, 1996). In the multistep process of transcriptional regulation of MMP genes, hormones may enhance or suppress *trans*-activation of MMP promoters, which occurs primarily at AP-1 sites (Benbow and Brinckerhoff, 1997), alter transcription of TIMPs, cytokines, or growth factors that in turn regulate MMP transcriptional activity, or bind to co-activators, co-repressors, and components of the general transcription apparatus (Schroen and Brinckerhoff, 1996). Many hormones or hormone-mimicking compounds have been shown to modulate expression of gelatinase A and B in different mammalian cell types *in vitro*. Some examples are included in Table 10. The general picture is that gelatinase A expression is consistently upregulated under influence of 17β-oestradiol in a receptor-dependent manner, which was suggested to involve the promoter AP-2 consensus site and signaling via MEK1/2-ERK activation (Wingrove *et al*., 1998; Guccione *et al*., 2002). Upregulation of gelatinase B was also induced by oestradiol in mesangial cells (Potier *et al*., 2001), although this hormone prevented LPS-induced production of gelatinase B in primary microglia cultures (Vegeto *et al*., 2001). In line with a tight regulation by other factors, gelatinase B appears to respond positively to triiodothyronine (Pereira *et al*., 1999), parathyroid hormone, 1,25-dihydroxyvitamin D3 (Meikle *et al*., 1992), and the relaxins H1 and H2 (Qin *et al*., 1997a; Qin *et al*., 1997b).

The production of both MMPs has been demonstrated to be inhibited by dexamethasone (Shapiro *et al*., 1991; Xie *et al*., 1994b; Houde *et al*., 1996; Mautino *et al*., 1997; Cha *et al*., 1998; Beppu *et al*., 2002), all-*trans*-retinoic acid (Nakajima *et al*., 1989; Braunhut and Moses, 1994; Fisher *et al*., 1996; Fisher *et al*., 1997; Vo *et al*., 1998; Tsang and Crowe, 2001), and progesterone (Marbaix *et al*., 1992) in various cells. Dexamethasone, a synthetic glucocorticoid that potently downregulates the immune response, was found to inhibit PMA-induced gelatinase B expression in fibrosarcoma cells by promoting translocation of the glucocorticoid receptor from the cytosol to the nucleus, which downmodulates AP-1 *trans*-activity (Cha *et al*., 1998). Additionally, dexamethasone blocked TNF-α-induced gelatinase B expression in SCC cells by targeting NF-κB (Beppu *et al*., 2002). Similarly,

Table 10. Regulation of gelatinase A and B expression by hormonal factors

Species and producer cell type[a]	Cell line and cell origin[b]	Inducer/repressor[b,c]	Effect on gelatinase A[b]	Effect on gelatinase B[b]	Induced regulatory elements/ pathways[b,d]	Required regulatory promoter elements[b,d]	References
Hormones							
Bovine endothelial cells	Aortic	TSP-1	NE	+	ND	ND	(Qian *et al.*, 1997)
Human carcinoma	ND-1; DU145; Tsu-Pr1 (prostatic)	Bombesin; calcitonin; neuromedin N; neurotensin	NE	+	ND	ND	(Sehgal and Thompson, 1998)
	Tera 2 (embryonic)	RA; RA/PMA	+	+	ND	ND	(Tienari *et al.*, 1994)
		RA/EGF	+	NE			
	PC-3ML (prostate)	Taxol	-	ND	ND	ND	(Stearns and Wang, 1992; Stearns and Wang, 1994)
	DU145 (prostate)	Calcitonin; bombesin; neurotensin; neuromedin N	NE	+	ND	ND	(Sehgal and Thompson, 1998)
	T47D; MCF-7; MDA-MB231 (breast)	E_2; progestin	NE	ND	ND	ND	(van den Brule *et al.*, 1992)
Human endometrium	Explants	Progesterone	-	-	ND	ND	(Marbaix *et al.*, 1992)
Human endothelial cells	Microvascular	Retinol	+	+	ND	ND	(Braunhut and Moses, 1994)
		RA	-	-			
Human epidermis	Skin	UVB/RA	NE	-	AP-1	[TRE]	(Fisher *et al.*, 1996; Fisher *et al.*, 1997)
Human fetal membrane	Explants	Relaxin H1, H2	NE	+	ND	ND	(Qin *et al.*, 1997a; Qin *et al.*, 1997b)
Human fibroblasts	WI-38	RA	-	ND	ND	ND	(Brown *et al.*, 1990)
Human fibrosarcoma	HT1080	RA+Bt_2cAMP	+	ND	cAMP	CRE (-302 bp)	(Hasan and Nakajima, 1999)
		PMA/(DEX, ursolic acid)	NE	-	c-Jun, AP-1	TRE	(Cha *et al.*, 1998)
Human granulosa-lutein cells	ND	E_2	+	ND	ND	ND	(Puistola *et al.*, 1995)
		E_2/GnRH-a	-	ND			
Human keratinocytes	Newborn foreskin	RA	+	NE	ND	ND	(Kobayashi *et al.*, 1998a)
Human macrophages	Alveolar	DEX	ND	-	ND	ND	(Mautino *et al.*, 1997)
		DEX; LPS/DEX	ND	-	ND	ND	(Shapiro *et al.*, 1991)
		$1,25(OH)_2D_3$	ND	-	ND	ND	(Lacraz *et al.*, 1994a)
		Staphylococcus aureus/$1,25(OH)_2D_3$	ND	-			
	PB	LPS/E_2	ND	-	ND	ND	(Vegeto *et al.*, 2001)
Human melanoma	HT-144	RA	+	ND	ND	ND	(Brown *et al.*, 1990)
Human monocytic leukaemia	U937; THP-1	$1,25(OH)_2D_3$	ND	NE	ND	ND	(Lacraz *et al.*, 1994a)

Table 10 (continued)

		PMA/1,25$(OH)_2D_3$	ND	-			
	THP-1	DEX	ND	NE	ND	ND	(Van Ranst *et al.*, 1991)
	U937	RA	NE	NE	ND	ND	(Watanabe *et al.*, 1993)
Human neuroblastoma	SKNBE	RA; RA+(PMA, TNF-α)	NE	+	ND	ND	(Chambaut-Guerin *et al.*, 2000)
Human osteoblasts	Bone	PTH; 1,25$(OH)_2D_3$	NE	+	ND	ND	(Meikle *et al.*, 1992)
Human skin	Skin	Clobetasol-17-propionate	NE	-	ND	ND	(Oikarinen *et al.*, 1993)
		RA	NE	NE			
Human smooth muscle cells	Artery (coronary and umbilical)	E_2	+	ND	ND	ND	(Wingrove *et al.*, 1998)
Human squamous cell carcinoma	SCC4	RA	ND	-	Ets-1, ERK1	PEA3 (-541, -554 bp)	(Vo *et al.*, 1998; Tsang and Crowe, 2001)
	SAS	TNF-α/DEX	NE	-	NF-κB	[NF-κB]	(Beppu *et al.*, 2002)
Murine macrophages	RAW264.7	CGP41251/DEX	ND	-	[NF-κB, RAR]	[NF-κB]	(Houde *et al.*, 1996)
		(LPS, CGP41251)+RA	ND	+			
	Peritoneal	LPS/DEX	-	-	ND	ND	(Xie *et al.*, 1994b)
Murine mesangial cells	Kidney	E_2	+	ND	AP-2, ERK	[AP-2]	(Guccione *et al.*, 2002)
Murine mesangial cells	Kidney	E_2	ND	+	[AP-1, NF-κB]	ND	(Potier *et al.*, 2001)
Rat adenocarcinoma	13762NF (mammary)	RA; 13-cis-RA; retinol; phenyl RA; TTNPB; TMMP RA; Ch55	-	-	ND	ND	(Nakajima *et al.*, 1989)
Rat microglia	Newborn brain	LPS/E_2	ND	-	ND	ND	(Vegeto *et al.*, 2001)
Rat osteoblasts	MC3T3	T_3	NE	+	Not PGE_2	ND	(Pereira *et al.*, 1999)
Rat osteosarcoma cells	Osteoblast; UMR106-01	PTH	NE	+	ND	ND	(Vernillo *et al.*, 1990)
		PTH/PHT	NE	-			
Prostaglandins/cAMP							
Human fibrosarcoma	HT1080	8-bromo-cAMP	+	NE	PKA	ND	(Tanaka *et al.*, 1995)
Human T lymphoblastoma	Tsup-1	PGE_2; LTB_4	+	+	ND	ND	(Leppert *et al.*, 1995a)
Human mesangial cells	Kidney	PMA/PGI_2	NE	-	PKA, c-Jun	ND	(Kitahara *et al.*, 2001)
Human monocytic leukaemia	THP-1	Bt_2cAMP	ND	NE	ND	ND	(Van Ranst *et al.*, 1991)
Rat hepatocytes	Fetal	PGE_2	+	+	PKA, p38 MAPK, PI-3K, NF-κB	ND	(Callejas *et al.*, 2001)
Rat mesangial cells	Glomerular	PGE_2; 8-bromo-cAMP	+	ND	cAMP	[AP-2]	(Zahner *et al.*, 1997)

[a]Species and producer cell types are listed in alphabetical order. [b]Abbreviations used are: AP-1/2, activating protein-1/2; Bt_2cAMP, dibutyryl cAMP; cAMP, adenosine-3',5'- cyclic monophosphate; c-Jun, product of *jun* oncogene from an avian sarcoma retrovirus, encoding a transcription factor; CGP41251, protein kinase C inhibitor; Ch55, 3,5-di-*tert*-butyl 4'-chalcone carboxylic acid; CRE, cAMP response element; DEX, dexamethasone; E_2, 17β-estradiol; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; Ets-1, transformation-specific protein produced by *ets* discovered in the E26 avian erythroblastosis virus, encoding a transcription factor; GnRH-a, gonadotropin-releasing hormone agonist; Jun, protein from an avian sarcoma retrovirus oncogene, causing fibrosarcoma tumors; LPS, lipopolysaccharide; LTB_4, leukotriene B_4; MAPK, mitogen-activated protein kinase; ND, not determined; NE, no effect; NF-κB, nuclear factor-kappa B; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D3; PB, peripheral blood; PEA3, polyomavirus enhancer A-binding protein-3; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; PHT, phenytoin; PI-3K, phophatidylinositol 3-kinase; PKA, protein kinase A; PMA, phorbol 12-myristate 13-acetate; PTH, parathyroid hormone; RA, all-*trans*-retinoic acid or tretinoin; RAR, RA receptor; T_3, triiodothyronine; TMMP, trimethylmethoxyphenyl; TNF-α, tumor necrosis factor-alpha; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; TRE, TPA response element; TSP-1, thrombospondin-1; TTNPB, tetrahydrotetramethyl naphthalenyl propenylbenzoic acid; UVB, ultraviolet B; +, inducing effect; -, repressive effect. [c]Whenever combined interacting agents are used, '+' indicates a synergistic action, whereas '/' separates the stimulating substance (in front) and the modulating compound (in the back). [d]Regulatory elements that are suggested by the authors to be involved in the induction/repression, but are not investigated, are put between brackets.

methylprednisolone, another synthetic glucocorticoid, was shown to inhibit the expression of gelatinase B in the injured spinal cord by suppression of the AP-1 and NF-κB transcription cascades via a glucocorticoid receptor mechanism (Xu *et al*., 2001a). The downmodulatory effect of retinoic acid on gelatinase B expression was attributed to reduced ERK1, Ets-1 (Tsang and Crowe, 2001), and AP-1 activity (Fisher *et al*., 1996), and at least involved the Ets-1 binding sites at positions –541 and –554 bp in the gelatinase B gene promoter region. A crosstalk between retinoic acid and integrin-dependent signaling was also observed (Vo *et al*., 1998; Tsang and Crowe, 2001). Retinoic acid has also been observed to exert a synergistic stimulatory effect on gelatinase B expression, in combination with PKC-modulating compounds (Houde *et al*., 1996). In addition, transfection experiments of human fibrosarcoma cells revealed enhanced transcription and basal promoter activity of the gelatinase A gene by a synergistic action of retinoic acid and dibutyryl cAMP. The distal AP-2 site in the enhancer element did not play a role, but a putative CREB-like element (consensus sequence 5'-TGACGTCC-3') at position –302 bp in the opposite strand could represent a functional mediator in this upregulation (Hasan and Nakajima, 1999).

2.1.3.7. Transcriptional Control by Other Factors

Another category of regulators of gelatinase B gene transcription are viral gene products and oncogenes. Many of these stimulate gelatinase B gene transcription, and often the TRE has been proposed as the regulatory interaction site (Table 11). In Section 2.1.3.4.2 we referred already to viral induction of gelatinase B to appraise the fact that virus infections are accompanied by cytokine induction *in vivo*. Cytokines are certainly involved, form the first day onward, in gelatinase B gene regulation after an acute viral infection, which most often resolves within a week. Here we want to stress the fact that specific viral products may have direct effects on gelatinase A and B expression. Viral oncogenes have received much attention a decade ago. Currently this interest has switched to tumor and metastasis suppressors and to the regulation of TIMP expression. Counterbalance of the activity of gelatinase A and B by upregulation of TIMPs may be a way how some metastasis suppressors or tumor-derived stimulatory factors (oncogene products) operate.

Similar direct effects, as with the products of viral or oncogenes, have also been observed with bacteria and bacterial products, such as lipoarabinomannan from *Mycobacteria* and LPS from *Escherichia coli*, with parasitic factors, plant products, including flavonoids and lectins, and with antibiotics (Table 12). To date, LPS or endotoxin is the most potent natural inducer of MMP biosynthesis and secretion by various cell types. Both gelatinase A and B are stimulated at the transcriptional and protein levels by LPS in monocytes and macrophages. LPS forms a high-affinity complex with LPS-binding protein (LBP) in human serum. This complex then binds to CD14 on the surface of monocytes and macrophages, modulating the expression of LPS-sensitive gene products. Intracellular signaling of LPS-stimulated gene expression occurs via activation of PI-3K, Jak-STAT, MAPKs, and NF-κB (Sweet and Hume, 1996; Kim and Koh,

Table 11. Regulation of gelatinase A and B expression by viral and oncogenes, and their products

Species and producer cell type[a]	Cell line and/or cell origin[b]	Inducer/repressor[b,c]	Effect on gelatinase A[b]	Effect on gelatinase B[b]	Induced regulatory elements/ pathways[b,d]	Required regulatory promoter elements[b,d]	References
Viral factors							
Human carcinoma	HeLa (cervical)	Mo-MuLV (infection); Mo-MuLV LTR (U3)-encoded *let* (transfection)	NE	+	c-Jun; AP-1	TRE (-79 bp)	(Weng *et al.*, 1995; Faller *et al.*, 1997)
	C33A (cervical epithelial)	EBV LMP-1; CTAR-1; CTAR-2	NE	+	AP-1; NF-κB	ND	(Takeshita *et al.*, 1999)
Human endothelial cells	HUVEC; HMEC-L	sHIV-1-Tat	NE	+	ND	ND	(Toschi *et al.*, 2001)
		sHIV-1-Tat+sbFGF	+	+			
Human fibroblasts	IMR-90 (lung)	SV40 (transformation) + (PMA, IL-1β, EGF)	NE	+	ND	ND	(Wilhelm *et al.*, 1989)
Human lymphocytes	PB; H9	HIV-1 (infection)	ND	+	ND	ND	(Weeks *et al.*, 1993a)
Human monocytes	PB	sHIV-1-Tat	ND	+	ND	ND	(Lafrenie *et al.*, 1996; Lafrenie *et al.*, 1997)
		HIV-1 (infection)	ND	+	ND	ND	(Dhawan *et al.*, 1992; Dhawan *et al.*, 1995)
Human monocytic leukaemia	U937	sHIV-1-Tat	ND	+	PTPases; NF-κB	ND	(Kumar *et al.*, 1999)
Human mononuclear cells	PB	HIV-1 (infection)	+	+	ND	ND	(Sundstrom *et al.*, 2001)
Human neuroectodermal cells	Dev	HTLV-1 (infection)	NE	+	[c-Fos; c-Jun]	[TRE]	(Giraudon *et al.*, 1995; Giraudon *et al.*, 1997)
Human T lymphocytes	Jurkat	sHIV-1-gp120	ND	+	p38 MAPK	ND	(Misse *et al.*, 2001)
Feline fibroblasts	AH927 (embryonic)	FeLV LTR (U3) (transfection)	NE	+	AP-1; MEK1/2	TRE (-79 bp)	(Ghosh and Faller, 1999)
Murine fibroblasts	Balb/c-3T3	FeLV LTR (U3) (transfection)	NE	+	AP-1; MEK1/2	TRE (-79 bp)	(Ghosh and Faller, 1999)

		Mo-MuLV (infection); Mo-MuLV LTR (U3)-encoded *let* (transfection)	NE	+	c-Jun; AP-1	TRE (-79 bp)	(Weng *et al.*, 1995; Faller *et al.*, 1997)
Rat glial cells	Embryonic	HTLV-1 (infection)	NE	+	[c-Fos; c-Jun]	[TRE]	(Giraudon *et al.*, 1995; Giraudon *et al.*, 1997)
Rat glioma	C6	sHIV-1-gp120	ND	+	p38 MAPK	ND	(Misse *et al.*, 2001)
Oncogene/oncogene products							
Human adenocarcinoma	OVCAR-3 (ovarian)	c-H-*ras* (transfection)	ND	+	Not MEK1	NF-κB (–600 bp); Sp1 (-563 bp); PEA3 (-541 bp); AP-1 (-79 p); RBE (-54 bp)	(Gum *et al.*, 1996)
Human epithelial cells	TBE-1 (bronchial)	c-H-*ras* (transfection)	+	ND	ND	ND	(Collier *et al.*, 1988)
		Ad5-*E1A* (transfection)	-	ND	AP-1	[TRE]	(Frisch *et al.*, 1990; Goldberg *et al.*, 1990)
Human fibroblasts	KMST-6 (embryonic)	c-H-*ras* (transfection)	NE	+	ND	ND	(Sato *et al.*, 1992)
	NIH-3T3	c-H-*ras* (transfection)	NE	+	ND	ND	(Ballin *et al.*, 1988)
Human fibrosarcoma	HT1080	c-Jun; JunB; v-Src (transfection)	ND	+	[AP-1; Sp1]	TRE (-79 bp); RBE (-54 bp)	(Sato *et al.*, 1993)
		c-Fos; ErbB-2 (transfection)	ND	NE			
		Ad5-*E1A* (transfection)	-	ND	AP-1	[TRE]	(Frisch *et al.*, 1990; Goldberg *et al.*, 1990)
Human hepatoma	Bel-7402	Ets-1 (transfection)	ND	+	Ets-1	ND	(Jiang *et al.*, 2001)
Human keratinocytes	HaCaT-A3 (epidermal)	c-H-*ras* (transfection) + TNF-α	NE	+	p38 MAPK; ERK1/2; c-Jun; c-Fos; JunB	ND	(Johansson *et al.*, 2000)
		c-H-*ras* (transfection) + TGF-β	+	+			
	RHEK-1 (epidermal)	c-H-*ras* (transfection) + EGF	ND	+	ND	ND	(Chen *et al.*, 1993)

Table 11 (continued)

Human melanoma	A2058	Ad5-*E1A* (transfection)	-	ND	AP-1	[TRE]	(Frisch *et al.*, 1990; Goldberg *et al.*, 1990)
Murine fibroblasts	NIH-3T3	c-H-*ras* (transfection)	+	ND	ND	ND	(Spinucci *et al.*, 1988)
Rat embryonic cells	2.10.10	c-H-*ras*+v-*myc* (transfection)	ND	+	ND	NF-κB (–600 bp); Sp1 (-563 bp); PEA3 (-541 bp); AP-1 (-79 p); RBE (-54 bp)	(Himelstein *et al.*, 1997)
Rat epithelial cells	NRK52E (kidney)	Activated c-H-*ras* (transfection)	NE	+	NF-κB	ND	(Yang *et al.*, 2001)
Rat fibroblasts	3Y1	c-H-*ras* (transfection)	+	NE	ND	ND	(Thant *et al.*, 1997)
		ConA+S17N *ras* (transfection)	-	-			
	Embryonic	c-H-*ras*, c-H-*ras*+v-*myc* (transfection)	ND	+	ND	ND	(Garbisa *et al.*, 1987; Bernhard *et al.*, 1990; Bernhard *et al.*, 1994)
		c-H-*ras*+Ad2-*E1A* (transfection)	ND	NE			
Metastasis-suppressor genes							
Human fibrosarcoma	HT1080	*KiSS-1* (transfection)	NE	-	NF-κB	[NF-κB]	(Yan *et al.*, 2001)
Tumor cell-derived stimulatory factors							
Human adenocarcinoma	MDA-435 (breast)	α-CD147; rCD147-Fc	-	NE	ND	ND	(Sun and Hemler, 2001)
		Purified CD147	+	NE			
Human fibroblasts	CCD-18 (colon)	TCSF	+	NE	ND	ND	(Kataoka *et al.*, 1993)
	Skin	EMMPRIN (CD147)	+	NE	ND	ND	(Guo *et al.*, 1997)

[a]Producer cell types are listed in alphabetical order. [b]Abbreviations used are: Ad2/5-*E1A*, adenovirus 2/5 type A E1A gene; AP-1, activating protein-1; bFGF, basic fibroblast growth factor; c-Fos, protein product of *fos* oncogene identified in a mouse osteosarcoma encoding a transcription factor; c-H-*ras*, cellular Harvey-*ras* oncogene; c-Jun, product of *jun* oncogene from an avian sarcoma retrovirus, encoding a transcription factor; ConA, concanavalin A; CTAR-1/2, C-terminal activation region-1/2; EBV, Epstein Barr virus; EGF, epidermal growth factor; EMMPRIN, extracellular matrix metalloproteinase inducer; ErbB-2, cell surface receptor for EGF; ERK, extracellular signal-regulated kinase; Ets-1, transformation-specific protein produced by *ets* discovered in the E26 avian erythroblastosis virus, encoding a transcription factor; FeLV, feline leukaemia virus; gp120, glycoprotein from HIV's envelope that binds to CD4; HIV-1, human immunodeficiency virus type-1; HTLV-1, human T cell lymphotropic virus type-1; IL-1β, interleukin-1beta; *KiSS-1*, metastasis-associated gene; LMP-1, latent membrane protein-1; LTR, long terminal repeat; MAPK, mitogen-associated protein kinase; MEK, MAPK/ERK kinase; Mo-MuLV, Moloney murine leukaemia virus; ND, not determined; NE, no effect; NF-κB, nuclear factor-kappa B; PB, peripheral blood; PEA3, polyomavirus enhancer A-binding protein-3; PMA, phorbol 12-myristate 13-acetate; PTPase, protein tyrosine phosphatase; RBE, retinoblastoma binding element; Sp1, stimulating protein-1; SV40, simian vacuolating virus n° 40; Tat, extracellular HIV protein; TCSF, tumor cell-derived collagenase stimulatory factor; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; TRE, TPA response element; v-*myc*, proto-oncogene found in the avian myelocytomatosis virus causing carcinomas and sarcomas; v-*src*, transforming (sarcoma inducing) gene of Rous sarcoma virus; +, inducing effect; -, repressive effect. [c]Whenever combined interacting agents are used, '+' indicates a synergistic action. The prefix 's' indicates soluble interacting agents, 'c-' means cellular, 'v-' means viral, 'α-' denotes an antibody, and 'r' stands for recombinant. [d]Regulatory elements that are suggested by the authors to be involved in the induction/repression, but are not investigated, are put between brackets.

Table 12. Regulation of gelatinase A and B expression by various factors

Species and producer cell type[a]	Cell line and/or cell origin[b]	Inducer/repressor[b,c]	Effect on gelatinase A[b]	Effect on gelatinase B[b]	Induced regulatory elements/ pathways[b,d]	Required regulatory promoter elements[b,d]	References
Bacterial/parasitical factors							
Bovine B cell lymphosarcoma	BL3; BL20	*Theileria annulata*	ND	+	c-Fos; AP-1; [NF-κB]	[TRE]	(Baylis *et al.*, 1995)
Human B lymphocytes	EBV-immortalized	LPS	ND	+	ND	ND	(Trocme *et al.*, 1998)
Human endothelial cells	HUVEC	LPS	NE (Activ)	NE	NF-κB	ND	(Kim and Koh, 2000)
Human epithelial cells	Bronchial	LPS	+	+	ND	ND	(Yao *et al.*, 1996; Yao *et al.*, 1997; Yao *et al.*, 1998)
Human macrophages	Alveolar	*Staphylococcus aureus*	ND	+	ND	ND	(Lacraz *et al.*, 1992; Lacraz *et al.*, 1994a; Lacraz *et al.*, 1995)
		LPS	+	+	ND	ND	(Welgus *et al.*, 1990)
		LPS	ND	+	PGE_2	ND	(Pentland *et al.*, 1995)
	Monocyte-derived	LPS	ND	+	ND	ND	(Saren *et al.*, 1996)
Human monocytes	PB	*Staphylococcus aureus*	ND	+	ND	ND	(Lacraz *et al.*, 1992; Lacraz *et al.*, 1994a; Lacraz *et al.*, 1995)
		LPS	ND	+	$cPLA_2$; PGE_2	ND	(Zhang *et al.*, 1998; Shankavaram *et al.*, 1998)
		ConA/*Vibrio cholera* toxin	NE	+	PGE_2; PGHS-2; cAMP	ND	(Corcoran *et al.*, 1994)
		ConA/*Bordetella pertussis* toxin	NE	-			
Human monocytic leukemia	THP-1	*Mycobacterium tuberculosis*; lipoarabinomannan; heat-killed *M. tuberculosis* H37Ra	NE	+	[c-Jun]	[TRE]	(Chang *et al.*, 1996)
		LPS	NE	+	ND	ND	(Van Ranst *et al.*, 1991; McMillan *et al.*, 1996b; Nelissen *et al.*, 2002b)
	U937	PMA+LPS	NE	+	ND	ND	(Saarialho-Kere *et al.*, 1993)
Human mononuclear cells	PB	LPS	NE	+	ND	ND	(Opdenakker *et al.*, 1991a; Nelissen *et al.*, 2002b)

Murine macrophages	Peritoneal	*Mycobacterium bovis* BCG; *M. tuberculosis* H37Rv	+	+	ND	ND	(Quiding-Jarbrink *et al.*, 2001)
Flavonoids							
Human carcinoma	MCF-7; MDA-MB231 (breast)	Genistein	-	-	c-Fos; c-Jun	TRE	(Shao *et al.*, 1998)
	A431 (skin)	Luteolin; quercetin; genistein	-	-	ND	ND	(Huang *et al.*, 1999)
Human fibrosarcoma	HT1080	PMA/nobiletin	NE	-	PI-3K; ERK/AP-1	ND	(Sato *et al.*, 2002)
Murine macrophages	Peritoneal	LPS/(DEX,genistein)	-	-	PTK	ND	(Xie *et al.*, 1994b)
Rabbit chondrocytes	Articular	IL-1α/nobiletin	NE	-	PGE_2	ND	(Ishiwa *et al.*, 2000)
Rabbit fibroblasts	Synovial	IL-1α/nobiletin	NE	-	PGE_2	ND	(Ishiwa *et al.*, 2000)
Lectins							
Human adenocarcinoma	MDA-MB-231 (breast)	ConA	NE (Activ)	ND	ND	ND	(Yu *et al.*, 1995; Yu *et al.*, 1997)
Human endothelial cells	HUVEC	ConA	NE (Activ)	NE	PKC	ND	(Foda *et al.*, 1996)
Human fibroblasts	Gingival (early passage)	ConA	+	ND	ND	ND	(Overall and Sodek, 1990)
Human B lymphocytes	EBV-immortalized	ConA	ND	+	ND	ND	(Trocme *et al.*, 1998)
Human monocytes	PB	ConA	ND	+	PGE_2/cAMP	ND	(Welgus *et al.*, 1990; Opdenakker *et al.*, 1991; Wahl and Corcoran, 1993; Corcoran *et al.*, 1994)
Human monocytic leukemia	THP-1	ConA	ND	+	ND	ND	(Van Ranst *et al.*, 1991)
Human mononuclear leukocytes	PB	UDA; CAA; Calsepa; Conarva; ConA; PHA-L4	ND	+	ND	ND	(Dubois *et al.*, 1998)
		DSA; VisAlbCBA; BPA; WGA; MAA	ND	-			
Rat astrocytes	Neocortical	TxLC-I; PHA-E4	+	NE	ND	ND	(Liuzzi *et al.*, 1999)
		WGA	+	+			
		MAA; DSA; BPA; VisAlbCBA; DBA	-	NE			
Rat fibroblasts	3Y1	ConA	+	+	ND	ND	(Thant *et al.*, 1997)
Rat microglia cells	Neocortical	PHA-E4; TxLC-I; Calsepa; HHA; ACA; PSA; WFA; XSA; ConA	NE	+	ND	ND	(Liuzzi *et al.*, 1999)
		VisAlbCBA; BPA; MAA; WGA; DSA	NE	-			

[a]Species and producer cell types are listed in alphabetical order. [b]Abbreviations used are: ACA, *Amaranthus caudatus* agglutinin; Activ, proteolytic activation of gelatinase to lower molecular weight forms; AP-1, activating protein-1; BCG, Bacille Calmette-Guerin; BPA, *Bauhinia purpurea* agglutinin; CAA, *Colchicum autumnale*; Calsepa, *Calystegia sepium* agglutinin; cAMP, adenosine-3',5'-cyclic monophosphate; c-Fos, protein product of *fos* oncogene identified in a mouse osteosarcoma encoding a transcription factor; c-Jun, product of *jun* oncogene from an avian sarcoma retrovirus, encoding a transcription factor; ConA, Concanavalin A; Conarva, *Convolvulus arvensis* agglutinin; cPLA2, cytoplasmatic phospholipase A2; DBA, *Dolichos biflorus* agglutinin; DEX, dexamethasone; DSA, *Datura stramonium* agglutinin; EBV, Epstein Barr virus; ERK, extracellular signal-regulated kinase; HHA, *Hippeastrum* hybrid agglutinin; IL-1α, interleukin-1alpha; LPS, lipopolysaccharide; MAA, *Maackia amurensis* agglutinin; ND, not determined; NE, no effect; NF-κB, nuclear factor-kappa B; PB, peripheral blood; PGE_2, prostaglandin E_2; PGHS-2, prostaglandin H synthase-2; PHA-E4, *Phaseolus vulgaris* agglutinin E; PHA-L4, *Phaseolus vulgaris* agglutinin L; PI-3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PSA, *Pisum sativum* agglutinin; PTK, protein tyrosine kinase; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; TRE, TPA response element; TxLC-I, *Tulipa* lectin; UDA, *Urtica dioica* agglutinin; VisAlbCBA, *Viscum album* chitin-binding agglutinin; WFA, *Wisteria floribunda* agglutinin; WGA, wheat germ (*Triticum aestivum*) agglutinin; XSA, *Xanthosoma sagittifolium* agglutinin; +, inducing effect; -, repressive effect. [c]Whenever combined interacting agents are used, '+' indicates a synergistic action, whereas '/' separates the activating substance (in front) and the modulating compound (in the back). The prefix 's' indicates soluble interacting agents, whereas 'r' stands for recombinant. [d]Regulatory elements that are suggested by the authors to be involved in the induction/repression, but are not investigated, are put between brackets.

2000). Similar to induction with PMA, many of the cellular signals mediated by LPS are PKC dependent, as was observed for LPS-enhanced gelatinase B production in mouse bone marrow-derived mast cells (Tanaka *et al*., 2001). Furthermore, activation of human monocytes with LPS has been found to result in the increased production of gelatinase B through a PGE_2-cAMP-dependent pathway. Tyrosine phosphorylation of $cPLA_2$ was shown to be one of the initial steps needed in this LPS-induced increase (Shankavaram *et al*., 1998). Finally, as observed for TGF-β-inducing effects, LPS can also regulate gene transcription of gelatinase B by modifying mRNA stabilization (see Section 2.1.3.3) (Saarialho-Kere *et al*., 1993; Yao *et al*., 1996). Again it is clear that indirect effects through cytokine induction have also been found with LPS as a bacterial product (McMillan *et al*., 1996b), and with lectins.

Because gelatinase B is regulated in a quite complex way (*vide supra*) and its induction is readily measured by zymography, it is not so surprising that this test was used as a read-out to measure the effects of physical/chemical stressing of cells. Thus, it was found that reactive oxygen species, UV- or X-ray radiation, shear stress, pH, osmotic effects, spindle inhibitors, and amyloid formation all influenced the balance between the gelatinases A and B (Table 13 and references). In line with this was the finding that Bcl-2 transfection, which constitutes a regulation mechanism for apoptosis, also induced gelatinase B (Ricca *et al*., 2000).

In conclusion, the transcriptional regulation of gelatinase B gene expression has been traced back to the molecular level in many studies and found to be rather complex. Many cytokines and cytokine inducers regulate the production of gelatinase B. Recently, hormones and cell adhesive effects have been added to a spectrum of soluble inducers. Within the context of living organisms these effector molecules cooperate and are counterbalanced by the regulation of transcription of the more constitutive gelatinase A gene and the TIMP genes.

2.2. Regulation of the Secretion of Gelatinase B by Neutrophils

One particular inflammatory cell type has to act without hesitation on rather simple alert signals: the neutrophilic granulocyte. These cells function in innate immunity by phagocytosis and killing of foreign invaders. Killing is either intracellular or extracellular by virtue of, for example, oxygen and nitrogen metabolites, enzymes such as lysozyme and antibiotic proteins such as defensins. In order to reach the inflammatory focus as first line defense cells, neutrophils are equiped with gelatinase B and other matrix (metallo)proteinases, such as neutrophil collagenase (MMP-8). Because these cells have a turnover of only a couple of days, they die in the periphery by a process called cytoclasis (apoptosis), and they are rapidly replaced by new cells originating from the bone marrow. Like in classic armies, this first line defense cell outnumbers in the circulation largely the other immune cells that are more specialized in their targeting mechanisms (e.g., lymphocytes) or endowed with more regulatory functions (monocytes/macrophages). Indeed, the circulating pool of neutrophils is about 75% of the white blood cells in man. Its specialization is evident from a high degree of differentia-

Table 13. Regulation of gelatinase A and B expression by various stress-related factors

Producer cell type[a]	Cell line and/or cell origin[b]	Inducer/repressor[b,c]	Effect on gelatinase A[b]	Effect on gelatinase B[b]	Induced regulatory elements/ pathways[b,d]	Required regulatory promoter elements[b,d]	References
Regulators of apoptosis							
Human adenocarcinoma	MCF7ADR (breast)	Bcl-2 (transfection)	ND	+	NF-κB	NF-κB (-600 bp)	(Ricca *et al.*, 2000)
Reactive oxygen species							
Bovine endothelial cells	Aortic	High glucose	ND	+	ROS	ND	(Uemura *et al.*, 2001)
Human endothelial cells	HUVEC	H_2O_2	+	+	ND	[NF-κB; TRE; AP-2]	(Belkhiri *et al.*, 1997)
Human fibroblasts	NB1RGB (dermal)	HXXO	+	ND	ND	ND	(Kawaguchi *et al.*, 1996)
Human rhabdomyosarcoma	SJRH30 (alveolar)	Hypoxia	NE	+	ND	ND	(Himelstein and Koch, 1998)
Human smooth muscle cells	Vascular	PMA/HXXO	NE (Activ)	NE (Activ)	ND	ND	(Rajagopalan *et al.*, 1996)
		H_2O_2	NE [(Des)activ]	NE			
		$ONOO^-$	NE (Activ)	NE			
Murine carcinoma	C87 (lung)	NAC	-	-	ND	ND	(Albini *et al.*, 1995)
Murine melanoma	K1735-M2; B16-F10	NAC	-	-	ND	ND	(Albini *et al.*, 1995)
Rabbit macrophages	Foam cells (atheroma)	NAC	NE	-	ROS	ND	(Galis *et al.*, 1998)
Rabbit chondrocytes	Articular	IL-1β/L-NMMA	-	-	NO	ND	(Tamura *et al.*, 1996)
Rat fibroblasts	Cardiac	HXXO; H_2O_2	+	+	ND	ND	(Siwik *et al.*, 2001)
	RFL-6 (lung)	SIN-1; HXXO/SNAP; $ONOO^-$	-	ND	ND	ND	(Owens *et al.*, 1997)
		O_2^-	+	ND			
Rat lung	Macrophages (alveolar); epithelial cells	Hyperoxia	+	+	NF-κB p65	ND	(Pardo *et al.*, 1998)
Rat mesangial cells	Glomerular	(IFN-γ, LPS)/L-NMMA	-	ND	ND	ND	(Trachtman *et al.*, 1996)
		SNAP	+	ND			
		IL-1β/(SNAP, DETA-NONOate)	-	-	NO	ND	(Eberhardt *et al.*, 2000a and 2000b)

Table 13 (continued)

		IL-1β/(HXXO, DMNQ)	ND	+	O_2^-; NF-κB p65; c-Jun; NF-κB; AP-1; JNK; ERK; p38 MAPK	NF-κB, TRE	(Eberhardt *et al.*, 2000b)
Rat smooth muscle cells	Vascular	IL-1β/DETA-NONOate	ND	-	O_2^-; ERK1/2; not p38 MAPK	ND	(Gurjar *et al.*, 2001)
		HXXO	ND	+			
		IL-1β/eNOS (gene transfer); IL-1β/(DETA-NONOate, 8-bromo-cGMP)	NE (Desactiv)	-	NO; cGMP	ND	(Gurjar *et al.*, 1999)
Irradiation							
Human epidermis	Skin	UVB	NE	+	NF-κB; AP-1	[NF-κB; TRE]	(Fisher *et al.*, 1996; Fisher *et al.*, 1997)
Human fibroblasts	NB1RGB (dermal)	UVA	NE	ND	ND	ND	(Kawaguchi *et al.*, 1996)
Human melanoma	SB-2 (cutaneous)	UVB	+	NE	ND	ND	(Singh *et al.*, 1995)
Rat astrocytes	Type-1	X-rays	+	ND	ND	ND	(Sawaya *et al.*, 1994)
Mechanical stress							
Rabbit chondrocytes	Articular	Shear stress	ND	+	JNK; Ras; Rac; Cdc42; c-Jun	TRE	(Jin *et al.*, 2000)
Other factors							
Human adenocarcinoma	MCF-7 (breast)	Calcium HA	+	+	ND	ND	(Morgan *et al.*, 2001)
	A549 (lung)	pH 6.8	+	+	ND	ND	(Kato *et al.*, 1992)
		pH5.9	-	-			
Human endothelial cells	HUVEC	High glucose	-	ND	PKC	ND	(Grigorova-Borsos *et al.*, 1996)
Human epithelial cells	Breast	Calcium HA	NE	+	ND	ND	(Morgan *et al.*, 2001)
Human fibroblasts	Synovial	Substance P	+	ND	ND	ND	(Hecker-Kia *et al.*, 1997)
Human fibrosarcoma	HT1080	pH 6.8	+	+	ND	ND	(Kato *et al.*, 1992)
		pH5.9	-	-			
Human keratinocytes	Newborn foreskin	Ca^{2+}	NE	+	ND	ND	(Kobayashi *et al.*, 1998a)
Human T lymphoblastoma	Tsup-1	VIP	ND	+	ND	ND	(Xia *et al.*, 1996)
Human melanoma	WM983a; WM239	Cytochalasin D	NE	-	ND	ND	(MacDougall and Kerbel, 1995)
		Colchicine	NE	NE			
Human monocytic leukaemia	THP-1	IFN-γ/CT_{105} Aβ precursor	ND	+	PTK; ERK; p38 MAPK	ND	(Chong *et al.*, 2001)
		Ca^{2+} ionophore	ND	NE	ND	ND	(Van Ranst *et al.*, 1991)
Human mononuclear cells	PB	Oxidized LDL	ND	+	NF-κB; AP-1	ND	(Xu *et al.*, 1999)

		Oxidized LDL/HDL	ND	-			
Human promyelocytic leukemia	HL-60	PMA/cytochalasin D	NE	-	ND	ND	(MacDougall and Kerbel, 1995)
Murine corneal cells	Eye	bFGF/curcuminoids	ND	-	AP-1	[TRE]	(Mohan *et al.*, 2000)
Pig macrophages	Alveolar	substance P	(+)	+	ND	ND	(D'Ortho *et al.*, 1995)
Rat astrocytes	Newborn brain	$A\beta_{1\text{-}38}$ peptide; $A\beta_{1\text{-}40}$ peptide	+	+	ND	ND	(Deb and Gottschall, 1996)
Rat mixed hippocampal cells	Fetal brain	$A\beta_{1\text{-}38}$ peptide; $A\beta_{1\text{-}40}$ peptide	+	+	ND	ND	(Deb and Gottschall, 1996)

[a]Species and producer cell types are listed in alphabetical order. [b]Abbreviations used are: Aβ, amyloid-β; Activ, proteolytic activation of gelatinase to lower molecular weight forms; AP-1/2, activating protein-1/2; Bcl-2, proto-oncogene, activated by chromosome translocation in human B cell lymphomas; bFGF, basic fibroblast growth factor; Cdc42, small GTPase, involved in cytoskeletal growth and organization; cGMP, 3',5'-cyclic guanosine monophosphate; c-Jun, product of *jun* oncogene from an avian sarcoma retrovirus, encoding a transcription factor; $CT_{105}A\beta$, 105-amino acid carboxyl-terminal fragment of amyloid-β precursor protein; Desactiv, disappearance of lower molecular weight activated forms; DETA-NONOate, NO donor; DMNQ, 2,3-dimethoxy-1,4-naphtoquinone (superoxide generator); eNOS, endothelial NO synthase; ERK, extracellular signal-regulated kinase; H_2O_2, hydrogen peroxide; HA, hydroxyapatite; HDL, high-density lipoprotein; HXXO, hypoxanthine/xanthine oxidase (superoxide generator); IFN-γ, interferon-gamma; IL-1β, interleukin-1beta; JNK, Jun N-terminal kinase; LDL, low-density lipoprotein; L-NMMA, N^G-monomethyl-L-arginine (inhibitor of NO synthase); LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NAC, N-acetyl-L-cysteine (ROS scavenger); ND, not determined; NE, no effect; NF-κB, nuclear factor-kappa B; NO, nitric oxide; O_2^-, superoxide; $ONOO^-$, peroxynitrite; PB, peripheral blood; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PTK, protein tyrosine kinase; Rac, small GTP-binding protein involved in regulating actin cytoskeleton; Ras, small GTP-binding onco-protein with GTPase activity (p21ras); ROS, reactive oxygen species; SIN-1, 6-morpholinosydnonimine (O_2^- +NO donor); SNAP, S-nitroso-N-acetyl-D,L-penicillamine (NO donor); TRE, TPA response element; UVA, ultraviolet A; UVB, ultraviolet B; VIP, vasoactive intestinal peptide; +, inducing effect; -, repressive effect. [c]Whenever combined interacting agents are used, '/' separates the stimulating substance (in front) and the modulating compound (in the back).
[d]Regulatory elements that are suggested by the authors to be involved in the induction/repression, but are not investigated, are put between brackets.

tion, as neutrophils synthesize and prepack gelatinase B in granules. This allows fast (< 15 min) release of large amounts of gelatinase B after appropriate stimuli, without the need of transcription and translation. Recently, Borregaard and co-workers, who have been studying the neutrophil for decades, defined gelatinase B as the most specific terminal marker of neutrophil differentiation (Cowland and Borregaard, 1999). What is not well appreciated until now is the fact that neutrophils do not make gelatinase A and — in addition — do not produce TIMPs. This implies that in the terminal differentiation of the neutrophil the genes for gelatinase A and TIMP-1 are completely switched off. In contrast, other leukocytes such as B and T lymphocytes (Weeks *et al*., 1993b; Trocmé *et al*., 1998) and monocytes (Opdenakker *et al*., 1991b), dendritic cells (Bartholomé *et al*., 2001) and natural killer cells constitutively produce gelatinase A, can be induced for *de novo* gelatinase B synthesis, and also produce TIMP-1 (Opdenakker *et al*., 2001).

In neutrophils, six different types of granules can be distinguished: dense peroxidase-positive granules rich in defensins, light peroxidase-positive granules low in defensins, dense peroxidase-negative granules with lactoferrin but without gelatinase B, intermediate peroxidase-negative granules with both lactoferrin and some gelatinase B, light peroxidase-negative granules with gelatinase B but without lactoferrin, and secretory vesicles with alkaline phosphatase (Gullberg *et al*., 1997). Peroxidase-positive granules are also denominated as primary or azurophil granules. Secondary or specific granules are identical to myeloperoxidase (MPO)-negative, lactoferrin–positive granules (Figure 6). Gelatinase B-containing granules, without lactoferrin, are also named tertiary granules. The different granules are synthesized subsequently during different stages of myeloid differentiation and each contain specific proteases as well as other enzymes and proteins (Cowland and Borregaard, 1999). Gelatinase B is synthesized mainly by the band and segmented neutrophils, which represent late stages in myeloid differentiation. It is then stored in the proform mainly in the tertiary or light peroxidase-negative granules without lactoferrin. As a consequence, the concentration of gelatinase B within the granules must be enormous and the question may be asked why it does not crystallize within such granules. Maybe the role of NGAL and the extensive glycosylation forms a means to prevent such crystal formation.

Given the fact that gelatinase B is prepacked in granules of a cell that is developmentally prohibited to synthesize gelatinase A or TIMP-1, several logical deductions can be made: (1) gelatinolysis by neutrophils, for example, permeation through basement membranes is mainly dependent on gelatinase B and irreversible, as it cannot be controlled by neutrophil TIMP-1. Gelatinase B thus contributes to the irreversible phase of leukocyte migration from the blood circulation. This irreversibility previously has been mainly attributed to adhesion molecules (which may induce gelatinase B). Obviously, also enzymes are involved; (2) a major control level of neutrophil function consists of degranulation, that is, the liberation of prepacked gelatinase B. Hence, the factors that induce this degranulation are crucial to understand the role of neutrophils in pathophysiology.

The secretion of the different neutrophil granules depends on the stimulus. As a general rule, the rank order of

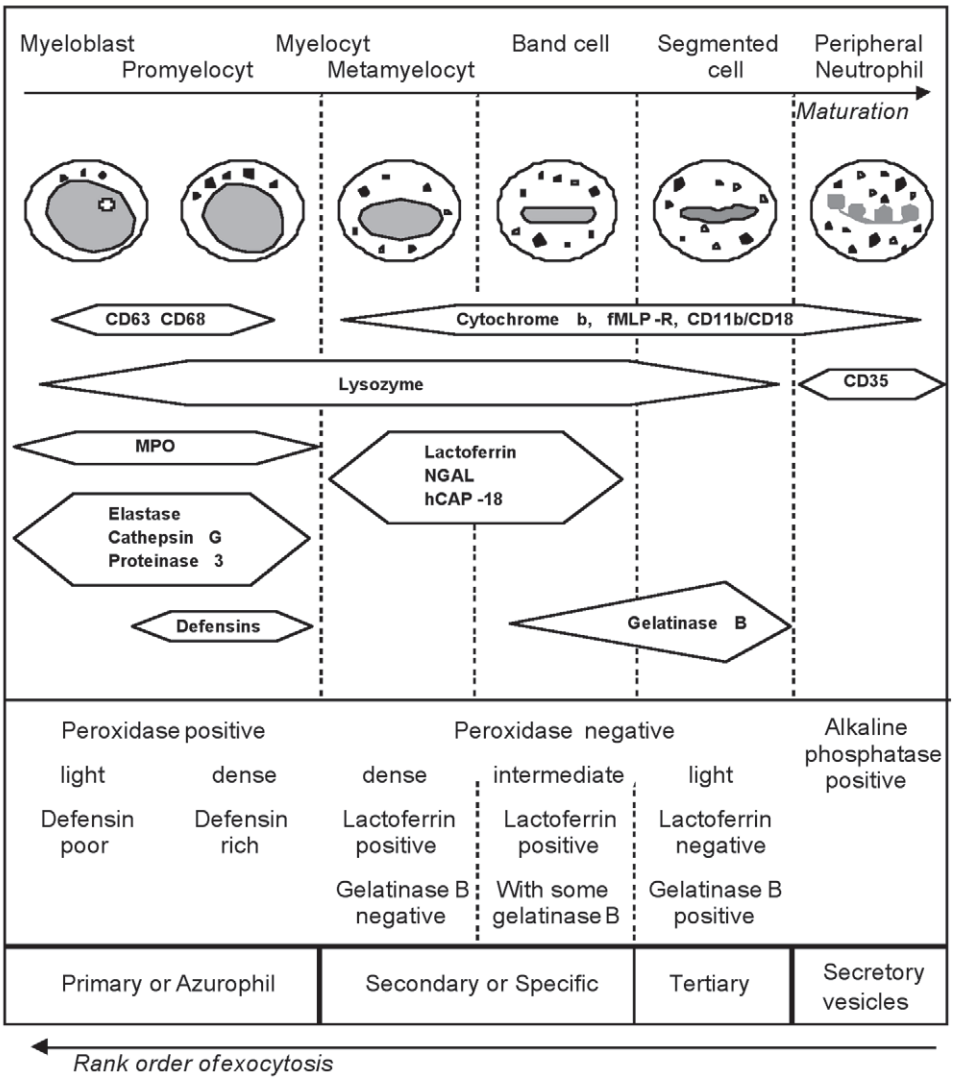

FIGURE 6. The granule diversity in neutrophils. During the maturation of neutrophils, different granule proteins are expressed according to the maturation stage, as indicated. The newly formed proteins are immediately stored in granules. Therefore, granules formed in different maturation stages have a different content and can be classified into primary (azurophil), secondary (specific), and tertiary granules and secretory vesicles. Further subdivision of the granules according to their content is indicated. In addition, the rank order of exocytosis is indicated at the bottom, because the latest formed granules will be mobilized more readily than the granules formed in an early stage. (Based on Cowland and Borregaard (1999) and Gullberg *et al.* (1997). MPO, myeloperoxidase; fMLP-R, fMLP-receptor; NGAL, neutrophil gelatinase B-associated lipocalin.)

exocytosis (Figure 6) is secretory vesicles, gelatinase B-containing vesicles, specific granules and azurophil granules (Sengelov *et al*., 1993), corresponding to the intracellular Ca^{2+} increase required for triggering the exocytosis. Different exogenous and endogenous stimuli have been found to trigger degranulation, the most potent one is probably PMA. The bacterial tripeptide formyl-methionyl-leucyl-phenylalanine (fMLP) and bacterial lipopolysaccharide also induce degranulation of gelatinase B-containing and specific granules (Opdenakker *et al*., 1991a).

Among the endogeneous physiological regulators of degranulation, the chemotactic factors, such as leukotriene B4 (LTB4), complement-derived C3a and C5a, and in particular chemotactic cytokines or chemokines, are crucial. All chemotactic factors for neutrophils activate these cells via a common pathway using serpentine G-protein-coupled receptor molecules. Serpentine receptors are rather primitive and respond to simple alerting signals that are active in vision (transducins), olfaction, stress (adrenergic receptors), and chemotaxis of all leukocyte types. These common receptor pathways in host defense are translated in molecular terms for neutrophil functions by the receptors for complement factors C3a and C5a, leukotrienes (e.g., LTB4), bacterial formylpeptides, and chemokines.

Degranulation of neutrophil gelatinase B under influence of chemokines was first documented for the chemokine interleukin-8 by Masure *et al*. (1991) and subsequently for granulocyte chemotactic protein-2 (GCP-2) (Proost *et al*., 1993b). In general, only the CXC chemokines containing the tripeptide ELR motif in front of the conserved CXC are active on neutrophils and trigger directional chemotaxis, neutrophil activation, and degranulation of gelatinase B-containing and specific granules (Wuyts *et al*., 1998; Masure *et al*., 1991). This degranulation may be upregulated by priming the neutrophils with TNF-α, whereas chemokine-induced release of azurophilic granules was only possible after pretreatment of the neutrophils with cytochalasin B (Brandt *et al*., 1992). The ELR-negative CXC-chemokine platelet factor-4 is shown to be able to trigger degranulation of specific granules, but only after priming of the neutrophils with TNF-α (Petersen *et al*., 1996). In addition, positive or negative feedback mechanisms occur, because gelatinase B processes different chemokines, resulting in potentiation of activity (e.g., IL-8) or in degradation (e.g., PF-4) (Van den Steen *et al*., 2000; see also above).

To investigate whether gelatinase B is necessary and/or sufficient for (neutrophil) chemotaxis *in vivo* various approaches have been followed: inhibition with monoclonal antibodies or gene knock-out technology. Because the mouse homologue of the most potent human neutrophil chemokine (IL-8) is still elusive or perhaps is GCP-2, the physiological role of gelatinase B has been studied in gelatinase B knock-out mice using mouse GCP-2 as a chemoattractant (D'Haese *et al*., 2000). It was found that gelatinase B indeed functions in *in vivo* chemotaxis, but that its role is compensated for during ontogenesis when it is deleted for life. Because other studies have not or only partially confirmed this observation, one is eagerly waiting to discover what the effect of GCP-2 is on chemotaxis in inducible gelatinase B knock-out mice.

When gelatinase B is produced and secreted by neutrophils, the monomeric form of the proenzyme is always accompanied by two other forms: a homodimer and a heterodimer with NGAL (see Section 1.1.2). The latter

molecule was purified from neutrophil supernatants and identified by amino acid sequence analysis (Kjeldsen *et al*., 1993; Triebel *et al*., 1992). Its function remains unknown. NGAL is made by neutrophils as a monomer, as dimer, and as complex with gelatinase B. In its covalent association with gelatinase B, it may hypothetically function, much like the attached oligosaccharides as a shield against autocatalysis or as protection against other neutrophil proteases as these degranulate during host defence (Tscheche *et al*., 2001). As the lipocalin has been shown to bind formylpeptides, it may also dampen the strong and irreversible degranulation of neutrophils by such bacterial peptides (Sengelov *et al*., 1994).

2.3. Activation of Progelatinase B

Gelatinase B is synthesized and secreted as zymogen or proenzyme, which remains inactive unless it is activated by the removal of the propeptide. This propeptide contains the conserved sequence PRCGXPD, of which the Cys is coordinated with the catalytic Zn^{2+}. After disruption of this coordination, for example, by proteolysis of the propeptide, a conformational change occurs and the Zn^{2+} becomes accessible for a hydrolytic water molecule and for the substrate, resulting in the activation of the enzyme (Van Wart and Birkedal-Hansen, 1990; Kleifeld *et al*., 2000). This mechanism is denominated as the "cysteine-switch mechanism". Different proteases are known to activate gelatinase B, for example, the serine proteases trypsin (Masure *et al*., 1990; Duncan *et al*., 1998), tissue kallikrein (Desrivières *et al*., 1993), cathepsin G (Sakata *et al*., 1989), mast cell chymase (Fang *et al*., 1996), and neutrophil elastase, which is present in azurophilic granules of neutrophils (Ferry *et al*., 1997). However, different MMPs can also activate each other, resulting in the creation of an activation cascade, or maybe rather an activation network. Figure 7 and Table 14 illustrate all activation ways, so far documented by experiment. Two major protease families merge into the MMP activation network: the plasminogen activator/plasmin system and the MT-MMPs. Plasminogen can be converted by tissue-type plasminogen activator (t-PA) or by urokinase (u-PA) into plasmin, itself being an activator for different MMPs. These MMPs can activate other MMPs, leading finally to the activation of gelatinase B, which is a terminal member (Cuzner and Opdenakker, 1999) of the activation network as can be derived from Figure 7. MT-MMPs contain the furin-sensitive motif in their propeptide, and might therefore be activated intracellularly. The MT-MMPs can activate some secreted MMPs through a complex with TIMP-2, for example, gelatinase A or collagenase-3 (MMP-13), both of which in turn can activate gelatinase B.

Proteolytic cleavage of the propeptide of gelatinase B occurs in two steps, with a first cleavage at Gln_{40}-Met_{41} and a second cleavage at Arg_{87}-Phe_{88}, as was shown for the activation by interstitial collagenase (MMP-1), gelatinase A, stromelysin-1, and collagenase-3 (Sang *et al*., 1995). The activation of gelatinase B by stromelysin-1 results in a further slow degradation of gelatinase B by cleavage at Pro_{428}-Glu_{429} (Shapiro *et al*., 1995). The effect of TIMP-1 on the activation of gelatinase B by stromelysin-1 is interesting. When the stromelysin-1 concentration is lower than the TIMP-1 concentra-

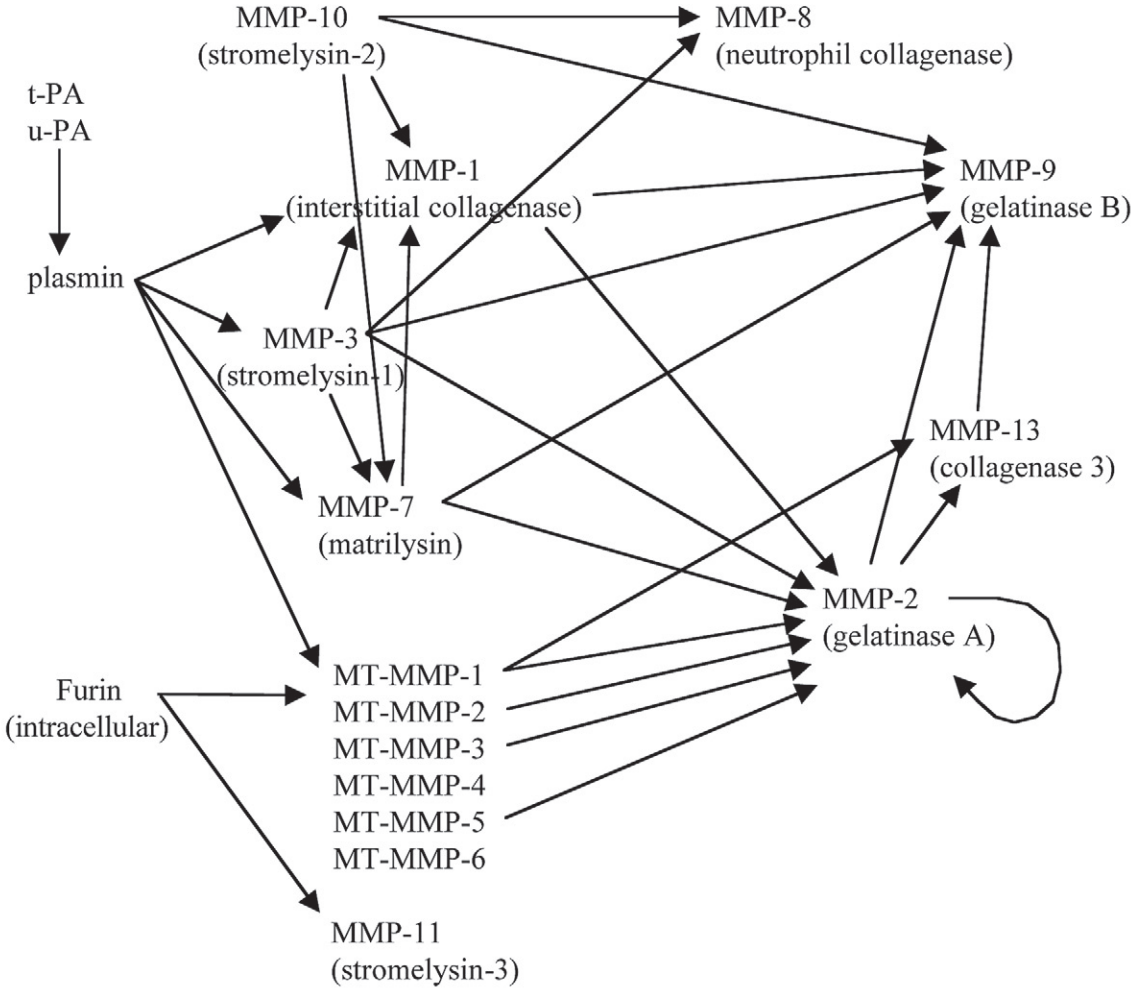

FIGURE 7. The activation network of gelatinase B by MMPs. Most soluble MMPs are secreted as latent proenzymes that need to be processed for activation. Possible pathways for the activation of gelatinase B by other MMPs are shown. (For references see Table 14.) Arrows from one enzyme to another indicate that the active form of the first enzyme converts the proform of the second enzyme to its active form, which is indicated.

Table 14. Direct and indirect activators of progelatinase B within the MMP network

Proenzyme	Activator	References
progelatinase B (proMMP-9)	matrilysin-1 (MMP-7)	(Imai *et al.*, 1995; Sang *et al.*, 1995)
	matrilysin-2 (MMP-26)	(Uria and Lopez-Otin, 2000)
	fibroblast collagenase (MMP-1)	(Sang *et al.*, 1995)
	collagenase 3 (MMP-13)	(Knauper *et al.*, 1997)
	stromelysin-1 (MMP-3)	(Ogata *et al.*, 1992)
	gelatinase A (MMP-2)	(Fridman *et al.*, 1995)
	stromelysin-2 (MMP-10)	(Nakamura *et al.*, 1998)
	tissue kallikrein (pig)	(Desrivières *et al.*, 1993)
	cathepsin G (rat)	(Sakata *et al.*, 1989)
	mast cell chymase (dog)	(Fang *et al.*, 1996)
	neutrophil elastase	(Ferry *et al.*, 1997)
	tumor trypsin-2 (human)	(Sorsa *et al.*, 1997)
neutrophil procollagenase (proMMP-8)	stromelysin-1 (MMP-3)	(Knauper *et al.*, 1993)
	stromelysin-2 (MMP-10)	(Knauper *et al.*, 1996a)
	cathepsin G	(Knauper *et al.*, 1990)
progelatinase A (proMMP-2)	stromelysin-1 (MMP-3)	(Miyazaki *et al.*, 1992; Crabbe *et al.*, 1994)
	Matrilysin-1 (MMP-7)	(Crabbe *et al.*, 1994)
	interstital collagenase (MMP-1)	(Crabbe *et al.*, 1994)
	MT-MMP-1	(Sato *et al.*, 1996a)
	MT-MMP-2	(Kolkenbrock *et al.*, 1997)
	MT-MMP-3	(Takino *et al.*, 1995)
	MT-MMP-5	(Llano *et al.*, 1999)
	autolytic, heparin-regulated	(Crabbe *et al.*, 1993)
	autolytic, TIMP-2 regulated	(Howard *et al.*, 1991)
	neutrophil elastase (if gelatin present, otherwise degradation)	(Rice and Banda, 1995)
	mast cell tryptase	(Lohi *et al.*, 1992)
interstitial fibroblast procollagenase (proMMP-1)	gelatinase A after trypsin	(Crabbe *et al.*, 1994)
	matrilysin (MMP-7)	(Imai *et al.*, 1995; Sang *et al.*, 1996)
	stromelysin 2 (MMP-10)	(Nicholson *et al.*, 1989; Windsor *et al.*, 1993)
	stromelysin11 (MMP-3)	(Suzuki *et al.*, 1990; Windsor *et al.*, 1993)
	plasmin	(Eeckhout and Vaes, 1977; Werb *et al.*, 1977)
	kallikrein	(Eeckhout and Vaes, 1977)
	lysosomal cathepsin B	(Eeckhout and Vaes, 1977)
	skin mast cell chymase	(Saarinen *et al.*, 1994)
	rat mast cell proteinase II	(Suzuki *et al.*, 1995)
	endothelial-cell-stimulating angiogenesis factor (ESAF)	(McLaughlin and Weiss, 1996)
prostromelysin-1 (proMMP-3)	plasmin	(Nagase *et al.*, 1990)
	plasma kallikrein	(Nagase *et al.*, 1990)
	neutrophil elastase	(Okada and Nakanishi, 1989)
	cathepsin G	(Okada and Nakanishi, 1989)
	mast cell tryptase	(Gruber *et al.*, 1989)
	rat mast cell proteinase I and II	(Suzuki *et al.*, 1995)
promatrilysin (proMMP-7)	plasmin	(Imai *et al.*, 1995)
	leukocyte elastase	(Imai *et al.*, 1995)
	stromelysin-1 (MMP-3)	(Imai *et al.*, 1995)
	stromelysin-2 (MMP-10)	(Nakamura *et al.*, 1998)
proMT-MMP-1 (proMMP-14)	furin (intracellular)	(Sato *et al.*, 1996a)
	plasmin	(Okumura *et al.*, 1997)
prostromelysin-3 (proMMP-11)	furin (intracellular)	(Pei and Weiss, 1995; Santavicca *et al.*, 1996)
procollagenase 3 (proMMP-13)	MT-MMP-1	(Knauper *et al.*, 1996b)
	gelatinase A	(Knauper *et al.*, 1996b)

tion, stromelysin-1 is inhibited and no activation occurs. When stromelysin-1 is present at a higher concentration than that of TIMP-1, gelatinase B becomes fully activated, as the TIMP-1 is displaced from gelatinase B onto stromelysin-1 (Ogata *et al*., 1995). The presence of Ca^{2+} is also important, for example, treatment of gelatinase B with trypsin in the presence of Ca^{2+} results in activation (cleavage at Arg_{87}-Phe_{88}), but in the absence of Ca^{2+} degradation of gelatinase B is observed (Bu and Pourmotabbed, 1995). Gelatinase A and gelatinase B were also shown to bind to insoluble elastin. When progelatinase B is bound to elastin, it remains completely unaffected by any enzymatic activator. In contrast, gelatinase A, bound to elastin, seems to undergo a fast autoactivation (Emonard and Hornebeck, 1997).

The activation of gelatinase B can also be performed by chemicals, of which organomercurials (e.g., 4-aminophenylmercuric acetate or APMA) are the most widely used. Treatment with organomercurials results in the stepwise fragmentation of the propeptide, yielding Met_{75} as final amino terminus of the enzyme, and in loss of the carboxyterminal hemopexin domain if Ca^{2+} is present (Triebel *et al*., 1992). Other chemicals known to activate gelatinase B are urea and detergents, which probably induce the disruption of the interaction of the Cys in the prodomain with the catalytic Zn^{2+} (Sopata and Maslinski, 1991). Interestingly, it was also found that gelatinase B can be activated by reactive oxygen species, such as hypochlorous acid. Reactive oxygen may be produced by activated neutrophils, constituting a possible physiological pathway for the activation of neutrophil gelatinase B (Peppin and Weiss, 1986). Finally, binding of progelatinase B to gelatin or type IV collagen can already confer some activity to the enzyme without proteolytic release of the propeptide (Bannikov *et al*., 2002).

In conclusion, the general picture of the activation network is one from plasminogen activation toward activation of progelatinase B as the final step (Figure 7). A reciprocal activation, from gelatinase B to plasminogen activators, seems not to occur (Ugwu *et al*., 2001).

2.4. Inhibition of Gelatinase B by TIMP

Once gelatinase B is secreted and activated, its activity can still be regulated by degradation or inhibition. Gelatinase B is inhibited by α_2-macroglobulin, the universal protease-inhibitor present in human serum (Birkedal-Hansen *et al*., 1993). However, the more specific TIMPs are shown to be important in regulating the activity of MMPs (Murphy and Docherty, 1992).

TIMPs are stable (glyco)proteins with a relative molecular weight of 20 to 30 kDa and they contain six conserved disulfide bridges (Murphy and Willenbrock, 1995). These disulfide bridges define six protein loops, of which the first three form an aminoterminal domain and the others comprise a carboxyterminal domain. These domains fold independently from each other, and the aminoterminal domain can function without the carboxyterminal domain (see also further). TIMP-1 is glycosylated at two sites, while TIMP-2 and –3 are not glycosylated.

Four different TIMP genes and proteins have been described in man, of which TIMP-1 binds with high affinity to gelatinase B, and TIMP-2 and –3 with

lower affinity. TIMP-2, –3, and –4 bind with high affinity to gelatinase A. TIMP-1 is an inducible protein, in contrast to TIMP-2, which is constitutively expressed (reviewed in Gomez *et al*., 1997). The inhibition by TIMPs follows the slow tight-binding kinetics and is highly complex as different binding sites for TIMPs are present on both gelatinases. Not only the activated gelatinase B can bind to different TIMPs, but the proenzyme is also able to bind TIMP-1 and TIMP-3 (Olson *et al*., 1997), whereas progelatinase A binds to TIMP-2, –3, and –4 (Olson *et al*., 1997; Butler *et al*., 1999; Bigg *et al*., 1997). The interaction between progelatinase B and TIMP-1 seems to occur mainly through the C-terminal domains of both molecules, because a C-terminal deletion mutant of TIMP-1 does not bind to progelatinase B (Murphy *et al*., 1991) and as C-terminal mutants of gelatinase B also do not bind TIMP-1 (Goldberg *et al*., 1992). Complexes of progelatinase B and TIMP-1 are able to inhibit other MMPs by the formation of a gelatinase B/TIMP-1/MMP complex, indicating that the inhibitory N-terminal domain of TIMP-1 is still available for interaction in the progelatinase B/TIMP-1 complex (Ogata *et al*., 1995). Inhibition of the activated gelatinase B, on the other hand, occurs through interaction between the N-terminal domains of TIMP-1 and the active site of the enzyme, as C-terminal deletion mutants of TIMP-1 retain their inhibitory activity against gelatinase B (Murphy *et al*., 1991). C-terminal deletion mutants of gelatinase B, lacking the hemopexin and collagen type V domains, are less effectively inhibited by TIMP-1, indicating that the C-terminal part is also involved (O'Connell *et al*., 1994). The C-terminal domains seem to be responsible for high-affinity interaction with TIMP-1 with a dissociation constant in the nanomolar range. In contrast, the N-terminal domains are responsible for low-affinity interaction with a dissociation constant in the micromolar range (Olson *et al*., 1997).

Interaction of gelatinase B with TIMP-2 is mediated by the N-terminal domains of the enzyme and not by the hemopexin domain, as deletion of the latter does not affect the binding of TIMP-2 to gelatinase B. Moreover, the inhibition of gelatinase B by TIMP-2 is less effective than by TIMP-1 (O'Connell *et al*., 1994). Also, no interactions of TIMP-2 with progelatinase B were observed, in contrast to the binding of TIMP-2 with progelatinase A (Olson *et al*., 1997).

TIMP-3 is an insoluble ECM-bound MMP-inhibitor with, like TIMP-2, a higher affinity for gelatinase A than for gelatinase B. It can bind to both progelatinases and activated gelatinases, and the carboxyterminal domains of both enzymes are important for the interaction with TIMP-3 (Butler *et al*., 1999). Also, negatively charged polysaccharides were shown to influence the interaction of TIMP-3 with gelatinases.

Interestingly, TIMP-1 was shown to possess completely different activities besides MMP inhibition. In fact, it was first isolated possessing erythroid potentiating activity (Docherty *et al*., 1985). Later it was also shown to stimulate the growth of keratinocytes (Bertaux *et al*., 1991), gingival fibroblasts and the Burkitt lymphoma cell line Raji, allowing the growth of the latter two cell types in basal media without serum (Hayakawa *et al*., 1992). These and other biological functions of TIMPs have been reviewed elsewhere (Gomez *et al*., 1997). It is an acceptable hypothesis that these effects may be ascribed to inhibition of the degradation of autocrine or paracrine growth factors by gelatinases or other MMPs.

2.5. Other Mechanisms for the Regulation of Gelatinase B Activity

Other regulation mechanisms of gelatinase B action are less well understood. For instance, the significance of the already discussed homodimerization and covalent complex formation with NGAL is not yet clear, although it was shown that dimerization has an influence on the activation rate (see above [Olson *et al*., 2000]). Glycosylation may also deserve a regulatory role, yet to be further explored. Both N- and O-linked glycans have indeed been shown to influence the functions of glycoproteins (reviewed in Rademacher *et al*., 1988; Rudd and Dwek, 1997; Van den Steen *et al*., 1998a). Further studies on the functional roles of the oligosaccharides of gelatinase B are in progress and show that the sialic acids of gelatinase B have an influence on the binding with TIMP-1 (Van den Steen *et al*., 2001).

Another interesting observation is that gelatinase B binds to different isoforms of CD44 at the cell surface (Bourguignon *et al*., 1998; Yu and Stamenkovic, 1999). This provides a means to localize the proteolytic activity to cell membranes and to prevent undesired activity at distant sites. Cell-membrane localization was found to have an essential role for, for example, u-PA, and a specific u-PA membrane receptor was found (Ploug *et al*., 1991; Roldan *et al*., 1990). Moreover, it was found that the expression of CD44 is correlated with the metastatic potential of cancer cells, as is also the case for the expression of gelatinase B (Bourguignon *et al*., 1998) (Himelstein *et al*., 1994). The α2(IV) chain of collagen IV, present close to the cell surface as a single chain, also binds progelatinase B (Olson *et al*., 1998). However, it is not yet clear whether this α2(IV) single-chain collagen is really associated with the cell membrane or rather with the surrounding extracellular matrix or is its function known. As a comparison, gelatinase A associates to cell surfaces by a number of different mechanisms. It can bind to the integrin $\alpha_v\beta_3$ through its carboxyterminal hemopexin-like domain (Brooks *et al*., 1996), or to MT1-MMP-associated tissue inhibitor of metalloproteinase (TIMP)-2 (Strongin *et al*., 1995). Unbound MT1-MMP may be responsible for the activation of progelatinase A in this ternary complex (Kinoshita *et al*., 1998) and of the $\alpha_v\beta_3$-bound gelatinase A (Hofmann *et al*., 2000). Via its fibronectin type II-like collagen-binding domain, the enzyme can bind to cell-associated chains of collagen types I, IV, and V, and elastin, forming a gelatinase A-collagen-β_1 integrin complex (Steffensen *et al*., 1998). For example, inactive gelatinase A was found to be bound to fibroblast-associated collagen, which could be displaced by fibronectin after contact with breast cancer cells, resulting in release of the enzyme (Saad *et al*., 2002). Retaining of gelatinase activity at the cell surface through the formation of these cell adhesion receptor/gelatinase complexes was demonstrated to promote collagen degradation and the invasive capacity of the cells. These processes may be further potentiated by enhanced *de novo* gelatinase A production that was found to be induced by αvβ3 and CD44 (Seftor *et al*., 1992; Takahashi *et al*., 1999).

Progelatinase B can bind to interstitial procollagenase, resulting in a stable complex (Goldberg *et al*., 1992). If TIMP-1 is added, this complex is displaced, resulting in the formation of a complex of TIMP-1 with progelatinase B, and in the release of procollagenase. However, when the

gelatinase B-collagenase complex is activated (e.g., by stromelysin), TIMP-1 can bind and inhibit the complex without disrupting it. This is in accordance with the finding that the complex is formed through the carboxyterminal domain of gelatinase B (Allan *et al*., 1995) and with the observed interaction of progelatinase B with TIMP-1 through the same carboxyterminal domain (see above). The activated complex of gelatinase B and interstitial collagenase is able to degrade completely fibrillar collagen (Goldberg *et al*., 1992).

When resting neutrophils are primed by moving from the blood stream into an inflammatory site or by exposure to LPS, they release gelatinase B (Masure *et al*., 1991) and become responsive to galectin-3 (oxidative burst) (Karlsson *et al*., 1998; Almkvist *et al*., 2001). Galectin-3, expressed at high levels by inflammatory macrophages and, at lower levels by the neutrophils themselves and other cells, may modulate the function of gelatinase B as well as the neutrophils en route to and at the inflammatory site. For example, galectin-3 may mediate binding of both neutrophils and gelatinase B to laminin, another ligand for galectin-3. On binding to terminal galactose (usually in the context of polylactosamine residues) in laminin, galectin-3 forms dimers (Ochieng *et al*., 1998) providing a second lectin binding site. This site can be occupied by gelatinase B via the terminal Galα1-3GlcNAc, Galβ1-4GlcNAc or Galβ1-3GalNAc residues on N- or O-linked glycans that are ligands for galectin 3 (Jin *et al*., 1995; Woo *et al*., 1990; Massa *et al*., 1993; Kuwabara *et al*., 1996). This binding event may augment the presentation of gelatinase B and initially restrict its diffusion from the inflammatory sites in the extracellular matrix at which it is secreted. However, gelatinase B cleaves the Ala62-Tyr63 bond in galectin 3 that has been proposed to be involved in the homodimerization of galectin-3 (Ochieng *et al*., 1994). These data suggest that although the cleaved galectin fragment binds more strongly to the sugars, the homodimeric galectin 3-mediated binding of gelatinase B to laminin may only be short-lived.

Insight into gelatinase B protein levels, regulated at the level of transcription, translation, and secretion, thus is clearly not enough to gain insight in its activity. Therefore, care schould be taken when interpreting the results of ELISA and zymography analyses of biological fluids, and these should be complemented with activity tests (see above). Indeed the presence of the gelatinase B protein does not necessarily indicate gelatinase B activity. For instance, most cell types secrete gelatinase B together with TIMP-1, and whether this results in gelatinase activity depends on the balance between the enzyme and its inhibitor and on the activation status of the enzyme. Neutrophils form a particular exception, as they do not produce TIMP-1 or gelatinase A and as they can activate the proenzyme themselves (see above, Opdenakker *et al*., 2001a; Peppin and Weiss, 1986). Insight into this balance between gelatinase B and TIMP and the activation status, together with other regulatory mechanisms such as cell surface localization, are crucial to understand the role of gelatinase B in different physiologic and pathologic processes as discussed in the next section.

3. THE ROLE OF GELATINASE B IN PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

Owing to its tightly regulated activity on extracellular substrates, as discussed

in the previous sections, gelatinase B has been implicated in various physiological and pathological functions. The role of this enzyme in physiology is mainly achieved by a remodeling of extracellular matrix components, while unbalanced degradation of these and other substrates often results in pathological conditions.

3.1. Physiological Functions

First, the role of gelatinase B in two physiological processes in human life, namely, reproduction, and growth and development will be described. Wound healing is a third example that will be discussed. Finally, the functions of gelatinase B in angiogenesis are exerted in physiological, as well as in pathological conditions, for example, neovascularization in tumors, and is discussed in both sections of physiology and pathology. Table 15 summarizes these functions of gelatinase B and presents the published phenotypes in gelatinase B-deficient mice (Table 15).

3.1.1. Reproduction

Matrix metalloproteases, and in particular gelatinase B, are involved in the female reproductive cycle at different stages. Remodeling of endometrial tissues is fundamental to the cyclical changes (Figure 8) that occur during the menstrual cycle, blastocyst implantation, and, in the absence of pregnancy, at menstruation. This field of action of gelatinase B also constitutes a well-known example of its transcriptional control by hormones. As an example, the chronological roles of gelatinase B in the menstrual cycle, implantation, parturition, and lactation are summarized, and the importance of regulatory mechanisms in enabling these cyclic changes is stressed.

During the normal menstrual cycle gelatinase B is produced by glandular epithelial cells starting at about day 7 and its levels increase until ovulation, when it contributes to the remodeling of the extracellular matrix during follicle growth (Song *et al*., 1999). MMP-19 and TIMP-1 seem to be more important in the release of the mature oocyte (Hägglund *et al*., 1999). From day 15 onward, extracellular secretion into the glandular lumen is detected by immunolocalization and reaches a maximum around day 22 (Jeziorska *et al*., 1996), although positive immunoreactivity of glandular epithelium was also found later — at menstruation — in another study (Skinner *et al*., 1999). Nevertheless, these cyclic changes of gelatinase B support a regulatory role by hormones in the female reproductive tract (Skinner *et al*., 1999). At menstruation, most of the enzyme is provided by polymorphonuclear cells, macrophages, and eosinophils (Jeziorska *et al*., 1996). The optimal presence of gelatinase B in human glandular secretion and in the uterine fluid during the periimplantation phase suggests a role for it in endometrial biology, not only in matrix remodeling during the menstrual cycle, but also in blastocyst recognition and implantation (Jeziorska *et al*., 1996). During early pregnancy, fetal cytotrophoblast cells penetrate the uterine epithelium and its basement membrane, and then invade the uterine endometrium. In mice, the observed strong expression of gelatinase B in the trophoblasts at days 5.5 and 7.5 may indicate a role for this enzyme in the invasion and subsequent implantation process of the early embryo. In particu-

Table 15. Gelatinase B in physiological and pathological processes and phenotypes of gelatinase B-deficient mice

Physiological functions	References	Phenotypes in knockout mice	References
Reproduction	Fisher *et al.*, 1989; Librach *et al.*, 1991; Behrendtsen *et al.*, 1992	impaired reproduction	Dubois *et al.*, 2000
Growth and development	Everts *et al.*, 1992;	delayed ossification	Vu *et al.*, 1998
		delayed osteoclast recruitment	Engsig *et al.*, 2000
	Wucherpfennig *et al.*, 1994	normal myelination	Oh *et al.*, 1999
		reduced angiogenesis	Bergers *et al.*, 2000
Leukocyte mobilization	Masure *et al.*, 1997; Pruijt *et al.*, 1999a and 2002	normal progenitor cell mobilization	Pruijt *et al.*, 2002
		impaired kit ligand processing and stem and progenitor recruitment	Heissig *et al.*, 2002
Inflammation	Welgus *et al.*, 1990; Cowland and Borregaard, 1999	normal neutrophil emigration	Betsuyaku *et al.*, 1999b
	Kobayashi *et al.*, 1999a	impaired neutrophil chemotaxis	D'Haese *et al.*, 2000
		impaired dermal dendritic and Langerhans cell migration	Ratzinger *et al.*, 2002
Wound healing	Matsubara *et al.*, 1991; Salo *et al.*, 1994; Young and Grinnell, 1994; Agren, 1994		
Pathological functions	**References**	**Phenotypes in knockout mice**	**References**
Premature rupture of membranes	Vadillo-Ortega *et al.*, 1996		
Bone remodeling	Ohashi *et al.*, 1996; Ueda *et al.*, 1996	resistance to necrotizing tail lesions	Dubois *et al.*, 1999
INFLAMMATION			
Chronic wounds	Wysocki *et al.*, 1993	resistance to necrotizing tail lesions	Dubois *et al.*, 1999
Blistering of the skin	Oikarinen *et al.*, 1993	resistance to bullous pemphigoid	Liu *et al.*, 1998
	Stähle-Bäckdahl *et al.*, 1994		
Anaphylactoid purpura	Kobayashi *et al.*, 1998b	shortened contact hypersensitivity	Wang *et al.*, 1999
Acute respiratory distress syndrome	Ricou *et al.*, 1996	normal bleomycin-induced fibrosing alveolitis, but diminished alveolar bronchiolization	Betsayaku *et al.*, 2000
LPS-induced lung injury		no protection	Betsayaku *et al.*, 1999c
Bronchiectasis	Sepper *et al.*, 1994		
Cystic fibrosis	Delacourt *et al.*, 1995		
Asthma	Dahlen *et al.*, 1999; Hoshino *et al.*, 1999; Yao *et al.*, 1999	protection against lung injury by immune complexes	Warner *et al.*, 2001
Pulmonary emphysema	Shapiro, 1994		
Silicosis	Pard *et al.*, 1999		
Periodontitis	Mäkelä *et al.*, 1994		
Inflammatory bowel disease	Bailey *et al.*, 1994	protection against experimental hepatitis	Wielockx *et al.*, 2001
Lupus nephritis	Nakamura *et al.*, 1993		
Heymann nephritis	McMillan *et al.*, 1996a		
Rheumatoid arthritis	Opdenakker *et al.*, 1991; Hirose *et al.*, 1992; Koolwijk *et al.*, 1995; Van den Steen *et al.*, 2002		
Sjögren's syndrome	Konttinen *et al.*, 1998		
Giant cell arteritis	Sorbi *et al.*, 1996		
Aneurysma	Thompson *et al.*, 1995; Freestone *et al.*, 1995		
Peripheral nerve injury	La Fleur *et al.*, 1996		
Guillain-Barré syndrome	Kieseier *et al.*, 1998; Hughes *et al.*, 1998		
Blood-brain barrier destruction	Rosenberg *et al.*, 1994; Mun-Bryce *et al.*, 1998	resistance against traumatic brain injury	Wang *et al.*, 2000

lar, it was suggested that the trophoblast cells of the implanting embryo used gelatinase B first for the degradation of the basement membrane collagen and then for the removal of denatured gelatinous fragments of stromal fibrillar collagen, which would pave the way for migration into the uterine decidual tissue (Reponen *et al.*, 1995). *In vitro* culture models of mouse blastocysts have shown that the expression of gelatinase B is upregulated in parallel with the differentiation of the trophoblast cells (Behrendtsen *et al.*, 1992). The invasive cytotrophoblast cells synthesize both metalloproteinases and u-PA (Librach *et al.*, 1991). *In vivo* experiments demonstrated the expression and activation of gelatinase B during

Table 15 (continued)

Multiple sclerosis	Gijbels *et al.*, 1992; Paemen *et al.*, 1994		
Experimental autoimmune encephalomyelitis (EAE)	Gijbels *et al.*, 1993	resistance of young mice to EAE	Dubois *et al.*, 1999
INFECTIOUS DISEASES			
HTLV-1 myelopathy	Giraudon *et al.*, 1998; Umehara *et al.*, 1998		
AIDS	Dhawan *et al.*, 1992; Weeks *et al.*, 1993a		
Viral and bacterial meningitis	Gijbels *et al.*, 1992; Paemen *et al.*, 1994		
Neuroborreliosis	Perides *et al.*, 1998		
Septic arthritis	Williams *et al.*, 1990		
Bacterial sepsis	Paemen *et al.*, 1997	resistance to LPS shock	Dubois *et al.*, 2002
DEGENERATIVE DISEASES			
Alzheimer's disease	Backstrom *et al.*, 1996		
Amyotrophic lateral sclerosis	Lim *et al.*, 1996		
VASCULAR DISEASES			
coronary atherosclerosis	Galis *et al.*, 1994		
myocardial infarction	Tyagi *et al.*, 1996	protection against cardiac rupture protection against reperfusion injury	Heymans *et al.*, 1999 Romanic *et al.*, 2002
cerebral infarction	Anthony *et al.*, 1997	resistance to focal ischemia and demyelination	Asahi *et al.*, 2001a; Asahi *et al.*, 2001b
arterial injury	Bendeck *et al.*, 1994		
CANCER			
Invasion	Stettler-Stevenson *et al.*, 1993; Himelstein *et al.*, 1994	reduced invasion	Coussens et al., 2000
Metastasis	Bourguignon *et al.*, 1998; Yu and Stamenkovic, 1999	reduction of metastasis	Itoh *et al.*, 1999a

Physiological and pathological functions of gelatinase B are indicated together with early literature references. These and more recent references are indicated in the text. For the phenotypes observed in gelatinase B knockout mice primary references have been included. It needs to be noticed that the knockout constructs and hence the phenotypes may differ between these studies.

colonization of the maternal decidua. mRNAs for stromelysin-1, stromelysin-3, gelatinase A, TIMP-1, and TIMP-2 were expressed in the undifferentiated stroma toward the outside of the decidua (Alexander *et al.*, 1996; Cañete-Soler *et al.*, 1995). Gelatinase B and TIMP-3 were both expressed in stromal cells at the site of early mouse embryo implantation (Reponen *et al.*, 1995), but the TIMP-3 mRNA, as well as the TIMP-3 protein, were only transiently present and declined from 6.5 days post-coitum onward, that is, after decidual formation, and at the initiation of the expansion and maintenance of decidual cells and the ingression of the implantation region into the decidua (Alexander *et al.*, 1996; Reponen *et al.*, 1995).

The invasion process, which enables implantation, has to be strictly regulated in space and in time. This regulation differentiates the normal process from malignancy. Indeed, the invasion has to be confined to the endometrial aspect of the myometrium and to continue only until midgestation. This control function is carried out in different ways and by specific effector molecules, of which TIMPs, hormones, and other circulating factors are well-known examples. In this respect, *in vitro* models showed that both TIMP-1 and an antigelatinase B-antibody inhibited the clearing of subjacent matrix by trophoblast cells (Librach *et al.*, 1991; Behrendtsen *et al.*, 1992). The transient expression of TIMPs, in particular TIMP-3, may also indicate a role in neutralizing excessive action of gelatinase B (Reponen *et al.*, 1995; Leco *et al.*, 1996; Riley *et al.*, 1999). It was also demonstrated that human cytotrophoblast cells, which are invasive only in the first trimester of gestation, express gelatinase B in active form only during the first trimester of the preg-

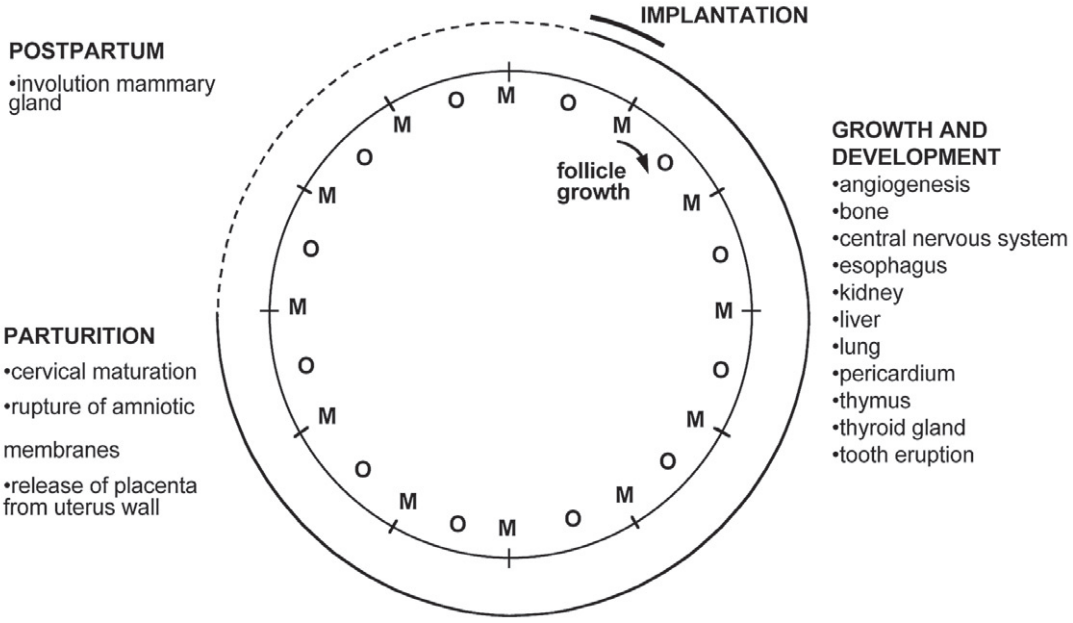

FIGURE 8. Roles of gelatinase B in the reproductive cycle, and in growth and development. Gelatinase B has been shown to intervene at different stages of the reproductive cycle through remodeling of tissues in implantation, parturition, and mammary gland involution. Also in growth and development, gelatinase B expression has been documented in various organs. The inner circle represents subsequent menstrual cycles, where gelatinase B is involved in ovulation (O) and menstruation (M). In pregnancy (outer cycle), implantation occurs during the second part of the menstrual cycle, followed by the development of the embryonic organs.

nancy (Fisher *et al*., 1989). The regulatory function of hormones in this context of temporally controlled expression of gelatinase B (*vide supra*) has been demonstrated by Shimonovitz *et al*. (Shimonovitz *et al*., 1998). Another example of temporal orchestration of invasiveness during implantation is the regulatory role of leukemia inhibitory factor and EGF on the expression of gelatinase B. At day 7, both soluble factors stimulated gelatinase B expression, whereas at day 9 or 10 EGF was found to have no effect but leukemia inhibitory factor decreased the production of gelatinase B (Harvey *et al*., 1995).

Besides a role in pregnancy via effects on ovulation and implantation, gelatinase B seems also to be involved in parturition. Indeed, the striking increase in gelatinase B expression in rat amnion and possibly the capsular region of the visceral yolk sac placenta approximately 12 h prior to delivery is responsible, in part, for the alterations in the structure of these fetal membranes before parturition (Lei *et al*., 1995). In humans, both gelatinase B protein levels and activity increase with labor in amniochorion, which may result in the degradation of the extracellular matrix of the fetal membranes and facilitate their rupture (Vadillo-Ortega *et al*., 1995). In addition, gelatinase B may play a role in the process of cervical maturation and dilatation (Osmers *et al*., 1995). Neutrophils seem to be the major cellular source of gelatinase B in this process (Winkler *et*

al., 1999). As already mentioned earlier, interleukin-8, the major neutrophil chemoattractant and activator, stimulates the quick release of progelatinase B from neutrophils (Masure *et al*., 1991). This enzyme is activated *in situ* and thus guarantees fast effects (Peppin and Weiss, 1986) without the need of transcriptional regulation, which would take at least 6 h to become full blown. Gelatinase B was also suggested to contribute to the release of the placenta from the uterus wall after parturition (Shimamori *et al*., 1995). Following birth, gelatinase B is involved in mammary gland involution when lactation is terminated (Werb *et al*., 1996).

In the male reproductive system, the testis is also a dynamic tissue showing continuous proliferation, differentiation, and migration of its cellular components, both during development and adult life (Sharpe *et al*., 1994). However, here the predominant gelatinase secreted by cultured peritubular cells and by Sertoli cells is gelatinase A (Hoeben *et al*., 1996). Perturbation of gelatinase B activity by genetic knock-out results in subfertility or infertility. Although these results prove the concept that this enzyme is important in fertility and sterility, its function can be compensated for. Which enzymes compensate at which specific stage is currently being investigated. However, this knowledge implies that the use of MMP inhibitors will have effects on reproduction and may be useful therapeutics (Dubois *et al*., 2000).

3.1.2. Growth and Development

Besides the localization of gelatinase B mRNA by *in situ* hybridization in the invading trophoblast cells and the yolk sac, a developmental study did not show any signal in the mouse embryo until day 11, when detectable reaction was present in the central nervous system (Cañete-Soler *et al*., 1995). Indeed, oligodendrocytes utilize gelatinase B to extend multiple processes toward the axons that are targeted for myelination. It was demonstrated that the temporal increase in gelatinase B expression in the murine white matter parallels the developmental milestones of myelination *in vivo* (Uhm *et al*., 1998; Oh *et al*., 1999). However, in gelatinase B-deficient mice maturation of oligodendrocytes and myelination are normal (Oh *et al*., 1999). Part of the complex process of neurogenesis is the formation of a complex vascular network, which consumes about 25% of the cardiac output after maturation. The expression of gelatinase B during brain development could well be associated with this process of brain vascularization (Cañete-Soler *et al*., 1995).

Apart from its presence in the central nervous system, gelatinase B expression appears in a precise temporal sequence in the liver, the developing bronchial epithelium and the primordial alveoli, the epithelium of the thyroid gland, the thymus, the trachea, the esophagus, the pericardium, and the endochondral plates of the bone (Cañete-Soler *et al*., 1995). During nephrogenesis, gelatinase B is limited to the invading vascular structures within immature glomeruli. It is not expressed by the differentiating epithelia of tubules or glomeruli, or in mature nephrons (Tanney *et al*., 1998). This is in line with the observation that kidneys of gelatinase B-deficient mice develop normally, histologically, as well as functionally (Liu *et al*., 2000b). In mouse tooth morphogenesis, gelatinase B was transiently expressed in the early dental mes-

enchyma surrounding the invaginating tooth bud. At the time of tooth eruption, and thus bone resorption, it was intensely expressed in osteoclasts (Sahlberg *et al*., 1999). The development of long bones by the process of endochondral ossification requires invasion by blood vessels, degradation of the cartilage matrix, and the deposition and subsequent remodeling of bone matrix. These processes are mediated by gelatinase B, which is secreted by the osteoclasts (Everts *et al*., 1992; Okada *et al*., 1995; Wucherpfennig *et al*., 1994). In fact, the mouse cDNA of gelatinase B was independently cloned from tumor cells (Tanaka *et al*., 1993; Masure *et al*., 1993) and from osteoclasts (Reponen *et al*., 1994). Gelatinase B was localized at the growth plate surface, forming the epiphysis/metaphysis interface, and within the epiphysis, at the edge of the marrow space. Both these sites are engaged in the resorption of endochondral cartilage. It has been hypothesized that gelatinase B attacks the edge of the endochondral cartilage and helps to solubilize the collagen-rich framework, which is then released for further digestion. This final step opens the way to invasion by capillaries, thereby making the replacement of cartilage by bone possible (Lee *et al*., 1999a). Convincingly, in gelatinase B-deficient mice this vascular invasion into the cartilage extracellular matrix is delayed, resulting in delayed ossification and formation of an excessively wide zone of hypertrophic cartilage (Vu *et al*., 1998). Similar growth plate alterations were reported when the angiogenic protein vascular endothelial growth factor (VEGF) was inactivated in a mouse model (Gerber *et al*., 1999). Thus, the synergistic effects between gelatinase B and VEGF in angiogenesis in growth plates has been explained by VEGF binding to extracellular matrix and being released by the action of gelatinase B (Bergers *et al*., 2000). Furthermore, VEGF is chemotactic for osteoclasts that can release gelatinase B (Engsig *et al*., 2000). Also in other physiological processes, for example, menstruation, placentation, embryo implantation, embryo growth, and wound healing, new blood vessels are required to supply oxygen and nutrients. The formation of collateral circulation is also necessary to limit damage in ischemic disease. In contrast and as discussed in more detail later, uncontrolled and persistent angiogenesis is a cause of many diseases. In proliferative diabetic retinopathy, for instance, which is characterized by preretinal neovascularization, progelatinase B was detected in the vitreous fluid of 73% of the patients and in only 8% of nondiabetic patients (Abu El-Asrar *et al*., 1998; Kosano *et al*., 1999). The intravitreous concentrations of VEGF were also elevated in patients with proliferative diabetic retinopathy, and the relation between this cytokine and gelatinase B may be similar as the mechanism outlined above (Kosano *et al*., 1999). In fact, all inflammatory and proliferative diseases are accompanied by angiogenesis, and gelatinase B is one of the MMPs that is expressed in tissues that are actively engaged in angiogenesis (Hiraoka *et al*., 1998).

3.1.3. Inflammation and Wound Healing

Gelatinase B is a secretion product of activated monocytes (Welgus *et al*., 1990; Opdenakker *et al*., 1991b; Opdenakker *et al*., 1991a) and a major component of the tertiary granules of human neutrophils (Hasty *et al*., 1990; Masure *et al*., 1991; Kjeldsen *et al*.,

1992; Cowland and Borregaard, 1999). Although at much lower levels, the enzyme is also produced by T cells and is induced by T cell activation (Weeks *et al*., 1993b; Leppert *et al*., 1995). All these cells are implicated in inflammation. Consequently, gelatinase B is postulated to play an important role in host defense. However, major immunodeficiencies have not yet been described in gelatinase B-deficient mice. It should be noticed that most knock-out mice are kept under specific pathogen-free (SPF) conditions, which is a privileged environment in terms of encountering pathogenic microorganisms. Therefore, we have placed a fraction of our gelatinase B-deficient mice (Dubois *et al*., 1999) in a conventional animal house environment. For up to 5 years no immunodeficiency was noticed in terms of opportunistic infections or spontaneous tumor development. The physiological aspects of inflammation, which we discuss here, are limited to wound healing because this constitutes an attempt of the organism to restore health. The implication of gelatinase B in inflammatory processes resulting in pathological conditions will be summarized below.

The *in vitro* observation that gelatinase B actively contributed to the wound repair process of the respiratory epithelium (Buisson *et al*., 1996) was in line with prior findings in several *in vivo* models, some of which are discussed in more detail. It was shown, for instance, that during wound healing in the human oral mucosa, gelatinase B was strongly expressed both in the epithelium and in the granulation tissue. This suggests a participation of gelatinase B in detaching keratinocytes from the basement membrane, promoting cell locomotion in wound matrix, and remodeling of the granulation tissue matrix. In this model, the expression of gelatinase A remained stable during wound healing (Salo *et al*., 1994). In fluid of burn blisters, gelatinase B was detected as early as 4 to 8 h after injury. Marked increases in gelatinase B levels, as well as the activation of the proenzyme occurred between day 0 and day 2, and may play a role in remodeling of denatured collagen (Young and Grinnell, 1994). In full- and partial-thickness skin wounds in pigs (Agren, 1994), in fluid of mastectomy wounds in humans (Wysocki *et al*., 1999), and in acetic acid-induced gastric ulcers in rats (Baragi *et al*., 1997), gelatinase B levels were highest in the early healing phase and then decreased as healing proceeded. Gelatinase A was more elevated than in uninjured skin, but remained fairly stable (Agren, 1994). In a rabbit model of corneal injury, gelatinase A participated in the prolonged process of collagen remodeling in the corneal stroma that eventually results in functional regeneration of the tissue. Gelatinase B expression did not correlate with stromal remodeling, but might play a role in controlling resynthesis of the epithelial basement membrane (Matsubara *et al*., 1991). The difference in gelatinase B/gelatinase A ratios during wound healing may be due to the nature of the wounded tissue. Injury to an avascular tissue, for example, the cornea, seems to be accompanied by higher levels of gelatinase A compared with gelatinase B, whereas the reverse is true for vessel-rich tissues such as the skin, in which the inflammatory response is more pronounced (Young and Grinnell, 1994).

A physiological role of gelatinase B, which also relates to inflammation and regeneration, is leukocyte recruitment and progenitor or stem cell mobilization. It was demonstrated in rhesus monkeys that interleukin-8-induced he-

matopoietic progenitor cell mobilization was accompanied by systemic release of gelatinase B. This mobilization of hematopoietic progenitor cells was prevented by pretreatment with an inhibitory antigelatinase B antibody (Fibbe *et al*., 1999; Pruijt *et al*., 1999a; Pruijt *et al*., 1999b). In the mouse it was found that neutrophils are indispensible for this IL-8-induced mobilization of hematopoietic progenitor cells. Because the mobilization was also observed in gelatinase B-deficient mice, gelatinase B seems dispensible or may be compensated for by other molecules (Pruijt *et al*., 2002). However, enzyme neutralization with antibodies is mechanistically quite different from a gene deletion, and this may account for these differences.

For the chemotaxis of inflammatory cells in gelatinase B-deficient mice, conflicting results have been published. Betsuyaku *et al*. (1999b) demonstrated that neutrophil migration under chemotactic pressure does not necessitate gelatinase B, whereas D'Haese *et al*. (2000) showed evidence that gelatinase B plays an assisting role in this process. In inflammatory processes in humans with the involvement of IL-8 as neutrophil attractant, this process may be reinforced because of the recent finding of potentiation of IL-8 by gelatinase B (Van den steen *et al*., 2000). Because of our findings that specific gelatinase B knockout mouse strains are leaky (unpublished results), and that non-leaky mice may develop compensatory mechanisms, the interpretation of knockout studies may be more complex than hithertio realized.

In Langerhans cell migration in *vivo*, gelatinase B was shown to participate, as an intradermal injection of a blocking monoclonal antibody prevented a hapten-induced decrease of Langerhans cell numbers in the epidermis and their accumulation in regional lymph nodes (Kobayashi *et al*., 1999). In conclusion, gelatinase B plays a major role as regulator and effector of immune functions (Opdenakker *et al*., 2001b) and in leukocyte biology (Opdenakker *et al*., 2001a)

3.2. Pathological Roles of Gelatinase B

The role of gelatinase B in various pathological conditions is discussed in view of the mechanism that underlies the damage, and most of these aspects are summarized in Table 15. The failure of regulatory mechanisms might lead to diminished or excessive production of gelatinase B and subsequently to restricted, extensive, or improperly timed degradation of extracellular matrices. Examples of this pathway are premature rupture of amniotic membranes and pathologic bone resorption. Other mechanisms of disease induction are inflammation, infection, vascular pathology, degeneration, and malignancy. Because specific pathologies are associated with excessive cellular gelatinase B mRNA expression, it was logical, as already mentioned, that the cDNA was cloned from such tissues or cells (Wilhelm *et al*., 1989; Hasty *et al*., 1990; Tanaka *et al*., 1993; Reponen *et al*., 1994a; Masure *et al*., 1993).

3.2.1. Premature Rupture of Amniotic Membranes

Premature rupture of membranes is associated with increased levels of

MMPs, particularly gelatinase B, and with reduced levels of TIMP-1 in amniotic fluid. This imbalance between gelatinase B and TIMP-1 may promote premature rupture of membranes (Vadillo-Ortega *et al*., 1996). The gelatinase B concentrations in the amniotic fluid were clearly more elevated in preterm labor resulting in preterm delivery than in term delivery. In preterm labor not resulting in delivery the gelatinase B levels were similar to those in term pregnancy without labor (Athayde *et al*., 1999).

3.2.2. Pathologic Bone Resorption

As gelatinase B is involved in normal bone development and continuous remodeling, inappropriate control of its synthesis, secretion, or activation may result in pathological bone resorption, as can be seen in *morbus Paget*, amyloidosis, hyperparathyroidism, giant cell tumors, osteolytic metastases, and bone resorption around total hip arthroplasties (Ohashi *et al*., 1996; Ueda *et al*., 1996; Vidovszky *et al*., 1998).

3.2.3. Inflammatory Diseases

The role of gelatinase B in inflammation, leading to a pathological state, may be direct, by tissue destruction, or indirect, by generation of an inflammatory signal or recruitment of inflammatory cells (Delclaux *et al*., 1996). The importance of the latter mechanism is not yet clear, as in one study neutrophil emigration in the lungs, peritoneum, and skin of adult gelatinase B-deficient mice seems not to require gelatinase B (Betsuyaku *et al*., 1999b). Similarly, neutrophil gelatinase B was found to be dispensible in transendothelial migration under flow *in vitro*, but it is unclear whether the used assay measured the migration through the basement membrane (Allport *et al*., 2002). We found that in young gelatinase B-deficient mice, migration of granulocytes toward the skin in response to intradermal injection of GCP-2 was impaired (D'Haese *et al*., 2000). More recent studies are in line with our observations and seem to favor a functional role of gelatinase B in neutrophil migration *in vivo* (Keck *et al*., 2002; Romanic *et al*., 2002). In the following sections, a number of inflammatory diseases are reviewed. For efficient comprehension and comparison, we have classified these according to the different organ systems.

3.2.3.1. Chronic Wound

As mentioned previously, the cellular and molecular events underlying traumatic or surgical wounds are situated at the overlap between physiology and pathology. In acute wounds, the pathophysiological changes are directed to restoration of the normal healthy state. However, if the healing mechanisms fail, these wounds become chronic. In acute wound fluid, gelatinase A and B levels have been found to be increased. In chronic wounds, these enzyme levels are still more elevated above control background levels. This may suggest that nonhealing ulcers develop an environment containing high levels of activated metalloproteinases, which may result in chronic tissue turnover and failure of wound closure (Weckroth *et al*., 1996; Wysocki *et al*., 1993; Bullen *et al*., 1995; Wysocki *et al*., 1999).

3.2.3.2. Inflammation of the Skin

In human skin, gelatinase B is mainly found in the epidermis and in endothelial cells, whereas gelatinase A is mostly expressed in fibroblasts. The ratio gelatinase B/gelatinase A varies considerably in blistering diseases, due to the level of blister formation, the degree of inflammation, and injury induced by blister formation (Oikarinen *et al*., 1993). In bullous pemphigoid, gelatinase B is present in blister fluid (Ståhle-Bäckdahl *et al*., 1994). The degradation of the extracellular domain of BP180, an autoantigen and a transmembrane hemidesmosome protein, by gelatinase B (Ståhle-Bäckdahl *et al*., 1994) may be a critical event in the pathogenesis of this autoimmune disease, as epidermal-dermal separation is an important feature in blister formation. The role of gelatinase B in this autoimmune disease is further strenghtened by the resistance of neonatal gelatinase B-deficient mice to bullous pemphigoid (Liu *et al*., 1998). In a case of anaphylactoid purpura, gelatinase B was also attributed a blister-inducing role, based on a high gelatinase B/gelatinase A ratio in the blister fluid when compared with that found in plasma (Kobayashi *et al*., 1998b).

Another indication for the inflammation-promoting role of gelatinase B in skin diseases is the frequent therapeutical use of topical glucocorticoids, which exhibit their action, at least in part, through gelatinase B. Indeed, as already mentioned in the section on gelatinase B gene regulation, glucocorticoids exert a directly inhibitory effect on the transcription of gelatinase B (Oikarinen *et al*., 1987; Oikarinen *et al*., 1986). Corticosteroids also inhibit the gelatinase B activation through an influence on the plasminogen-plasmin cascade (Andreasen *et al*., 1987).

An immunological skin disorder in which gelatinase B was shown to play a critical role in its resolution is contact hypersensitivity (Wang *et al*., 1999). Indeed, it was demonstrated in gelatinase B-deficient mice that gelatinase B is necessary for timely resolution of the reaction to antigenic challenge.

3.2.3.3. Inflammation of the Pulmonary Tract

Gelatinase B has been implicated in the pathophysiology of different lung diseases. During the early phase of the Acute Respiratory Distress Syndrome (ARDS), gelatinase B levels in bronchoalveolar lavage fluid were higher than in control patients, but plasma levels were not different. This suggests a local action of the enzyme. The gelatinase B/TIMP-1 ratio remained elevated in late phases of prolonged ARDS. These high intrapulmonary levels of gelatinase B may reflect an increased turnover of extracellular matrix in acute lung injury (Ricou *et al*., 1996).

High levels of gelatinases A and B were also found in the bronchoalveolar lavage fluid of patients with bronchiectasis, a condition that is caused by an inflammatory destruction of the extracellular matrix components of the bronchial wall. Furthermore, the level of degradative potential, measured by zymography, correlated with the severity of the disease, suggesting a role for these enzymes in the inflammatory destruction (Sepper *et al*., 1994).

In sputum supernatants of patients with cystic fibrosis, which is character-

ized by inflammation and subsequent destruction of small bronchioles, and later of larger airways, gelatinase B activity was increased and correlated with high levels of substrate degradation products in bronchial secretions (Delacourt *et al*., 1995).

In a mouse model it was shown that bleomycin-induced fibrosing alveolitis develops irrespective of the presence of gelatinase B. However, gelatinase B seems to be required for alveolar bronchiolization, perhaps by facilitating a migration of Clara cells and other bronchial cells into the regions of alveolar injury (Betsuyaku *et al*., 1999b). In another model with immunoglobulin G immune complexes, gelatinase B deficiency conferred protection against lung injury (Warner *et al*., 2001). Gelatinase B may also display a pathogenic function in asthma (Dahlen *et al*., 1999; Hoshino *et al*., 1999; Yao *et al*., 1999), in pulmonary emphysema (Betsuyaku *et al*., 1999a; Shapiro, 1994) and in lung silicosis (Pardo *et al*., 1999). Acute lung injury, induced by endotoxin, was not mediated by gelatinase B (Betsuyaku *et al*., 1999c). In contrast, recently we showed that a different strain of gelatinase B-deficient mice are protected against systemic endotoxin (Dubois *et al*., 2002 submitted).

3.2.3.4. Inflammation of the Gastrointestinal Tract

In oral rinses of patients with periodontitis, higher levels of gelatinase A and B were detected than in those of healthy subjects. Moreover, the levels of gelatinase A and B increased with periodontal disease activity (Mäkelä *et al*., 1994). Increased numbers of polymorphonuclear leukocytes that were positive for gelatinase B immunostaining were found in inflammatory bowel diseases, such as Crohn's disease (Bailey *et al*., 1994). Hepatitis and liver failure accompany viral infections and toxic insults of the liver. This pathology is in part mediated by TNF- and is observed in mouse models of toxic hepatitis. Gelatinase B-deficient mice were resistant to induction of hepatitis by tumor necrosis factor. Moreover, metalloproteinase-inhibiting drugs also prevented hepatitis (Wielockx *et al*., 2001).

3.2.3.5. Inflammation of the Renal Tract

In an animal model of lupus nephritis, mRNA levels for gelatinase B were increased, and this suggests that enhanced expression of gelatinase B may contribute to the evolution of glomerular injury. The beneficial effect of methylprednisolone in this pathology may be associated with its ability to suppress the expression of mRNA for metalloproteinases and their inhibitors (Nakamura *et al*., 1993).

In a rat model of Heymann nephritis, gelatinase B mRNA and protein were increased when compared with levels in normal rats. The correlation between this enhanced expression and proteinuria may even suggest a causative link with changes in glomerular capillary permeability (McMillan *et al*., 1996a). However, gelatinase B was shown not to be involved in the progression of glomerulonephritis in a mouse model of Alport syndrome (Andrews *et al*., 2000).

3.2.3.6. Inflammation of the Joint

In inflammatory arthritis, for example, rheumatoid arthritis, high levels of gelatinase B have been detected by zymography and other techniques in synovial fluid (Opdenakker *et al*., 1991a). These studies were corroborated later and gelatinase B was also found, by zymography, to be increased in serum (Koolwijk *et al*., 1995; Ahrens *et al*., 1996; Gruber *et al*., 1996). Elevated synovial fluid levels of gelatinase B antigen correlate positively with the extent of joint involvement and severity of disease. This indicates that gelatinase B may be a useful marker of progressive inflammatory disease associated with abnormally high matrix turnover and cartilage destruction, which is pathognomonic for rheumatoid arthritis (Ahrens *et al*., 1996). In rheumatoid arthritis, the positive correlation between neutrophil counts in the synovial fluid and gelatinase B activity suggests that the enzyme is released from activated, infiltrating neutrophils (Hirose *et al*., 1992). We found that gelatinase B production in synovial fluid in joint disease was predominantly by neutrophils, whereas cell types of the macrophage/antigen-presenting cell lineage, which produce less gelatinase B, may be predominant in osteoarthritis and traumatic synovitis (Grillet *et al*., 1997). Indeed, in case of acute joint trauma, chondromatosis, villonodular synovitis or a cyst of a bursa, high numbers of strongly immunopositive neutrophils were observed in addition to weaker staining macrophages. In rheumatoid arthritis there was no tissue immunostaining if neutrophil infiltration was absent, and in other cases of chronic synovitis a strong gelatinase B expression was observed in dendritic cells (Grillet *et al*., 1997). The analysis of gelatinase B activity may help to discriminate between rheumatoid arthritis and osteoarthritis on a biochemical basis, as was shown recently, when we analyzed the degradation of denatured type II collagen (Van den Steen *et al*., 2002).

Another concern of rheumatologists is Sjögren's syndrome, in which saliva was demonstrated to contain higher levels of gelatinase B. The associated ultrastructural changes of the basal lamina suggest a role for gelatinase B in glandular alterations in Sjögren's syndrome and thus may constitute a pathogenic clue for this autoimmune disease (Konttinen *et al*., 1998).

3.2.3.7. Inflammation of Blood Vessels

In giant cell arteritis, increased serum levels of gelatinase B were found. The detection of gelatinase B mRNA in the lamina media of the inflamed vasculature suggests that degradation of intercellular matrix may play a role in the pathogenesis of giant cell arteritis. This mRNA was mainly detected in cells that resemble smooth muscle cells and fibroblasts. Myeloperoxidase activity did not correlate with the serum gelatinase activity or antigen levels, indicating that polymorphonuclear cells are probably not responsible for elevated gelatinase B titers (Sorbi *et al*., 1996).

When tissues of athero-occlusive disease and abdominal aortic aneurysms were compared by zymography and immunohistochemistry with normal tissues, gelatinase B was present in the occlusive, and at most elevated levels in the aneurysmic vessel walls. The localization of gelatinase B in macrophages in the damaged wall of aneurysmal

aortas suggests that chronic release of this metalloproteinase contributes to extracellular matrix degradation in abdominal aortic aneurysms (Thompson *et al*., 1995). Indeed, gelatinase B was found at higher levels in large aneurysms, whereas gelatinase A was the principal gelatinase in small aneurysms. Thus, it is possible that gelatinase B is important in the transformation of a slowly growing small aneurysm to a dangerous, fast-growing aneurysm (Freestone *et al*., 1995; Sakalihasan *et al*., 1996). Also at the mRNA level, gelatinase B was present at significantly higher levels in aneurysmal than in normal aorta, particularly in the adventitial macrophages in areas of neovascularization (McMillan *et al*., 1995). The critical role of gelatinase B in a mouse model of aortic aneurysm disease was proven by bone marrow transplantation experiments from wild-type to gelatinase B-deficient mice and *vice versa* (Pyo *et al*., 2000). These studies suggest differences in the regulated expression of the gelatinase B gene in aneurysm vs. control, in other words in the gene promoter activity. In Section 2.1.2, we discussed the associations of a SNP and a microstallite DNA in the gelatinase B gene (which influences promoter activity) with atherosclerotic disease (Zhang *et al*., 1999b; St Jean *et al*., 1995). The dinucleotide microsatellite polymorphism was shown to result in small differences in gelatinase B expression within the intracranial vasculature, leading to increased susceptibility to formation of intracranial aneurysms (Peters *et al*., 1999). In view of the higher expression of gelatinase B in aneurysms (McMillan *et al*., 1995; Peters *et al*., 1999) and the functional studies of the gelatinase B promoter, epistasis may be important in the pathogenesis. The potential role of the gelatinase B gene in multiple sclerosis may be explained in a similar way (Nelissen *et al*., 2000).

3.2.3.8. Inflammation of the Nervous System

Neuroinflammatory diseases are another clinical field in which gelatinase B is supposed to exert (detrimental) effects. Gelatinase B has been detected in the peripheral and central nervous systems, as well as in the protective barrier between the nervous compartment and the blood.

In the peripheral nervous system, gelatinase B appeared to be induced in crush and distal segments of mouse sciatic nerve after injury (La Fleur *et al*., 1996). In Experimental Allergic Neuritis (EAN), the animal model for the Guillain-Barré syndrome, quantitative polymerase chain reaction analysis revealed an upregulation of gelatinase B mRNA with peak levels concurrent with maximal disease severity. Immunohistochemically, gelatinase B was localized primarily around blood vessels within the epineurium and endoneurium in diseased, but not normal rat sciatic nerve (Kieseier *et al*., 1998a; Hughes *et al*., 1998). In the Guillain-Barré syndrome, positive immunoreactivity for gelatinase B was also found in sural nerve biopsies and was corroborated by the demonstration of increased mRNA expression in comparison with noninflammatory neuropathies (Kieseier *et al*., 1998b). Plasma levels of gelatinase B are also elevated in the Guillain-Barré syndrome and correlate with disease severity (Créange *et al*., 1999). In chronic inflammatory demyelinating polyneuropathy and nonsystemic

vasculitic neuropathy, two chronic inflammatory polyneuropathies that have been linked to an autoimmune response of unknown cause against antigens of the peripheral nervous system, gelatinase B expression was strongly enhanced in nerve tissue and the enzyme was mainly produced by T cells, whereas macrophages contributed only to a minor extent (Leppert *et al*., 1999). The finding that gelatinase B is upregulated during inflammatory (demyelinating) diseases of the peripheral nervous system, and its predominant localization around blood vessels, suggests that gelatinase B may play a crucial role in the disruption of the blood-nerve barrier, allowing mononuclear cells and other circulating factors access to the nerve (Kieseier *et al*., 1998a).

Similar damage and inflammation may account for the outcome of brain trauma. In a mouse model of experimental brain injury, gelatinase B was increased. Moreover, the neurological outcome as measured by motor neuron activity showed less deficiencies in gelatinase B knockout mice vs. littermate controls. Consequently, gelatinase B contributes to the pathology of traumatic brain injury (Wang *et al*., 2000). More studies have been done on the blood-brain barrier. In a model of LPS-injured brain, gelatinase B was shown to play a role in regulating the size-dependent opening of the blood-brain barrier during acute neuro-inflammation. Intracerebral injection of LPS was followed by an increase in barrier permeability for small and large molecules. This correlated with an upregulated production of gelatinase B during the initial 24 h after LPS injury. Treatment with a synthetic inhibitor of matrix metalloproteases resulted in a decrease in barrier permeability, but only for small molecules (Mun-Bryce and Rosenberg, 1998). Hemorrhagic injury also induces gelatinase B. The subsequent degradation of the extracellular matrix leads to opening of the blood-brain barrier, with secondary brain edema and cell death (Rosenberg *et al*., 1994). The use of MMP inhibitors, such as BB-94 (Batimastat), reduced the capillary damage, caused by intracerebral infusion of TNF-α, and resulted in delayed production of vasogenic edema (Rosenberg *et al*., 1995). The association between increased gelatinase B levels in the cerebrospinal fluid from patients with multiple sclerosis and a disturbed blood-brain barrier, as demonstrated by Magnetic Resonance Imaging (MRI), also points to a role for gelatinase B in opening of the blood-brain barrier (Rosenberg *et al*., 1996a).

These observations followed a number of studies on the prototype of the neuroinflammatory disorders: multiple sclerosis. Although neutral enzymes had been suggested to play a role in MS (Cuzner *et al*., 1975), only after the discovery of gelatinase B as a parameter in rheumatoid arthritis (Opdenakker *et al*., 1991a), the enzyme was identified in multiple sclerosis (Gijbels *et al*., 1992). In an extensive early study (Paemen *et al*., 1994), gelatinase B was detected in the cerebrospinal fluid of patients with optic neuritis, multiple sclerosis, and other inflammatory neurologic diseases, but not in normal controls, and its levels were correlated with the IgG index in MS. In contrast, gelatinase A was constitutively present in all samples. More recently, it was found that these gelatinase B levels were similar in relapses and in the clinically stable phase of relapsing-remitting multiple sclerosis (Leppert *et al*., 1998), although serum levels of gelatinase B have been shown to be more elevated during relapse (Lee *et al*., 1999b; Lichtinghagen *et al*., 1999).

In primary progressive multiple sclerosis, gelatinase B was only increased in 57% of the cerebrospinal fluid samples, and the levels were below those encountered in the relapsing-remitting form. The sustained increase of gelatinase B in clinically stable multiple sclerosis supports the concept that multiple sclerosis is associated with ongoing proteolysis that may result in progressive tissue damage (Leppert *et al*., 1998). Immunohistochemical reaction with gelatinase B-specific monoclonal antibodies (Paemen *et al*., 1995) was documented within human MS plaques in concert with the expression of other metallo- and serine proteases and specific inhibitors (Cuzner *et al*., 1996; Maeda and Sobel, 1996). Besides these findings on gelatinase B in the cerebrospinal fluid and in the histology of the central nervous system, a positive correlation was also observed for gelatinase B and TIMP-1 expression in the blood of multiple sclerosis patients. Indeed, numbers of gelatinase B and TIMP-1 mRNA-expressing blood cells were higher in MS patients than in patients with other neurological diseases or healthy subjects (Özenci *et al*., 1999). Gelatinase B has also been shown to degrade the collagen type IV in the basement membranes of the endothelial walls. This suggests that gelatinase B might play a key role in the destruction of the blood-brain barrier (Mun-Bryce and Rosenberg, 1998; Rosenberg *et al*., 1996a; Rosenberg *et al*., 1994; Rosenberg *et al*., 1995), which normally preserves the immunologically privileged status of the central nervous system (Leppert *et al*., 1996; Rosenberg *et al*., 1996a). Other possible functions of gelatinase B are activation or degradation of disease-modifying cytokines (Schönbeck *et al*., 1998) and direct damage of central nervous system cells. Finally, the beneficial effect of interferon-β on MS may be mediated by a control of the protease balance, the net activity of proteases and protease inhibitors (Opdenakker and Van Damme, 1994), for example, by the inhibition of the gelatinase B expression resulting in reduction of T lymphocyte infiltration into the central nervous system (Leppert *et al*., 1996; Stüve *et al*., 1996) or inhibition of the migration of activated leukocytes through the blood-brain barrier (Lou *et al*., 1999). The first experimental evidence for an effect of interferon-β on the protease balance (net effect of gelatinases and TIMPs) was demonstrated recently. Gelatinase activity, which is upregulated by inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1 was dose-dependently suppressed with interferon-β (Bartholomé *et al*., 2001).

In murine Experimental Autoimmune Encephalomyelitis (EAE), which is used as animal model for multiple sclerosis, gelatinase B was also increased in the cerebrospinal fluid of diseased mice (Gijbels *et al*., 1993). Recently, it was demonstrated that levels of gelatinase B mRNA were increased in adoptive transfer EAE at times of maximum disease severity. Positive immunochemical staining with an gelatinase B-specific monoclonal antibody was observed along the meninges, around blood vessels and within the parenchyma in diseased, but not in normal animals (Kieseier *et al*., 1998b). EAE experiments in which gelatinase B-deficient mice (Dubois *et al*., 1999) or protease inhibitors (Brosnan *et al*., 1980; Gijbels *et al*., 1994; Inuzuka *et al*., 1988; Hewson *et al*., 1995; Norga *et al*., 1995; Clements *et al*., 1997) were used also illustrate that central nervous system inflammation is mediated — at least in part — by proteases.

Besides these results from *in vivo* observations in humans and in EAE models, biochemical studies showed that gelatinase B is capable to cleave human and animal myelin basic protein (MBP) into peptide fragments of which at least one coincided with a documented major MBP-autoantigen (Proost *et al*., 1993; Gijbels *et al*., 1993; Opdenakker *et al*., 1994), indicating that gelatinase B may participate in the generation of immunodominant epitopes (Figure 9).

3.2.4. Infectious Diseases

Inflammation is often the result of infection and in most instances the microbial etiological agent is known. In human T-cell lymphotrope virus-1 (HTLV-1)-myelopathy (HAM: HTLV-1-associated myelopathy or TSP: tropical spastic paraparesis), for instance, the HTLV-1 causes spastic paraparesis. Gelatinase B was found at high levels in cerebrospinal fluids and sera of all patients with HTLV-1-myelopathy, whereas TIMP-1 and TIMP-2 expression was found to remain constant. The presence of gelatinase B in these patients may be a marker of the intense matrix remodeling, associated with inflammation and neurodegeneration (Giraudon *et al*., 1998; Umehara *et al*., 1998). In addition, immunohistochemistry demonstrated more gelatinase B-positive peri- or intravascular mononuclear cells in active spinal cord lesions (Umehara *et al*., 1998).

In HIV infection gelatinase B was also detected in the cerebrospinal fluid of 40% of seropositive patients, and at significantly higher levels in patients with neurologic deficits (Sporer *et al*., 1998), in particular HIV dementia (Conant *et al*., 1999). The induction of gelatinase B may be caused either directly by the virus, as has been shown for measles virus and Newcastle disease virus (Opdenakker *et al*., 1991a), or indirectly through release of inflammatory mediators in response to HIV infection of the brain (Sporer *et al*., 1998). In *in vitro* models, it was demonstrated that HIV-infected monocytes (Dhawan *et al*., 1992) and lymphocytes (Weeks *et al*., 1993a) secreted increased amounts of gelatinase B. Furthermore, HIV-1-infected lymphocytes are more invasive when tested in a reconstituted basement membrane. This suggests an increased ability of these cells to leave the circulation and to migrate into target tissues (Weeks *et al*., 1993a; Chapel *et al*., 1994).

In viral or bacterial meningitis, gelatinase B was elevated, whereas it was not present in the cerebrospinal fluid of healthy controls (Gijbels *et al*., 1992). Similar observations were made in central nervous system syphilis, tuberculosis, and Lyme disease (Paemen *et al*., 1994), and were confirmed in later studies (Kolb *et al*., 1998; Paul *et al*., 1998; Kieseier *et al*., 1999; Perides *et al*., 1998). The gelatinase B levels seemed to be correlated with the neutrophil cell number in the cerebrospinal fluid, and thus gelatinase B might be involved in the opening of the blood-brain barrier, which then allows the neutrophils to cross the basement membrane of the brain capillaries.

Trachoma is a chronic follicular keratoconjunctivitis caused by *Chlamydia trachomatis* in which increased levels of gelatinase B and numbers of inflammatory cells containing gelatinase B suggest that this enzyme plays a role in the pathogenesis of conjunctival scarring (Abu El-Asrar *et al*., 2000).

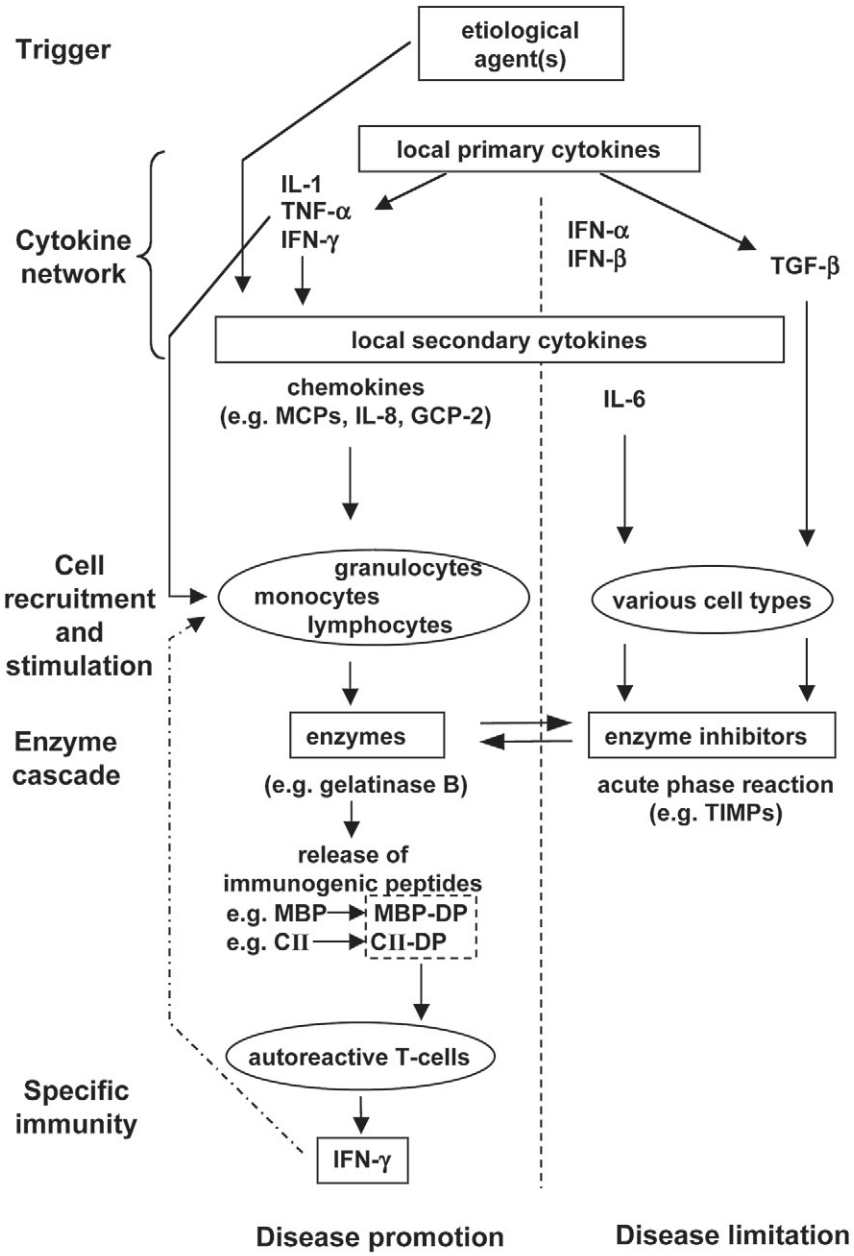

FIGURE 9. Extracellular proteolysis by gelatinase B in the generation of immunodominant epitopes. Gelatinase B activity is controlled by mechanisms including cytokine-induced gene transcription, activation by the protease network, and modulation by enzyme inhibitors. Net activity of gelatinase B contributes to extracellular degradation of proteins into peptides and results in a molar excess of antigens, compared with the intact protein. Uptake and processing of this enhanced quantum of peptides by antigen-presenting cells leads to an increased probability of presentation and activation of autoreactive T-cells and contributes to autoimmunity (Opdenakker and Van Damme, 1994; Van den Steen *et al.*, 2002). The diagram illustrates experimentally proven molecular interactions, such as cytokine, protease, and inhibitor inductions and proteolysis by gelatinase B, but does not take into account all aspects of posttranslational modifications. For instance, natural collagen II peptides, excised by gelatinase B, may be glycosylated (Van den Steen *et al.*, 2002). CII, collagen type II; IL-, interleukin-; TNF-α, tumor necrosis factor-α; IFN-, interferon-; MCP, monocyte chemotactic protein; GCP-2, granulocyte chemotactic protein-2; TGF, tranforming growth factor; PAI, plasminogen activator inhibitor; MBP, myelin basic protein; DP, degradation products.

In staphylococcal septic arthritis, the rapid loss of proteoglycan and persistent degradation of cartilage is due to the production and activation of chondrocyte proteases, for example, gelatinase B (Williams *et al*., 1990).

In sepsis, the release of proteases from polymorphonuclear leukocytes, and the degradation of connective tissue structures and soluble proteins by proteolysis or oxidation, is believed to be an important factor contributing to multiple organ failure (Jochum *et al*., 1984; Nuijens *et al*., 1992). A role for gelatinase B in bacteraemia was demonstrated in baboons (Paemen *et al*., 1997). Indirect evidence for a function of gelatinase B in sepsis may be provided by IL-8, which has been shown to be elevated in sepsis (Hack *et al*., 1992; Van Zee *et al*., 1991; Endo *et al*., 1995). IL-8 is one of the most potent neutrophil chemoattractants (Van Damme *et al*., 1988), and activation leads to almost immediate degranulation of gelatinase B from neutrophils (Masure *et al*., 1991) in human. Most recently, we found that gelatinase B-deficient mice are resistant to endotoxin-induced septic shock (Dubois *et al*., 2002). This supports the concept that specific gelatinase B inhibition may constitute a therapy of sepsis and septic shock.

3.2.5. Degenerative Diseases

In Alzheimer's disease (Backstrom *et al*., 1996) and in amyloid-positive brain specimens of aged dogs (Lim *et al*., 1997) levels of the latent form of gelatinase B are increased. The enzyme is secreted by neurons and is capable of degrading $A\beta_{1\text{-}40}$, thereby reducing the probability of accumulation of the peptide in the senile plaques (Lim *et al*., 1996). The lack of activation of gelatinase B might cause accumulation of $A\beta_{1\text{-}40}$, which might contribute to the pathogenesis of Alzheimer's disease, and to the impairment of memory and behavior.

In another neurodegenerative disease, amyotrophic lateral sclerosis (ALS), gelatinase B expression was detected in the pyramidal neurons in the motor cortex, and in the motor neurons in the thoracic and lumbar spinal cord. In the latter areas of the central nervous system, considerable numbers of neurons degenerate in this disease. Levels of gelatinase B were also elevated in the frontal and occipital cortices, of which the former may be involved in cognitive dysfunction in ALS (Lim *et al*., 1996).

3.2.6. Vascular Diseases

In coronary atherosclerotic lesions, gelatinase B was found to be highly expressed. Gelatinase B as well as stromelysin-1 and interstitial collagenase (MMP-1) was overexpressed in regions of foam cell accumulation, whereas normal arteries stained uniformly for gelatinase A and TIMPs (Galis *et al*., 1994), the latter of which may play an important regulatory role in arterial wall homeostasis. Indeed, in porcine coronary arteries, a higher intrinsic gelatinolytic activity and a rapid cell outgrowth was seen in the adventitia, whereas preferential expression of TIMPs was present in the media that exhibited slower cell outgrowth. Impairment of TIMP synthesis may thus contribute to the pathogenesis of coronary lesion formation (Shi *et al*., 1999). Nevertheless, the intracellular localization of gelatinase B was most frequently documented in coronary atherectomy specimens

from patients with atherosclerosis and angina with acute ischemia, when compared with those without acute ischemia. This suggests that active synthesis of gelatinase B by macrophages and smooth muscle cells is strongly associated with the clinical syndrome of unsTable angina, possibly by metalloproteinase-induced matrix degradation, which promotes plaque rupture (Brown *et al*., 1995). Also at the DNA level, an association was found between the gelatinase B SNP and the severity of coronary atherosclerosis, but no association was detected with myocardial infarction (see Section 2.1.2; Zhang *et al*., 1999b).

During myocardial infarction, myocardial MMPs and TIMPs are induced at the gene level. TIMP-1 was reduced and gelatinase B was increased at the protein level in the infarcted tissue (Tyagi *et al*., 1996). Gelatinase B-deficient mice were partially protected against ventricular enlargement, collagen accumulation and cardiac rupture, which are complications of acute myocardial infarction. Temporary TIMP-1 gene transfer in these mice prevented cardiac rupture completely and did not abort infarct healing (Heymans *et al*., 1999). In addition, ischemia- and reperfusion-induced expression of progelatinase B and active gelatinase B were significantly reduced in mice lacking one gelatinase B allele. Less neutrophils were detected in the infarction area after ischemia-reperfusion in knock-out vs. wild-type mice. These data indicate that gelatinase B might be a target for treatment of acute myocardial infarction (Romanic *et al*., 2002).

In cerebral infarction in humans, gelatinase B expression was mainly present in neutrophils in acute infarcts up to 1 week following the vascular event (Anthony *et al*., 1997). In rats, an increase of gelatinase B occurred 12 h after middle cerebral artery occlusion. Secondary vasogenic edema was maximal 1 to 2 days after a stroke, which coincided with elevated gelatinase B. This suggests a role for gelatinase B in secondary tissue damage and vasogenic edema (Rosenberg *et al*., 1996b; Gasche *et al*., 1999). A role of gelatinase B in hemorrhagic transformation after focal cerebral ischemia was also illustrated in non-human primates (Heo *et al*., 1999).

After balloon catheter injury of the carotid artery of the rat, the production of an 88-kDa gelatinase was induced and continued during the period of migration of smooth muscle cells from the media to the intima. This suggests that gelatinase expression directly facilitates smooth muscle cell migration within the media and into the intima and plays a role in neointimal formation that characterizes arterial tissue remodeling after injury (Bendeck *et al*., 1994; Meng *et al*., 1999), although the inhibition of smooth muscle cell migration seems not to be sufficient to inhibit lesion growth. Lesion size eventually reaches control levels via increased smooth muscle cell replication (Bendeck *et al*., 1996). Gelatinase B was also detected after perivascular injury in mice, and this is mainly in macrophages in the adventitia (Lijnen *et al*., 1999). Gelatinase B levels were increased after focal ischemia (Asahi *et al*., 2000). In line with this observation, gelatinase B-deficient mice were protected against focal cerebral ischemia. Previously we discussed that myelin basic protein is a substrate of gelatinase B (Proost *et al*., 1993), and in the ischemia study also less myelin degradation was observed in the knock-out mice (Asahi *et al*., 2001b). The activity of tetracyclines in reducing postthrombotic infarction areas is also in line with the mentioned studies in

knockout mice and constituted an early demonstration that gelatinase B is also disease promoting (Yrjanheikki *et al*., 1998; Yrjanheikki *et al*., 1999).

3.2.7. Proliferative Diseases

Gelatinase B has been implicated in tumor cell invasion and metastasis due to its ability to degrade basement membrane collagens (Himelstein *et al*., 1994). MMPs participate in several steps in tumor progression, including invasion, metastasis, and angiogenesis. The literature on MMPs in tumor biology is extensive and best summarized in reviews (Stetler-Stevenson *et al*., 1993; Powell and Matrisian, 1996; Egeblad and Werb, 2002). The latter review emphasizes the bivalent role played by all MMPs in tumor biology, much like its Yin/Yang function in inflammation (Yong *et al*., 2001). Here we give some examples of tumors in which specifically gelatinase B expression has been shown to be elevated. Further, we focus on the producer cells (stromal or tumor cells), on the correlation between gelatinase B levels and histologic grade in some tumors, and on the role of gelatinase B in angiogenesis, which renders tumor growth possible, and in invasion and metastasis.

Increased expression of gelatinase B has been described in brain tumors and cerebrospinal fluid of patients with brain tumors (Rao *et al*., 1993; Friedberg *et al*., 1998), in bladder cancer (Davies *et al*., 1993), basal cell and squamous cell cancers of the skin (Karelina *et al*., 1993; Pyke *et al*., 1992), malignant pigment lesions of the skin (van den Oord *et al*., 1997), squamous cell carcinomas of the lung (Cañete-Soler *et al*., 1994), colon and breast carcinomas (Zucker *et al*., 1993), endometrial carcinoma (Takemura *et al*., 1992), ovarian cancer (Takemura *et al*., 1994), prostatic carcinoma (Hamdy *et al*., 1994), pancreatic cancer (Gress *et al*., 1995), and gastric cancer (Nomura *et al*., 1996). In malignant fibrous histiocytomas and benign dermatofibromas, the synthesis of the mRNAs for both gelatinases was quantitatively similar, suggesting that no correlation exists between the biological behavior of the tumors and the synthesis of these enzymes (Soini *et al*., 1993). In contrast, gelatinase B expression levels were correlated with the histologic grade of human malignant lymphomas (Kossakowska *et al*., 1993), and with the aggressiveness of prostatic adenocarcinoma (Hamdy *et al*., 1994), gastric cancer (Torii *et al*., 1997), gliomas (Rao *et al*., 1996), and bladder malignancy (Davies *et al*., 1993).

By analogy with other protease receptors on cancer cells (e.g., the urokinase receptor), it has been attempted to define (an) elusive gelatinase B receptor(s). Only in proliferative diseases has it been shown that gelatinase B may exert its action through a receptor. CD44 may serve as an gelatinase B docking molecule to retain proteolytic activity on the cell surface, and the CD44/gelatinase B complex formation is associated with tumor invasiveness and angiogenesis *in vivo* (Bourguignon *et al*., 1998; Yu and Stamenkovic, 1999; Wallach-Dayan *et al*., 2001).

In bladder tumors, as well as in many other cancers described above, MMP expression was not necessarily localized in the tumor cells, but rather was found in the surrounding stromal cells and inflammatory cells of host origin, especially at the tumor-stroma interface (Pyke *et al*., 1992; Himelstein *et al*., 1994; Soini *et al*., 1994; Nielsen *et al*., 1996)

and particularly in stromal cells closest to the invasion front. Often the detection declined with increasing distance from the tumor (Davies *et al*., 1993). The involvement of cancer and/or stromal cells may be tumor-type dependent, as the gelatinase B mRNA was found in tumor and stromal cells of squamous cell lung carcinomas, whereas it was not found in the adenocarcinomas of the lung, or in the surrounding stroma. In gastric cancer gelatinase B expression was recognized in cells of the cancer stroma, but not in cancer cells (Torii *et al*., 1997). In skin cancer, mRNA for gelatinase B was present in a subpopulation of tissue macrophages in all squamous cell and most of the basal cell carcinomas, whereas malignant cells showed a signal for gelatinase B only in most of the squamous cell and in none of the basal cell carcinomas (Pyke *et al*., 1992). These phenotypic differences among carcinoma cells may be caused in part by alteration of the aforementioned $(CA)_n$ dinucleotide repeat in the promoter region of gelatinase B (Shimajiri *et al*., 1999). Additional evidence for the importance of gelatinase B production by other cells than the cancer cells themselves was provided in a mouse model with B16-BL6 melanoma cells or Lewis lung carcinoma cells (Itoh *et al*., 1999a). In this study, host-derived gelatinase B turned out to be important in the process of metastasis. This may indicate that stromal cells are actively involved in the generation and regulation of extracellular proteolysis during cancer cell invasion. This was also confirmed for gelatinase A by demonstration of an important role for host gelatinase A in tumor progression (Itoh *et al*., 1998). In a mouse model of oncogene-induced multistage tumorigenesis, it was demonstrated that gelatinase B from bone marrow-derived host cells contributed to skin carcinogenesis. Gelatinase B deficiency decreased the incidence of invasive tumors but resulted in higher tumor histology grade (Coussens *et al*., 2000). This study is in line with the observation that tumors, which produce more neutrophil chemokines and thus recruit more host cell gelatinase B, are more invasive (Opdenakker *et al*., 1992). This so-called countercurrent principle involves gelatinase B and implies angiogenesis (Figure 10) (Van Coillie *et al*., 2001).

Besides invasion of the surrounding tissues and metastasis, angiogenesis is a crucial element in tumor growth. The capacity of both primary and metastatic tumors to grow in size beyond the limits of oxygen diffusion requires the establishment of a neovasculature. Indeed, faster growing and highly invasive and metastatic tumors need more vessels to convey nutrients and oxygen and to remove catabolites. This process of angiogenesis involves the migration of stimulated endothelial cells and subsequent tube formation and depends on a tightly controlled proteolysis of the components of the extracellular matrix. As in physiological conditions, angiogenesis *in vitro* is mediated, in part, by gelatinase B, because it was demonstrated on Matrigel that gelatinase B is important in endothelial cell morphogenesis, leading to tube formation (Schnaper *et al*., 1993). In endometrial carcinoma, it was demonstrated that angiogenesis and overexpression of gelatinase B mRNA occurred simultaneously (Iurlaro *et al*., 1999). The finding that gelatinase B expression is localized to the perivascular smooth cells and pericytes at the proliferating borders in gliomas provides further support for the importance of this enzyme in angiogenesis (Forsyth *et*

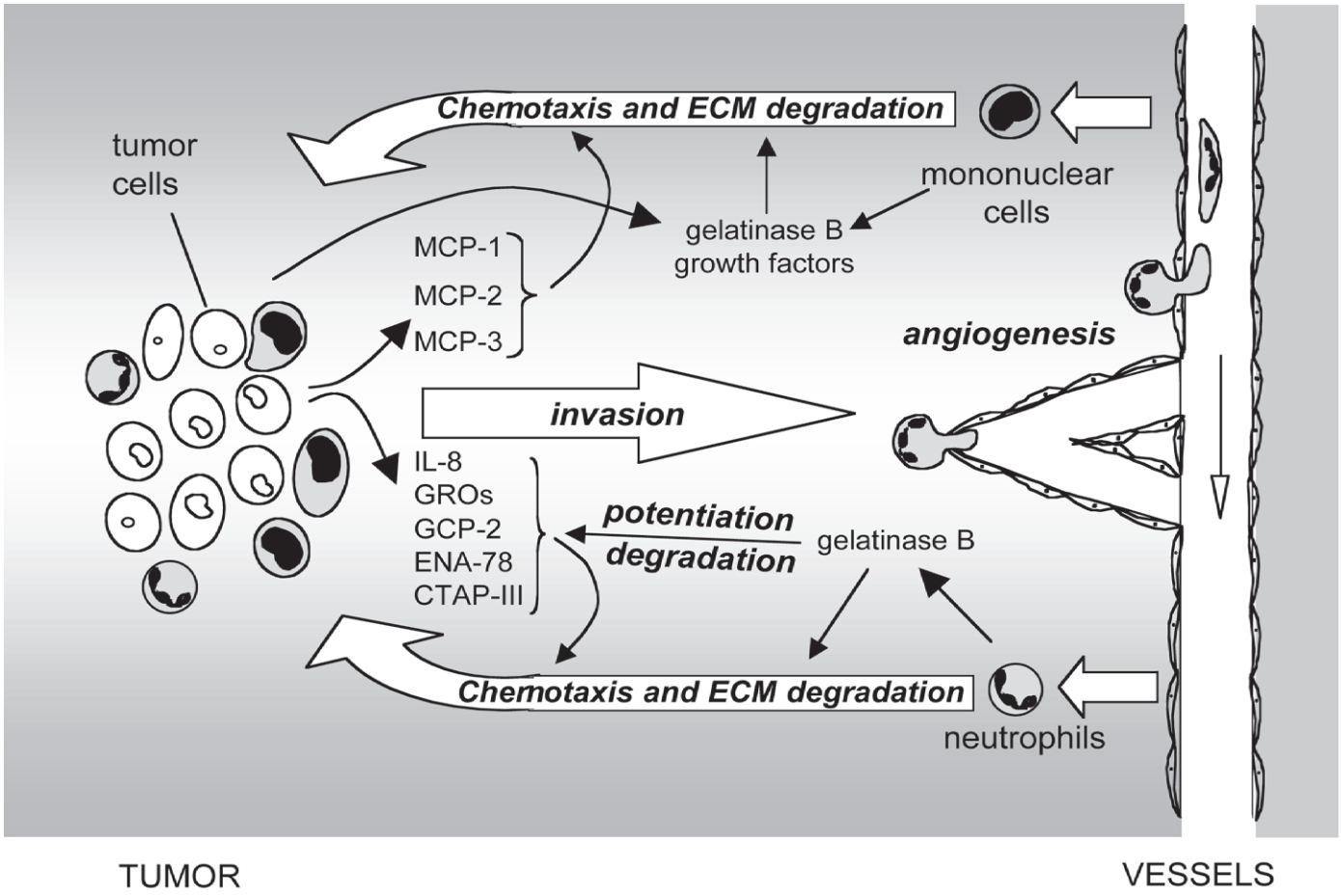

FIGURE 10. Leukocyte gelatinase B assists in the migration of cancer cells. Cancer cells secrete chemokines or instruct stromal cells to produce chemokines. This results in the attraction and activation of leukocytes such as neutrophils and monocytes. By secreting gelatinase B (and possibly also other proteases) on their way, the leukocytes degrade the extracellular matrix and create a channel towards the cancer cells. The cancer cells use this path in the opposite direction, resulting in the directional migration towards blood vessels (invasion and metastasis) (Opdenakker and Van Damme, 1992; Coussens *et al.*, 2000; Van Coillie *et al.*, 2001). In addition, gelatinase B processes different CXC-chemokines, resulting in positive or negative feedback loops, depending on the chemokine, and both gelatinase B and ELR-containing CXC-chemokines also promote angiogenesis. Gelatinase B may also be produced at the invasion front by the cancer cells or by stromal cells.

al., 1999). Moreover, synthetic inhibitors of gelatinase A and B prevent tumor growth and invasion through a tumor targeting, antiangiogenic, and antiinvasive action (Koivunen *et al*., 1999; Eccles *et al*., 1996). Another argument for a role of gelatinase B in angiogenesis has been provided by a study in mycosis fungoides, a lymphoproliferative disease of T cell lineage. Here it was shown that the microvessel area in skin tissue, which was used as a measure for angiogenesis, and the percentage of lesions expressing gelatinase B mRNA increased in parallel with tumor progression (Vacca *et al*., 1997). However, the exact contribution of gelatinase B in angiogenesis has not yet been elucidated. *In vitro* experiments showed that TGF-β and PMA, two factors associated with tumor progression are able to cooperate to induce gelatinase B expression and to decrease the expression of TIMP (vide supra). The resulting proteolytic potential did not correlate with motility and thus migration capacity of the endothelial cells, suggesting that gelatinase B contributes in a complex way to the angiogenic process (Puyraimond *et al*., 1999).

Although gelatinase B has been recognized as a promoter of tumor growth both by degrading matrix barriers and by enhancing angiogenesis, it also is one of the MMPs that generates angiostatin out of plasminogen. Angiostatin results in limiting tumor neovascularization by the inhibition of proliferation of microvascular endothelial cells (Cornelius *et al*., 1998). Probably the role and mechanisms of action of each MMP and each TIMP in angiogenesis are different depending on the tissue/cell specificity, the stages of endothelial differentiation, local microenvironmental factors, and tumor-host interactions (Sang, 1998).

4. CONCLUSIONS AND FUTURE DIRECTIONS

About 3000 entries on gelatinase B or MMP-9 exist already in the PubMed data library (http://www.ncbi.nlm.nih.gov). Only a fraction of these have been discussed in detail here and, where possible, reference has been made to existing reviews. In this review, the emphasis has been focussed on structure, regulation, and biological functions. The structure and regulation of gelatinase B is complex, and its functions are far from completely understood, posing challenges for the future. Even apparently simple issues, such as the definition of the natural substrates in physiology and pathology remain elusive. For instance, how can one prove *in vivo* the cleavage of supposed substrates, such as denatured collagens? By reviewing the literature, we have tried to demonstrate that in many pathological conditions secretion of gelatinase B is detrimental, and that its activity needs to be tempered in cancer, inflammation, and autoimmune and cardiovascular disorders. The prospect of treating these diseases has led many academic and industrial scientists to generate highly active and selective inhibitors of gelatinase B. The problems of such enterprises are not simple, as reviewed recently for the whole family of matrix metalloproteinases (Coussens *et al*., 2002). The search for potent gelatinase B inhibitors will remain a major challenge, and effective therapies will probably only be achieved following the development and combination of new technologies. Rational drug design will be aided by improved methods for structural analysis that make it reasonable to expect the challenge of determining the three-dimensional structure of gelatinase B to be met.

Two recent publications (Rowsell *et al*., 2002; Elkins *et al*., 2002) demonstrate the efforts in this direction. Although generating crystals of natural human progelatinase B with all its N- and O-linked sugars constitutes a major challenge for X-ray crystallography, so far even the structures of recombinant variants of the intact molecule remain elusive. Moreover, the role of glycosylation of this biologically important molecule is not yet well understood, not least because of the difficulties in obtaining reasonable quantities of natural material. Biological studies of gelatinase B are gradually moving from *in vitro* to *in vivo* research. This is demonstrated by the ever-increasing number of important findings in gelatinase B-deficient mice. The essence of the science here depends on making comparisons, because differences in phenotypes may be caused not only by differences in knock-out constructs, but also by strain variations. Such comparisons will form an ideal platform from which to study the molecular interactions between genetic and epigenetic influences. In other words, it is to be expected that the functional role will be better defined and that we will understand whether or why gelatinase B is indeed an Achilles' heel in multifactorial diseases such as cancer and autoimmunity.

ACKNOWLEDGMENTS

Our research on gelatinase B has been generously supported by the Fund for Scientific research (FWO-Vlaanderen), the Cancer Research Foundation of Fortis AB, the Belgian Federation against Cancer and the Geconcerteerde OnderzoeksActies. The authors thank also Dr. Maria Hedlund for helpful discussions and Dr. Mark R. Wormald for his help with the molecular modeling.

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