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Membrane Proteins with Soluble Counterparts: Role of Proteolysis in the Release of Transmembrane Proteins

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Many proteins lead a dual existence as both membrane-bound and soluble isoforms. One that has recently received a great deal of attention is the amyloid precursor protein (APP),[1] a C-terminally anchored transmembrane protein that is released by a specific proteolytic cleavage near the membrane anchor to generate a soluble form (Weidemann et al., 1989; Palmert et al., 1989). While the functions of either form remain speculative, it has been postulated that inappropriate or aberrant cleavage of the APP leads to the generation of β-amyloid deposits that are associated with Alzheimer's disease and Down syndrome (Selkoe, 1990). This has prompted considerable interest in the proteases that may be involved in this process since they would be prime targets for the design of therapeutic agents. Proteolytic processing of membrane-bound proteins is not limited to APP, however, and may actually be an important control mechanism of fundamental significance to cell biology. Moreover, it falls within the general phenomenon of proteins with membrane-bound and soluble isoforms, which is surprisingly widespread and extends to virtually all structural and functional classes of integral membrane proteins, including examples as diverse as angiotensin-converting enzyme (ACE) (Ehlers et al., 1991), the neural cell adhesion molecule (NCAM) (Nybroe et al., 1989), and the newly described c-*kit* ligand (Martin et al., 1990). It appears to be a useful and commonly employed device that allows cells to multiply the functional roles of proteins with a minimum investment in energy and structural complexity.

In general terms, soluble and membrane-bound isoforms of the same protein can be generated by one of two mechanisms (Figure 1): first, by separate biosynthetic pathways, either by alternative pre-mRNA splicing of a common transcript or by transcription of closely related but distinct genes; and second, by posttranslational release of the extracellular domain of membrane proteins by hydrolytic cleavage of the membrane anchor. Hydrolysis involves either cleavage of the glycolipid of glycosylphosphatidylinositol- (GPI-) anchored proteins or limited proteolysis of the extracellular domain of transmembrane (TM) proteins at a site adjacent to the membrane-spanning sequence.

The mechanisms and biological significance of alternative splicing and of GPI anchoring have been extensively reviewed (Leff et al., 1986; Breitbart et al., 1987; Low & Saltiel, 1988; Ferguson & Williams, 1988) and will not be considered further except insofar as they pertain to the generation of soluble and membrane isoforms of proteins. Unlike GPI hydrolysis, which was quickly recognized as a potential means by which certain membrane proteins could be selectively released (Ferguson & Williams, 1988), proteolysis is often regarded only as a means to remove and degrade membrane proteins as part of their normal turnover. However, in recent years numerous instances have been reported in which membrane proteins are apparently specifically released[2] by proteolysis in a directed and sometimes regulated manner to produce active, soluble forms. These include proteins that are sufficiently diverse in structure and function to suggest that limited proteolysis may constitute a general mechanism in the release of membrane proteins to generate soluble isoforms with defined functions. In addition

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[1] Abbreviations: ACE, angiotensin-converting enzyme; APP, amyloid precursor protein; CAM, cell adhesion molecule; NCAM, neural cell adhesion molecule; CSF-1, colony-stimulating factor; EGF, epidermal growth factor; GPI, glycosylphosphatidylinositol; GPI-PL, GPI-specific phospholipase; PI-PLC, phosphatidylinositol-specific phospholipase C; Ig, immunoglobulin; NEP, neutral endopeptidase 24.11; MPSP, membrane-protein-solubilizing protease; PCE, prohormone-converting enzyme; PKC, protein kinase C; NGF, nerve growth factor; TGF-α, transforming growth factor α; TM, transmembrane; TNF, tumor necrosis factor; VSG, variant surface glycoprotein.

[2] In this context, the terms "release" and "solubilization" are used in preference to "shedding". Shedding has been widely used in the literature to denote the loss of any kind of cell-surface-associated protein (peripheral and integral membrane proteins) by any means (hydrolytic cleavage, nonhydrolytic dissociation, or budding off of membrane vesicles) (Black, 1980). Thus the term is too vague for the purposes of this review, which focuses only on integral membrane proteins and the specific hydrolytic release of their extracellular domains to generate soluble isoforms.

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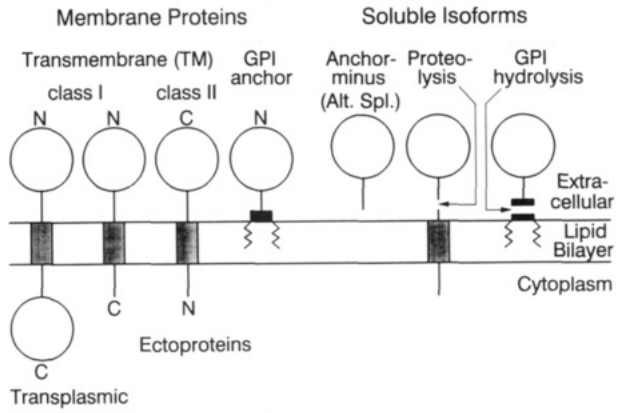

FIGURE 1: Integral membrane proteins with soluble isoforms: topologies and solubilizing mechanisms. Proteins of interest (see Table I) are depicted as domains (circles) linked to transmembrane (TM) sequences (shaded) or glycosylphosphatidylinositol (GPI) moieties (solid black with tails); connecting stalks and cytoplasmic tails are shown as straight lines. Orientation of the N- and C-termini of TM proteins (class I and II) is as described by von Heijne and Gavel (1988). Proteins with intra- and extracellular domains of roughly equal size are termed transplasmic; those with cytoplasmic tails ~40 residues in length or less are ectoproteins. Soluble isoforms consist of the extracellular (or intravesicular) domains of membrane proteins and are generated as anchor-minus forms by alternative splicing (alt spl) or hydrolytically by proteolysis or GPI hydrolysis.

to the APP, other examples include the Mel-14 neutrophil adhesion protein (Kishimoto et al., 1989) and the tumor necrosis factor (TNF) receptor (Loetscher et al., 1990; Lantz et al., 1990; Kohno et al., 1990).

In the ensuing discussion we will explore the scope and diversity of these proteins, discuss the significance of membrane-bound and soluble isoforms of the same polypeptide, and emphasize the importance of limited proteolysis in this process.

Proteins with Membrane-Bound and Soluble Isoforms: Scope and Diversity

It is readily apparent from Table I that the scope of these proteins is remarkable. This diversity encompasses virtually all types of membrane proteins, grouped by either structural or functional criteria, resident on a wide variety of cell types. Most of the proteins listed are traditionally regarded as integral membrane proteins, but in all cases there is good evidence for the existence of soluble counterparts in vivo, generally from their detection in various bodily fluids and secretions, such as blood and seminal plasma, cerebrospinal fluid, amniotic fluid, urine, and milk. The mechanisms generating the soluble forms in vivo are usually inferred indirectly from data provided by cell culture systems, cDNA sequences, susceptibility to cleavage and release by phospholipases or proteases, or inhibition of membrane protein release by appropriate hydrolase inhibitors.

It may be instructive to consider in more detail each group of proteins represented in Table I and to discuss examples of individual proteins for which insights have been gained into the respective roles of the membrane and soluble isoforms or for which the solubilizing mechanism is particularly interesting.

Receptors. In recent years it has been recognized that a number of receptors, mostly cytokine receptors, have soluble counterparts in various body fluids. The soluble forms, usually called binding proteins, have been shown to consist of the extracellular domains of their respective receptors. It is likely that these soluble isoforms regulate the effects of cognate ligands by acting as soluble inhibitors. Moreover, in cases where the binding proteins are produced by proteolytic release, the cells are desensitized through loss of their cell-surface receptors. In some instances these events are regulated by activation of protein kinase C (PKC), as in the proteolytic release of the TNF and the colony-stimulating factor 1 (CSF-1) receptors (Lantz et al., 1990; Downing et al., 1989).

The insulin-like growth factor (IGF) receptors are instructive because, whereas no soluble forms are known, a family of soluble IGF-binding proteins exists, the members of which are structurally unrelated to the receptors (Brown et al., 1989). Therefore, in contrast to the receptors listed in Table I, soluble isoforms of the ligand-binding domain of the IGF receptor are not used as binding proteins, but instead distinct proteins have evolved for this purpose (Tollefsen et al., 1989). It remains to be established why in some cases separate polypeptides are required for receptors and binding proteins, whereas in others membrane-bound and soluble isoforms of the same protein are sufficient.

Receptor Ligands. Considerable interest has been generated by the recent finding that certain growth factors, classically thought of as soluble autocrine factors, not only exist as membrane-bound proteins but also are active as such (Steele, 1989; Massagué, 1990) (Table I). These factors may participate in a novel mode of intercellular communication restricted to adjacent cells that is akin to cell–cell adhesion, termed "juxtacrine" stimulation (Massagué, 1990). Cleavage, for instance, of pro-TGF-α is not aimed at generating the active form of the factor from a precursor but at changing from one active form (membrane-bound) to another (soluble) (Massagué, 1990). This is given credence by the observation that proteolytic cleavage of pro-TGF-α to generate soluble TGF-α is activated by, inter alia, a phorbol ester mediated, PKC-dependent mechanism (similar to the activation of the proteolytic release of receptor ectoplasmic domains noted above) (Pandiella & Massagué, 1991). For TNF it has been proposed that the cell-surface form mediates localized cytotoxicity because cell to cell contact is required, whereas widespread release of soluble TNF will produce systemic effects (Kriegler et al., 1988).

The mechanism for regulating the proteolytic release of membrane-bound CSF-1 is unusual and differs from that for TGF-α. Instead of a means for activating the responsible protease(s), alternative forms of the membrane-bound CSF-1 are produced by alternative splicing that differ in the sequence of the ectoplasmic domain adjacent to the TM region, and these different juxtamembrane sequences are differentially susceptible to proteolysis (Cerretti et al., 1988; Stein et al., 1990). This mechanism has also been observed for the c-*kit* ligand (Flanagan et al., 1991). Alternative juxtamembrane sequences in membrane proteins with soluble isoforms may be a useful regulatory device in modulating the cell-surface expression, rate of release, and production of soluble forms of such proteins.

Cell Adhesion Molecules. Cell–cell and cell–matrix adhesive interactions are critical to all developmental processes and it is not surprising that a large variety of proteins mediate these interactions, although it now seems clear that these apparently diverse proteins can be grouped into four superfamilies (Springer, 1990; Albelda & Buck, 1990). Cells of the immune system in particular require a mechanism for the rapid transition between adherent and nonadherent states, and therefore many of the cell adhesion molecules (CAMs) undergo rapid modulation of both surface expression and release (Springer, 1990). A good example is the murine neutrophil Mel-14 antigen (gp100$^{Mel\text{-}14}$ neutrophil adhesion protein), which is thought to be involved in mediating the initial adhesion between neutrophils and endothelial cells adjacent to

inflammatory sites. Activation of adherent neutrophils by chemotactic factors results in a rapid proteolytic release of Mel-14, providing a rapid deadhesion mechanism allowing extravasation to occur (Kishimoto et al., 1989). Activation of neutrophils can be reproduced in vitro by incubation with phorbol esters, once again suggesting that the proteolytic release is activated by a PKC-dependent mechanism, as noted for the TNF and CSF-1 receptors and pro-TGF-α, above.

The complexity of the interplay between soluble and membrane isoforms of these proteins is best illustrated by the neural cell adhesion molecule (NCAM): alternative splicing generates class I ectoproteins and transplasmic proteins, GPI-anchored proteins, and anchorless secreted isoforms (Cunningham et al., 1987; Gower et al., 1988). Moreover, neuronal culture media contain not only the latter secreted isoforms but also solubilized forms of the TM and GPI-linked proteins (Nybroe et al., 1989). These data indicate that neural and muscle cells require a complex array of membrane and soluble NCAMs and make use of at least three mechanisms for producing soluble forms. Some or all of these isoforms may be exploited by malignant cells, such as small-cell lung cancer cells, during metastatic dissemination (Doyle et al., 1990).

Leukocyte Antigens. The classical example of a protein that is both membrane-bound and soluble, each form having distinct physiological roles, is the immunoglobulin (Ig). The TM and secreted isoforms of Ig are produced by alternative splicing of the 3′ end of a common pre-mRNA (Rogers et al., 1980; Early et al., 1980; Wall & Kuehl, 1983). Several other leukocyte antigens have membrane and soluble isoforms generated by a similar mechanism (Table I).

The class I MHC proteins are more complicated. Although the details are not entirely clear, the evidence suggests that in addition to an alternatively spliced, anchor-minus secreted form (Haga et al., 1991), some class I proteins, notably Qa-2, are synthesized as both TM and GPI-anchored forms, and either can be spontaneously released (Soloski et al., 1986; Robinson, 1987; Stiernberg et al., 1987), particularly by activated cells (Soloski et al., 1986). These antigens further exemplify the complex interplay between membrane and soluble isoforms of the same protein and the multiple mechanisms employed to generate them.

The mechanism for solubilizing the IgG Fc receptor (FcR III, CD16) is cell-specific. On neutrophils, CD16 is GPI-linked and is released after neutrophil stimulation, presumably by activation of an endogenous GPI-specific phospholipase (GPI-PL), suggesting a role for CD16 release in inflammatory reactions (Huizinga et al., 1988) (compare this to the proteolytic release of Mel-14 from activated neutrophils). On natural killer cells CD16 is a class I TM ectoprotein, which in culture is also spontaneously released, presumably proteolytically (Lanier et al., 1989 a,b).

Lastly, the IgE receptor (FcE II, CD23) is interesting because it is the only antigen in this group that is anchored in the cell membrane by an uncleaved signal peptide (class II TM ectoprotein) (Ikuta et al., 1987). Moreover, it is cleaved into biologically active soluble fragments (IgE-binding factors) by an autoproteolytic mechanism (Letellier et al., 1990).

Ectoenzymes. Mammalian cells express a large variety of ectoenzymes, and particularly dense populations are found on the brush border membranes of kidney, intestine, and choroid plexus (Kenny, 1986; Kenny et al., 1989). It is remarkable that of these only a small number have been documented to exist as soluble isoforms in vivo (Table I). It would appear that for the most part a sharp distinction exists between membrane-bound ectoenzymes and secreted soluble enzymes, and these differ in structure and function. However, there are some notable exceptions.

Angiotensin-converting enzyme (ACE) is a class I TM ectoprotein (Soubrier et al., 1988) that is expressed predominantly on the surfaces of fluid-bathed cells [reviewed in Ehlers and Riordan (1990)]. All body fluids contain soluble ACE, most likely derived from the membrane-bound enzyme by proteolytic release (Ehlers et al., 1991; Wei et al., 1991). Despite its abundance in some fluids, e.g., prostatic and seminal fluid (Cushman & Cheung, 1971; Yokoyama et al., 1982; Lanzillo et al., 1985), and guinea pig serum (Soffer, 1981), the specific functions of soluble ACE are unknown. In general terms it may be said that ACE, as well as other enzymes in this group, is released to provide a soluble form that can act at a distance from the site of production. Alternatively, anchoring of ectoenzymes may be a means to generate (or degrade) high local concentrations of effectors, whereas release abrogates this localized activity. It is of interest that activated monocytes and their derivatives (macrophages and granuloma epithelioid cells) express and release large quantities of ACE (Okabe et al., 1985; Weinstock & Boros, 1982) that are presumed to play a significant role in some forms of granulomatous inflammation (Weinstock, 1986), spilling over into the serum of affected individuals (Silverstein et al., 1976; Okabe et al., 1985).

Among proteins with membrane and soluble isoforms, the cholinesterases constitute one of the most complex systems described to date (Chatonnet & Lockridge, 1989; Inestrosa & Perelman, 1989). In addition to integral membrane forms linked by both conventional GPI anchors as well as by an undefined hydrophobic 20-kDa anchor, there are also peripheral membrane forms that interact with cell-surface-associated proteoglycans via a collagen-like tail. Soluble cholinesterases arise by alternative splicing that generates anchor-minus forms, and presumably also by GPI hydrolysis and possibly proteolysis of membrane-bound protein. This rich diversity must underlie a requirement for precise control of the distribution between soluble and bound forms of these enzymes, which in turn presumably subserves the requirements for choline metabolism of particular cells (Chatonnet & Lockridge, 1989; Inestrosa & Perelman, 1989).

Miscellaneous Proteins. Included here (Table I) is an eclectic group of proteins that do not conveniently fit into the categories discussed above but that nevertheless provide some particularly interesting examples of membrane proteins with soluble counterparts. Foremost among these is the much-studied amyloid precursor protein (APP). As noted earlier, APP is the precursor of β-amyloid protein or A4, the major protein component of cerebral amyloid deposits in patients with Alzheimer's disease (Selkoe, 1990). APP is a class I TM ectoprotein resident on cell surfaces in brain and peripheral tissues (Kang et al., 1987) and is constitutively cleaved near the membrane anchor to release a soluble form of the extracellular domain both in vitro and in vivo (Weidemann et al., 1989; Palmert et al., 1989). Of great interest is that the $\sim$40-residue β-amyloid protein is composed of portions of the ectoplasmic and TM domains of APP (Kang et al., 1987) and therefore cannot arise from the secreted APP, which has lost both the TM and cytoplasmic domains (Sisodia et al., 1990; Esch et al., 1990). This implies that the β-amyloid protein does not result from the constitutive cleavage of APP but rather from aberrant processing. It remains to be established whether this aberrant processing primarily stems from an overproduction of APP [as may be the case in Down syndrome (Selkoe, 1990)], a mutation at or near the constitutive cleavage

Table I: Membrane Proteins with Soluble Isoforms[a]

protein	topology[b]	anchor[c]	cellular distribution	solubilizing mechanism[d]			ref[e]
				alt spl	GPI-hydr	proteol	
Receptors							
TNF receptor	transplasmic	TM, internal (class I)	most cells			+(A)[f]	1
NGF receptor	transplasmic	TM, internal (class I)	neurons, neural crest derivatives			+	2
CSF-1 receptor	transplasmic	TM, internal (class I)	monocytes, macrophages			+(A)	3
transferrin receptor	ectoprotein	TM, N-term. (class II)	most cells			+	4
EGF receptor	transplasmic	TM, internal (class I)	epithelial cells	+			5
GH[g] receptor	transplasmic	TM, internal (class I)	most cells			+	6
poly-Ig receptor	transplasmic	TM, internal (class I)	epithelial cells			+	7
folate receptor	ectoprotein	TM, C-term. (class I)	most cells			+	8
Receptor Ligands							
TGF-α	ectoprotein	TM, C-term. (class I)	most cells			+	9
c-*kit* ligand	ectoprotein	TM, C-term. (class I)	stromal cells	+[h]		+	10
TNF	ectoprotein	TM, N-term. (class II)	monocytes, macrophages			+	11
CSF-1	ectoprotein	TM, C-term. (class I)	mesenchymal cells	+[h]		+	12
Cell Adhesion Molecules							
Mel-14 (gp 100)	ectoprotein	TM, C-term. (class I)	murine neutrophils			+(A)	13
Leu-8	ectoprotein	TM, C-term., and GPI	human lymphocytes		+	+?	14
ELAM-1	ectoprotein	TM, C-term. (class I)	endothelial cells			+?(A)	15
GMP-140	ectoprotein	TM, C-term. (class I)	endothelial cells, platelets	+			16
glycoprotein Ib	ectoprotein	TM, C-term. (class I)	platelets			+(?A)	17
NCAM	ectoprotein and transplasmic	TM, C-term. and internal (class I), and GPI	neurons, glia, muscle cells	+	+	+	18
Leukocyte Antigens							
immunoglobulin	ectoprotein	TM, C-term. (class I)	B-cells	+			19
CD8 (T8/Leu 2)	ectoprotein	TM, C-term. (class I)	T-cells	+		+	20
class I MHC	ectoprotein	TM, C-term. (class I)	all cells	+	+(A)	+	21
Tac (IL 2 receptor)	ectoprotein	TM, C-term. (class I)	lymphocytes			+(A)	22
IL 4 receptor	transplasmic	TM, internal (class I)	B- and T-cells, monocytes, mast cells	+			23
CD16 (FcR III)	ectoprotein	TM, internal, and GPI	granulocytes, macrophages, T-cells		+(A)	+	24
CD23 (IgE receptor)	ectoprotein	TM, N-term. (class II)	lymphocytes, eosinophils, platelets			+	25
CD14	ectoprotein	GPI	monocytes and macrophages		+	+?	26
Ectoenzymes							
ACE	ectoprotein	TM, C-term. (class I)	endothelial and epithelial cells, monocytes			+	27
NEP 24.11	ectoprotein	TM, N-term. (class II)	epithelial cells, lymphocytes			+?	28
DPIV	ectoprotein	TM, N-term. (class II)	epithelial cells, lymphocytes			+?	29
sialyltransferase	ectoprotein	TM, N-term. (class II)	most cells			+	30
DβM	ectoprotein	TM, N-term. (class II)	chromaffin cells			+	31
carboxypeptidase H	ectoprotein	TM, N-term. (class II)	neurons, endocrine cells			+	32
cholinesterase	ectoprotein	GPI and ?TM	neurons, muscle cells, etc.	+	+?	+?	33
Viral Membrane Proteins							
gp55 (SFFV)	ectoprotein	TM, C-term. (class I)	erythroblasts			+	34
VSV glycoprotein	ectoprotein	TM, C-term. (class I)	epithelial cells	+?		+	35
Miscellaneous Proteins							
APP	ectoprotein	TM, C-term. (class I)	brain, muscle, and epithelial cells			+	36
β-glycan	ectoprotein	TM (class I or II?)	most cells	+?		+	37
syndecan	ectoprotein	TM, C-term. (class I)	epithelial and plasma cells			+	38
DAF	ectoprotein	GPI		+	+		39
VSG	ectoprotein	GPI	*Trypanosoma brucei*		+(A)	+?(A)	40
CEA	ectoprotein	GPI and TM, C-term. (I)	epithelial cells	+	+	+?	41
prion protein	ectoprotein and transplasmic	GPI and TM, internal (IV)	neurons		+	+?	42
GP-2	ectoprotein	GPI	pancreatic acinar cells		+		43

[a] Integral membrane proteins that also exist in vivo as biologically active soluble isoforms. [b] Proteins are classified as either ectoproteins or transplasmic proteins, depending on the relative proportions of the ectoplasmic, transmembrane (TM), and cytoplasmic domains, as illustrated in Figure 1. [c] TM proteins are classified according to von Heijne and Gavel (1988); in some cases both TM and GPI-anchored isoforms occur. [d] Mechanisms generating the soluble isoforms include alternative splicing (alt spl) producing anchor-plus and anchor-minus forms, GPI-hydrolysis (GPI-hydr) of GPI-linked proteins, and limited proteolysis (proteol) of the ectoplasmic domains of TM proteins. [e] References: (1) Loetscher et al., 1990; Lantz et al., 1990; Kohno et al., 1990; Schall et al., 1990; Porteu & Nathan, 1990; (2) Zupan et al., 1989; (3) Downing et al., 1989; (4) Chitambar & Zivkovic, 1989; (5) Ullrich et al., 1984; Weber et al., 1984; (6) Leung et al., 1987; Trivedi & Daughaday, 1988; (7) Mostov et al., 1980; Mostov & Deitcher, 1986; Ahnen et al., 1986; (8) Elwood, 1989; Elwood et al., 1991; (9) Massagué, 1990; Pandiella & Massagué, 1991; (10) Flanagan & Leder, 1990; Martin et al., 1990; Huang et al., 1990; Anderson et al., 1990; Flanagan et al., 1991; (11) Kriegler et al., 1988; Perez et al., 1990; Cseh & Beutler, 1989; (12) Rettenmier et al., 1987; Cerretti et al., 1988; Stein et al., 1990; (13) Kishimoto et al., 1989; (14) Camerini et al., 1989; (15) Bevilacqua et al., 1989; (16) Johnston et al., 1989; Johnston et al., 1990; (17) Clemetson et al., 1981; Lopez et al., 1987; Titani et al.,

Table I (Continued)

1987; (18) Nybroe et al., 1989; Cunningham et al., 1987; Gower et al., 1988; (19) Rogers et al., 1980; Early et al., 1980; Wall & Kuehl, 1983; (20) Fujimoto et al., 1983, 1984; Littman, 1987; (21) Robinson, 1987; Haga et al., 1991; Stiernberg et al., 1987; (22) Rubin et al., 1985; Nelson et al., 1986; Robb & Kutny, 1987; Loughnan et al., 1988; (23) Mosley et al., 1989; (24) Huizinga et al., 1988; Lanier et al., 1989a,b; (25) Letellier et al., 1990; Ikuta et al., 1987; (26) Haziot et al., 1988; (27) Ehlers et al., 1991; Wei et al., 1991; Krulewitz et al., 1984; (28) Erdös et al., 1985; Spillantini et al., 1990; Komada et al., 1986; Malfroy et al., 1987; (29) Ogata et al., 1989; Sanda et al., 1989; (30) Paulson et al., 1987; Lammers & Jamieson, 1988, 1989; (31) Taljanidisz et al., 1989; (32) Rodriguez et al., 1989; Parkinson, 1990; (33) Chatonnet & Lockridge, 1989; Inestrosa & Perelman, 1989; (34) Gliniak & Kabat, 1989; (35) Little & Huang, 1978; Chatis & Morrison, 1983; Garreis-Wabnitz & Kruppa, 1984; (36) Kang et al., 1987; Weidemann et al., 1989; Sisodia et al., 1990; Esch et al., 1990; (37) Andres et al., 1989; (38) Weitzhandler et al., 1988; Bernfield & Sanderson, 1990; (39) Caras et al., 1987; (40) Ferguson & Williams, 1988; Bülow et al., 1989; (41) Sack et al., 1988; Barnett et al., 1989; Zheng et al., 1990; (42) Hay et al., 1987; Stahl et al., 1990; Yost et al., 1990; Lopez et al., 1990; (43) Fukuoka et al., 1991. [f] Solubilization is absolutely or relatively dependent on cellular activation (A). [g] Abbreviations not defined elsewhere: GH, growth hormone; ELAM-1, endothelial leukocyte adhesion molecule 1; GMP-140, granule membrane protein 140; IL 2 and 4, interleukin 2 and 4; FcR III, IgG FC receptor; DPIV, dipeptidyl peptidase IV; DβM, dopamine β-monooxygenase; gp55, leukemogenic glycoprotein encoded by Friend spleen focus-forming virus; VSV, vesicular stomatitis virus, DAF, decay accelerating factor; CEA, carcinoembryonic antigen; GP-2, zymogen membrane glycoprotein. [h] Alternative splicing generates alternative juxtamembrane regions (not anchor-plus and anchor-minus forms).

site in APP [as in Dutch hereditary cerebral hemorrhage with amyloidosis (Levy et al., 1990) and familial Alzheimer's disease (Goate et al., 1991)], or an abnormally low activity of the constitutive (normal) protease. It may well be that Alzheimer's and related diseases are the first conditions to be described that are related to and, at least in part, result from an abnormality in the solubilization of a membrane protein.

Another cellular protein of unknown function that appears to contribute to neurodegenerative diseases associated with amyloidosis is the prion protein (Prusiner, 1991). This protein can exist as both a GPI-linked ectoprotein and a class IV transplasmic protein, and a secretory form probably arises by GPI hydrolysis, although proteolysis also remains a possibility (Hay et al., 1987; Stahl et al., 1990). Unlike the normal cellular prion protein isoform, the scrapie isoform differs from the former by an undefined posttranslational modification, is resistant to cleavage by PI-PLC, and is not spontaneously released in soluble form (Stahl et al., 1990). It is therefore conceivable that, as with APP, secondary aberrant processing occurs with pathological consequences.

Hydrolysis as a Solubilizing Mechanism: Potential Advantages

Both the general utility of generating membrane-bound and soluble isoforms of the same polypeptide and the specific functions of these isoforms from various classes of proteins have been discussed. In this context, the effectiveness of alternative splicing to produce anchor-minus secreted forms of membrane proteins can be readily appreciated (Figure 1). However, there are specific benefits to first producing a membrane-anchored protein and then hydrolytically releasing it. These advantages, some of which have already been alluded to, can be summarized as follows:

(1) A specific population of membrane proteins can be rapidly released in a directed and regulated manner, offering a means for rapidly changing the phenotype of a cell. Examples include the Mel-14 neutrophil adhesion protein that is released from activated neutrophils while the surface expression of its partner Mac-1 is increased (Kishimoto et al., 1989), the TNF receptor, which is released from cells exposed to TNF or phorbol esters (Lantz et al., 1990), and shedding of the coat of differentiating *Trypanosoma brucei* by release of VSG (Bülow et al., 1989).

(2) Hydrolytic release constitutes a fast and effective means for *simultaneously* removing a surface-bound protein and generating the soluble isoform. This is of general utility for both receptors and CAMs: Cells are desensitized to the cognate ligand that at the same time is also inactivated by binding to the released ectoplasmic domain, thus leading to a rapid abrogation of the receptor-stimulation or cell-adhesion event.

(3) Hydrolytic release allows for rapid switching from a restricted, localized action of the membrane form to a regional or systemic action of the soluble form. This applies particularly to the membrane-bound cytokines (Massagué, 1990; Kriegler et al., 1988).

(4) In cases where there is an apparently constitutive release of membrane proteins, this may represent a cost-effective means for the cell to generate a steady supply of membrane and soluble isoforms, as opposed to investing in the additional machinery required to produce these isoforms by separate biosynthetic pathways. Examples include APP and ACE (see Table I for references).

Proteolysis versus GPI Hydrolysis. Classically, the proteolytic release of membrane proteins has carried the connotation that it is merely a means whereby cells rid themselves of spent proteins that have outlived their usefulness; in this context the term shedding is quite appropriate. In many cases, including for some of the proteins listed in Table I, the proteolytic—and presumably also GPI hydrolytic—release of membrane proteins likely constitutes, at least in part, the normal turnover of such proteins. This notwithstanding, however, it is abundantly clear that there are many instances in which, as outlined in the preceding discussion, the proteolytic release of membrane proteins subserves specific functions other than simple disposal.

Unlike proteolysis, the hydrolytic cleavage of GPI anchors and consequent solubilization of GPI-anchored proteins has indeed been proposed as one of the functions of this unusual anchor (Low & Saltiel, 1988; Ferguson & Williams, 1988), and it has been suggested that these proteins may be subject to selective release by GPI-PLs. However, depending on the site of action of the GPI-PL—on the cell surface or in an endocytotic compartment—it is not clear how specific this action would be, since activation of extracellular GPI-PLs could result in the release of most or all GPI-anchored proteins. In contrast, the proteolytic cleavage of juxtamembrane sequences of TM proteins would potentially be highly specific if there are particular sequence motifs recognized by particular sequence-specific proteases. At present, the diversity of GPI-PLs and membrane-protein-solubilizing proteases (MPSPs) is unknown.

Potential Membrane-Protein-Solubilizing Proteases (MPSPs)

Despite the large number of membrane proteins released proteolytically (Table I), no convincing candidate for MPSP has been unequivocally identified and isolated. This is no doubt due, in part, to certain inherent difficulties. First, the conclusion that a membrane protein is proteolytically released is usually based on indirect evidence, and the precise cleavage sites have been identified in a few instances only, such as APP (Esch et al., 1990) and TGF-α (Massagué, 1990).

Second, many of these proteins are susceptible to cleavage and release by nonspecific proteases. For example, ACE is readily released by trypsin, which cleaves at a site that is likely quite close to the endogenous cleavage site (Ehlers et al., 1991). Similarly, murine surface CD8, CSF-1, and syndecan are also easily released by trypsin (Littman, 1987; Rettenmier et al., 1987; Weitzhandler et al., 1988). This does not necessarily rule out the existence of sequence-specific MPSPs. Instead, the vulnerability of membrane proteins to proteolytic attack may merely indicate that in these proteins there is an exposed, juxtamembranous stalk region that contains a sequence motif recognized by an MPSP and also hydrolyzed by less specific proteases. Of course it is equally possible that some MPSPs are themselves relatively nonspecific and will act on any membrane proteins with exposed stalks. Indeed, in mutant Chinese hamster ovary cells that express a defect in protein O–glycosylation, cell-surface proteins are rapidly released by proteolytic cleavage, likely due to the absence of protective, stalk-associated O-linked sugars (Kozarsky et al., 1988; Reddy et al., 1989). Regulation of the proteolytic release of membrane proteins would then necessarily depend on other mechanisms, such as activation of the MPSP or its restriction to a defined compartment (see later).

Third, it is not clear whether the putative MPSPs will fall into a particular mechanistic class, forming a family of proteases that differ from one another only with regard to cleavage-site specificities, or comprise a mixture of unrelated proteases. Whereas the former would be intellectually more satisfying, the extant data favor the latter. Thus, in addition to an example of autoproteolysis [IgE receptor (Lettelier et al., 1990), which in itself negates the idea of a universal MPSP], evidence for metalloproteases and sulfhydryl, aspartyl, and serine proteases has been reported. The only common feature is that all putative MPSPs are also membrane proteins, although not necessarily at the cell surface. With these reservations in mind, possible candidate MPSPs will be considered below.

(*a*) *Prohormone-Converting Enzyme* (*PCE*). One of the most obvious candidates for the role of MPSP would appear to be an endoprotease similar or identical to the eukaryotic PCE, such as KEX2 from yeast (Thomas et al., 1988) and its human counterpart PACE (paired basic amino acid cleaving enzyme) (Wise et al., 1990). These are membrane-bound Ca^{2+}-dependent serine proteases that cleave after di-, tri- or tetrabasic sites in various prohormones (Fisher & Scheller, 1988). If PCE is indeed an MPSP, cleavage of cell surface proteins would have to occur after endocytosis, as the PCE is localized intracellularly in the secretory pathway (Fisher & Scheller, 1988). Review of all the proteins in Table I that are proteolytically released indicates that only three contain dibasic sites within 30 residues of their TM domains: the TNF receptor (Schall et al., 1990), leukemogenic membrane glycoprotein (Wolff et al., 1983), and preprocarboxypeptidase H (Rodriguez et al., 1989); of these only the last appears to be a credible substrate for the PCE. Carboxypeptidase H is itself a component of the secretory pathway, existing as both membrane-bound and soluble forms in the secretory vesicle (Fricker et al., 1990). Although controversial, recent evidence indicates that the membrane form is likely a class II TM protein and that a 56-kDa soluble form arises after cleavage of the signal peptide, while a second, 53-kDa soluble form is produced by proteolysis at a string of five arginine residues by the PCE (Parkinson, 1990).

(*b*) *Signal Peptidase*. The signal peptidase is a prototypical cellular processing protease. It is membrane-bound and recognizes a quasi-specific sequence motif in the region adjacent to the membrane-spanning signal peptide. Thus, it would appear to be an ideal candidate MPSP, particularly in the case of class II TM proteins, i.e., those that are anchored by uncleaved signal peptides. As noted above, one of the soluble forms of carboxypeptidase H is likely generated by a signal peptidase like enzyme (Parkinson, 1990). However, the cleavage sites known for other class II TM proteins—sialyltransferase, dopamine β-monooxygenase, and TNF—are unlike those processed by the signal peptidase (Paulson et al., 1987; Taljanidisz et al., 1989; Perez et al., 1990).

(*c*) *Lysosomal Endopeptidases*. Lysosomal enzymes, known for their ferocious degradative capacities, would seem to be unlikely candidates for the purpose of releasing membrane proteins by a single, specific cleavage, and yet two lysosomal-like enzymes have been implicated: cathepsin D and elastase.

Lammers and Jamieson (1988) made the interesting proposal that Golgi-membrane-bound sialyltransferase, which is also found in various secretions and body fluids (Paulson et al., 1987), is an acute-phase reactant that during inflammation is released from the liver into the plasma following cleavage by a cathepsin D like activity. Since cathepsin D is lysosomal, these authors suggest that during inflammation cathepsin D is inefficiently targeted, with some passing into the trans-Golgi network where the sialyltransferase is located. Activation of cathepsin D, an aspartyl protease, is achieved by Golgi-membrane proton pumps that can sufficiently lower the intra-Golgi pH (Lammers & Jamieson, 1988, 1989). It is significant that this cleavage is specific, since another Golgi enzyme, galactosyltransferase, is not released under the same conditions (Lammers & Jamieson, 1988). Moreover, inspection of the cleavage site (Paulson et al., 1987) reveals that it involves an asparagine residue. Others have noted that the asymmetric cleavage undergone by all lysosomal enzymes usually occurs after a residue with an amide side chain (asparagine or glutamine) (Yonezawa et al., 1988; Erickson, 1989). Whether cathepsin D is responsible for the limited proteolysis of lysosomal enzymes, including itself, and of sialyltransferase remains to be established. In its favor is evidence that cathepsin D is membrane-bound in endosomes (Diment et al., 1988), consistent with the membrane-bound activity that releases sialyltransferase (Lammers & Jamieson, 1988) and with MPSPs in general; against cathepsin D are reports that leupeptin, a cysteine protease inhibitor, blocks the cleavage of some lysosomal enzymes [reviewed in Erickson (1989)]. It should be noted that the release of APP also occurs after cleavage at a glutamine residue (Esch et al., 1990). The extent to which the proteolytic processing of these various proteins is related is likely an important question.

A second lysosomal protease of interest is elastase. Pro-TGF-α undergoes two posttranslational cleavages, one of which releases it from the membrane. Both cleavages occur at Ala-Val sites that are typical of the specificity of elastase-like proteases (Massagué, 1990). Similar cleavage sites are also involved in the release of human TNF (Perez et al., 1990) and the c-*kit* ligand (Martin et al., 1990). Apart from their presence in the azurophil granules and phagosomes of neutrophils, elastases have also been identified in the cytoplasm and on the cell membrane of various other cells, including monocytes, macrophages, and smooth muscle cells (Janoff, 1985). Inspection of the juxtamembrane sequences of other proteins listed in Table I reveals no other obvious elastase-susceptible sites, and thus this kind of cleavage site may be restricted to some of the membrane-bound growth factors.

(*d*) *Metal-Dependent Proteases.* Glycocalicin, the water-soluble extracellular domain of the α subunit of glycoprotein Ib of platelets, is released from membrane-bound glycoprotein Ib after cleavage by a Ca^{2+}-activated thiol protease (Clemetson et al., 1981), most likely calpain (Titani et al., 1987). Since calpain is cytosolic (Suzuki et al., 1987), this requires lysis of platelets (Lopez et al., 1987) and therefore calpain would not appear to be a generally useful MPSP. However, calpain is known to associate with the cell membrane (Hatanaka et al., 1984) and to cleave membrane proteins such as the EGF receptor (Cassel & Glaser, 1982). Moreover, calpain has been implicated in the release of surface-bound fibronectin (Schollmeyer, 1986 a,b), which can be speculated to involve an internal cleavage and release of either integrins or cell-surface proteoglycans that bind fibronectin (Ruoslahti, 1988).

A membrane-bound metalloprotease has been reported to cleave the membrane anchor of the folate receptor to generate soluble folate binding protein (Elwood et al., 1991). Similarly, an endogenous, EDTA-sensitive, membrane-associated hydrolase releases ACE in a soluble form from pig kidney membranes (Hooper et al., 1987). The precise cleavage sites have not been determined in either case. Lastly, a Ca^{2+}-activated protease may participate in the generation of the amyloid β-protein but is as yet incompletely characterized (Abraham et al., 1991).

(*e*) *Protein Kinase C Activated Proteases.* As indicated in Table I, proteolytic release of several membrane proteins is enhanced after activation of the host cell, and in four cases this is a PKC-dependent process: Mel-14 antigen (Kishimoto et al., 1989), TNF receptor (Lantz et al., 1990), CSF-1 receptor (Downing et al., 1989), and pro-TGF-α (Pandiella & Massagué, 1991). Theoretically, this phenomenon may be due to activation of a protease, increased exposure of the membrane protein to the protease, or conversion of the membrane protein to a better substrate (Pandiella & Massagué, 1991). In the case of pro-TGF-α and the CSF-1 receptor, the last possibility was discounted (Pandiella & Massagué, 1991; Downing et al., 1989), leaving two interesting alternatives: direct activation of the MPSP by phosphorylation or increased access to the substrate. The latter can be envisaged to entail fusion of MPSP-containing vesicles with the cell membrane or endocytotic cycling of the membrane protein into such a compartment. Whatever mechanism is responsible, the activation phenomenon lends support to the concept that the proteolytic release of membrane proteins can be a specific and regulated event.

Conclusions

The evidence presented indicates that there is an astounding array of proteins that exist as membrane-bound and soluble isoforms, of great structural and functional diversity. We have suggested that this phenomenon is of general utility to cells in allowing them to generate membrane and soluble isoforms from the same polypeptide but that there are also many instances where the specific release of membrane proteins serves specialized functions.

Of the mechanisms generating soluble isoforms from membrane proteins, proteolytic release may hold the greatest interest if it can be shown that sequence-specific processing enzymes selectively release individual target proteins [we have employed the term membrane-protein-solubilizing protease (MPSP) for this putative class of proteases]. This would certainly not be the first instance where limited proteolysis plays a key role in physiological regulation (Neurath, 1989). Moreover, the interplay of normal and aberrant proteolytic processing of membrane proteins will likely become an area of enormous interest if the current hypothesis that Alzheimer's disease results from the aberrant processing of APP proves to be correct (Selkoe, 1990). Indeed, amyloidosis, a collection of etiologically unrelated diseases of which Alzheimer's is one, has been described as "a final common pathway for protein deposition in tissues" and can be regarded as the end result of a sequence of events by which circulating and locally overproduced proteins are proteolytically processed and deposited as insoluble amyloid fibrils (Stone, 1990).

Finally, soluble forms of membrane proteins have been proposed to be of potential therapeutic value, irrespective of whether such soluble forms occur naturally. For instance, it is well known that viruses make use of resident cell-surface proteins as cellular receptors, and thus the administration of soluble forms of these proteins may block their infectivity. This is indeed the case for HIV-1 and rhinovirus infections, which are blocked by soluble CD4 and ICAM-1 antigens, respectively (Capon et al., 1989; Marlin et al., 1990). Similarly, a soluble form of the Mel-14 leukocyte CAM inhibits neutrophil-mediated inflammation (Watson et al., 1991), mimicking the spontaneous release of Mel-14 by activated neutrophils that prevents their entry into normal tissues (Kishimoto et al., 1989).

References

Abraham, C. R., Driscoll, J., Potter, H., Van Nostrand, W. E., & Tempst, P. (1991) *Biochem. Biophys. Res. Commun. 174*, 790–796.

Ahnen, D. J., Singleton, J. R., Hoops, T. C., & Kloppel, T. M. (1986) *J. Clin. Invest. 77*, 1841–1848.

Albelda, S. M., & Buck, C. A. (1990) *FASEB J. 4*, 2868–2880.

Anderson, D. M., Lyman, S. D., Baird, A., Wignall, J. M., Eisenman, J., Rauch, C., March, C. J., Boswell, H. S., Gimpel, S. D., Cosman, D., & Williams, D. E. (1990) *Cell 63*, 235–243.

Andres, J. L., Stanley, K., Cheifetz, S., & Massagué, J. (1989) *J. Cell Biol. 109*, 3137–3145.

Barnett, T. R., Kretschmer, A., Austen, D. A., Goebel, S. J., Hart, J. T., Elting, J. J., & Kamarck, M. E. (1989) *J. Cell Biol. 108*, 267–276.

Bernfield, M., & Sanderson, R. D. (1990) *Philos. Trans. R. Soc. London B327*, 171–186.

Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A., & Seed, B. (1989) *Science 243*, 1160–1165.

Black, P. H. (1980) *Adv. Cancer Res. 32*, 75–199.

Breitbart, R. E., Andreadis, A., & Nadal-Ginard, B. (1987) *Annu. Rev. Biochem. 56*, 467–495.

Brown, A. L., Chiarotti, L., Orlowski, C. C., Mehlman, T., Burgess, W. H., Ackerman, E. J., Brani, C. B., & Rechler, M. M. (1989) *J. Biol. Chem. 264*, 5148–5154.

Bülow, R., Nonnengässer, C., & Overath, P. (1989) *Mol. Biochem. Parasitol. 32*, 85–92.

Camerini, D., James, S. P., Stamenkovic, I., & Seed, B. (1989) *Nature 342*, 78–82.

Capon, D. J., Chamow, S. M., Mordenti, J., Marsters, S. A., Gregory, T., Mitsuya, H., Byrn, R. A., Lucas, C., Wurm, F. M., Groopman, J. E., Broder, S., & Smith, D. H. (1989) *Nature 337*, 525–531.

Caras, I. W., Davitz, M. A., Rhee, L., Weddell, G., Martin, D. W., Jr., & Nussenzweig, V. (1987) *Nature 325*, 545–549.

Cassel, D., & Glaser, L. (1982) *J. Biol. Chem. 257*, 9845–9848.

Cerretti, D. P., Wignall, J., Anderson, D., Tushinsky, R. J., Gallis, B. M., Stya, M., Gillis, S., Urdal, D. L., & Cosman, D. (1988) *Mol. Immunol. 25*, 761–770.

Chatis, P. A., & Morrison, T. G. (1983) *J. Virol. 45*, 80–90.
Chatonnet, A., & Lockridge, O. (1989) *Biochem. J. 260*, 625–634.
Chitambar, C. R., & Zivkovic, Z. (1989) *Blood 74*, 602–608.
Clemetson, K. J., Naim, H. Y., & Lüscher, E. F. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2712–2716.
Cseh, K., & Beutler, B. (1989) *J. Biol. Chem. 264*, 16256–16260.
Cunningham, B. A., Hemperly, J. J., Murray, B. A., Prediger, E. A., Brackenbury, R., & Edelman, G. M. (1987) *Science 236*, 799–806.
Cushman, D. W., & Cheung, H. S. (1971) *Biochim. Biophys. Acta 250*, 261–265.
Diment, S., Leech, M. S., & Stahl, P. D. (1988) *J. Biol. Chem. 263*, 6901–6907.
Downing, J. R., Roussel, M. F., & Sherr, C. J. (1989) *Mol. Cell. Biol. 9*, 2890–2896.
Doyle, L. A., Borges, M., Hussain, A., Elias, A., & Tomiyasu, T. (1990) *J. Clin. Invest. 86*, 1848–1854.
Early, P., Rogers, J., Davis, M., Calame, K., Bond, M., Wall, R., & Hood, L. (1980) *Cell 20*, 313–319.
Ehlers, M. R. W., & Riordan, J. F. (1990) in *Hypertension: Pathophysiology, Diagnosis, and Management* (Laragh, J. H., & Brenner, B. M., Eds.) pp 1217–1231, Raven Press, New York.
Ehlers, M. R. W., Chen, Y.-N. P., & Riordan, J. F. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 1009–1013.
Elwood, P. C. (1989) *J. Biol. Chem. 264*, 14893–14901.
Elwood, P. C., Deutsch, J. C., & Kolhouse, J. F. (1991) *J. Biol. Chem. 266*, 2346–2353.
Erdös, E. G., Schulz, W. W., Gafford, J. T., & Defendini, R. (1985) *Lab. Invest. 52*, 437–447.
Erickson, A. H. (1988) *J. Cell. Biochem. 40*, 31–41.
Esch, F. S., Keim, P. S., Beattie, E. C., Blacher, R. W., Culwell, A. R., Oltersdorf, T., McClure, D., & Ward, P. J. (1990) *Science 248*, 1122–1124.
Ferguson, M. A. J., & Williams, A. F. (1988) *Annu. Rev. Biochem. 57*, 285–320.
Fisher, J. M., & Scheller, R. H. (1988) *J. Biol. Chem. 263*, 16515–16518.
Flanagan, J. G., & Leder, P. (1990) *Cell 63*, 185–194.
Flanagan, J. G., Chan, D. C., & Leder, P. (1991) *Cell 64*, 1025–1035.
Fricker, L. D., Das, B. & Angeletti, R. H. (1990) *J. Biol. Chem. 265*, 2476–2482.
Fujimoto, J., Levy, S., & Levy, R. (1983) *J. Exp. Med. 158*, 752–766.
Fujimoto, J., Stewart, S. J., & Levy, R. (1984) *J. Exp. Med. 160*, 116–124.
Fukuoka, S.-I., Freedman, S. D., & Scheele, G. A. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 2898–2902.
Gamble, J. R., Skinner, M. P., Berndt, M. C., & Vadas, M. A. (1990) *Science 249*, 414–417.
Garreis-Wabnitz, C., & Kruppa, J. (1984) *EMBO J. 3*, 1469–1476.
Gliniak, B. C., & Kabat, D. (1989) *J. Virol. 63*, 3561–3568.
Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., & Hardy, J. (1991) *Nature 349*, 704–706.
Gower, H. J., Barton, C. H., Elsom, V. L., Thompson, J., Moore, S. E., Dickson, G., & Walsh, F. S. (1988) *Cell 55*, 955–964.
Haga, J. A., She, J.-X., & Kao, K.-J. (1991) *J. Biol. Chem. 266*, 3695–3701.
Hatanaka, M., Yoshimura, N., Murakami, T., Kannagi, R., & Murachi, T. (1984) *Biochemistry 23*, 3272–3276.
Hay, B., Prusiner, S. B., & Lingappa, V. R. (1987) *Biochemistry 26*, 8110–8115.
Haziot, A., Chen, S., Ferrero, E., Low, M. G., Silber, R., & Goyert, S. M. (1988) *J. Immunol. 141*, 547–552.
Hooper, N. M., Keen, J., Pappin, D. J. C., & Turner, A. J. (1987) *Biochem. J. 247*, 85–93.
Huang, E., Nocka, K., Beier, D. R., Chu, T.-Y., Buck, J., Lahm, H.-W., Wellner, D., Leder, P., & Besmer, P. (1990) *Cell 63*, 225–233.
Huizinga, T. W. J., van der Schoot, C. E., Jost, C., Klaassen, R., Kleijer, M., Kr. von dem Borne, A. E. G., Roos, D., & Tetteroo, P. A. T. (1988) *Nature 333*, 667–669.
Ikuta, K., Takami, M., Kim, C. W., Honjo, T., Miyoshi, T., Tagaya, Y., Kawabe, T., & Yodoi, J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 819–823.
Inestrosa, N. C., & Perelman, A. (1989) *Trend. Pharmacol. Sci. 10*, 325–329.
Janoff, A. (1985) *Annu. Rev. Med. 36*, 207–216.
Johnston, G. I., Cook, R. G., & McEver, R. P. (1989) *Cell 56*, 1033–1044.
Johnston, G. I., Bliss, G. A., Newman, P. J. & McEver, R. P. (1990) *J. Biol. Chem. 265*, 21381–21385.
Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K., & Müller-Hill, B. (1987) *Nature 325*, 733–736.
Kenny, J. (1986) *Trends Biochem. Sci. 11*, 40–42.
Kenny, A. J., O'Hare, M. J., & Gusterson, B. A. (1989) *Lancet ii*, 785–787.
Kishimoto, T. K., Jutila, M. A., Berg, E. L. & Butcher, E. C. (1989) *Science 245*, 1238–1241.
Kohno, T., Brewer, M. T., Baker, S. L., Schwartz, P. E., King, M. W., Hale, K. K., Squires, C. H., Thompson, R. C., & Vannice, J. L. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 8331–8335.
Komada, Y., Peiper, S., Tarnowski, B., Melvin, S., Kamiya, H., & Sakurai, M. (1986) *Leuk. Res. 10*, 665–670.
Kozarsky, K., Kingsley, D., & Krieger, M. (1988) *Proc. Natl. Sci. U.S.A. 85*, 4335–4339.
Kriegler, M., Perez, C., DeFay, K., Albert, I., & Lu, S. D. (1988) *Cell 53*, 45–53.
Krulewitz, A. H., Baur, W. E., & Fanburg, B. L. (1984) *Am. J. Physiol. 247*, C163–C168.
Lammers, G., & Jamieson, J. C. (1988) *Biochem. J. 256*, 623–631.
Lammers, G., & Jamieson, J. C. (1989) *Biochem. J. 261*, 389–393.
Lanier, L. L., Phillips, J. H., & Testi, R. (1989a) *Eur. J. Immunol. 19*, 775–778.
Lanier, L. L., Cwirla, S., Yu, G., Testi, R., & Phillips, J. H. (1989b) *Science 246*, 1611–1613.
Lantz, M., Gullberg, U., Nilsson, E., & Olsson, I. (1990) *J. Clin. Invest. 86*, 1396–1402.
Lanzillo, J. J., Stevens, J., Dasarathy, Y., Yotsumoto, H., & Fanburg, B. L. (1985) *J. Biol. Chem. 260*, 14938–14944.
Leff, S. E., Rosenfeld, M. G., & Evans, R. M. (1986) *Annu. Rev. Biochem. 55*, 1091–1117.
Letellier, M., Nakajima, T., Pulido-Cejudo, G., Hofstetter, H., & Delespesse, G. (1990) *J. Exp. Med. 172*, 693–700.
Leung, D. W., Spencer, S. A., Cachianes, G., Hammonds, R. G., Collins, C., Henzel, W. J., Barnard, R., Waters, M. J., & Wood, W. I. (1987) *Nature 330*, 537–543.

Levy, E., Carman, M. D., Fernandez-Madrid, I. J., Power, M. D., Lieberburg, I., van Duinen, S. G., Bots, G. Th. A. M., Luyendijk, W., & Frangione, B. (1990) *Science 248*, 1124–1126.

Little, S. P., & Huang, A. S. (1978) *J. Virol. 27*, 330–339.

Littman, D. R. (1987) *Annu. Rev. Immunol. 5*, 561–584.

Loetscher, H., Pan, Y.-C. E., Lahm, H.-W., Gentz, R., Brockhaus, M., Tabuchi, H., & Lesslauer, W. (1990) *Cell 61*, 351–359.

Lopez, C. D., Yost, C. S., Prusiner, S. B., Myers, R. M., & Lingappa, V. R. (1990) *Science 248*, 226–229.

Lopez, J. A., Chung, D. W., Fujikawa, K., Hagen, F. S., Papayannopoulou, T., & Roth, G. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5615–5619.

Loughnan, M. S., Sanderson, C. J., & Nossal, G. J. V. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3115–3119.

Low, M. G., & Saltiel, A. R. (1988) *Science 239*, 268–275.

Malfroy, B., Schofield, P. R., Kuang, W.-J., Seeburg, P. H., Mason, A. J., & Henzel, W. J. (1987) *Biochem. Biophys. Res. Commun. 144*, 59–66.

Marlin, S. D., Staunton, D. E., Springer, T. A., Stratowa, C., Sommergruber, W., & Merluzzi, V. J. (1990) *Nature 344*, 70–72.

Martin, F. H., Suggs, S. V., Langley, K. E., Lu, H. S., Ting, J., Okino, K. H., Morris, C. F., McNiece, I. K., Jacobsen, F. W., Mendiaz, E. A., Birkett, N. C., Smith, K. A., Johnson, M. J., Parker, V. P., Flores, J. C., Patel, A. C., Fisher, E. F., Erjavec, H. O., Herrera, C. J., Wypych, J., Sachdev, R. K., Pope, J. A., Leslie, I., Wen, D., Lin, C.-H., Cuppies, R. L., & Zsebo, K. M. (1990) *Cell 63*, 203–211.

Massagué, J. (1990) *J. Biol. Chem. 265*, 21393–21396.

Mosley, B., Beckmann, P., March, C. J., Idzerda, R. L., Gimpel, S. D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J. M., Smith, C., Gallis, B., Sims, J. E., Urdal, D., Widmer, M. B., Cosman, D., & Park, L. S. (1989) *Cell 59*, 335–348.

Mostov, K. E., & Deitcher, D. L. (1986) *Cell 46*, 613–621.

Mostov, K. E., Kraehenbuhl, J.-P., & Blobel, G. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7257–7261.

Nelson, D. L., Rubin, L. A., Kurman, C. C., Fritz, M. E., & Boutin, B. (1986) *J. Clin. Immunol. 6*, 114–120.

Neurath, H. (1989) *Trends Biochem. Sci. 14*, 268–271.

Nybroe, O., Linnemann, D., & Bock, E. (1989) *J. Neurochem. 53*, 1372–1378.

Ogata, S., Misumi, Y., & Ikehara, Y. (1989) *J. Biol. Chem. 264*, 3596–3601.

Okabe, T., Yamagata, K., Fujisawa, M., Watanabe, J., Takaku, F., Lanzillo, J. J., & Fanburg, B. L. (1985) *J. Clin. Invest. 75*, 911–914.

Palmert, M. R., Podlisny, M. B., Witker, D. S., Oltersdorf, T., Younkin, L. H., Selkoe, D. J., & Younkin, S. G. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 6338–6342.

Pandiella, A., & Massagué, J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 1726–1730.

Parkinson, D. (1990) *J. Biol. Chem. 265*, 17101–17105.

Paulson, J. C., Weinstein, J., Ujita, E. L., Riggs, K. J., & Lai, P.-H., (1987) *Biochem. Soc. Trans. 15*, 618–620.

Perez, C., Albert, I., DeFay, K., Zachariades, N., Gooding, L., & Kriegler, M. (1990) *Cell 63*, 251–258.

Porteu, F., & Nathan, C. (1990) *J. Exp. Med. 172*, 599–607.

Prusiner, S. B. (1991) *Science 252*, 1515–1522.

Reddy, P., Caras, I., & Krieger, M. (1989) *J. Biol. Chem. 264*, 17329–17336.

Rettenmier, C. W., Roussel, M. F., Ashmun, R. A., Ralph, P., Price, K., & Sherr, C. J. (1987) *Mol. Cell. Biol. 7*, 2378–2387.

Robb, R. J., & Kutny, R. M. (1987) *J. Immunol. 139*, 855–862.

Robinson, P. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 527–531.

Rodriguez, C., Brayton, K. A., Brownstein, M., & Dixon, J. E. (1989) *J. Biol. Chem. 264*, 5988–5995.

Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L., & Wall, R. (1980) *Cell 20*, 303–312.

Rubin, L. A., Kurman, C. C., Fritz, M. E., Biddison, W. E., Boutin, B., Yarchoan, R., & Nelson, D. L. (1985) *J. Immunol. 135*, 3172–3177.

Ruoslahti, E. (1988) *Annu. Rev. Biochem. 57*, 375–413.

Sack, T. L., Gum, J. R., Low, M. G., & Kim, Y. S. (1988) *J. Clin. Invest. 82*, 586–593.

Sanda, M. G., Pierson, R., Smith, C., Reemtsma, K., & Rose, E. (1989) *Transplant. Proc. 21*, 2525–2526.

Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H. W., Gatanaga, T., Granger, G. A., Lentz, R., Raab, H., Kohr, W. J., & Goeddel, D. V. (1990) *Cell 61*, 361–370.

Schollmeyer, J. E. (1986a) *Exp. Cell Res. 162*, 411–422.

Schollmeyer, J. E. (1986b) *Exp. Cell Res. 163*, 413–422.

Selkoe, D. J. (1990) *Science 248*, 1058–1060.

Silverstein, E., Friedland, J., Lyons, H. A., & Gourin, A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2137–2141.

Sisodia, S. S., Koo, E. H., Beyreuther, K., Unterbeck, A., & Price, D. L. (1990) *Science 248*, 492–495.

Soffer, R. L. (1981) in *Biochemical Regulation of Blood Pressure* (Soffer, R. L., Ed.) pp 123–164, Wiley, New York.

Soloski, M. J., Vernachio, J., Einhorn, G., & Lattimore, A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 2949–2953.

Soubrier, F., Alhenc-Gelas, F., Hubert, C., Allegrini, J., John, M., Tregear, G., & Corvol, P. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 9386–9390.

Spillantini, M. G., Sicuteri, F., Salmon, S., & Malfroy, B. (1990) *Biochem. Pharmacol. 39*, 1353–1356.

Springer, T. A. (1990) *Nature 346*, 425–434.

Stahl, N., Borchelt, D. R., & Prusiner, S. B. (1990) *Biochemistry 29*, 5405–5412.

Stein, J., Borzillo, G. V., & Rettenmier, C. W. (1990) *Blood 76*, 1308–1314.

Stiernberg, J., Low, M. G., Flaherty, L., & Kincade, P. W. (1987) *J. Immunol. 38*, 3877–3884.

Stone, M. J., (1990) *Blood 75*, 531–545.

Suzuki, K., Imajoh, S., Emori, Y., Kawasaki, H., Minami, Y., & Ohno, S. (1987) *FEBS Lett. 220*, 271–277.

Taljanidisz, J., Stewart, L., Smith, A. J., & Klinman, J. P. (1989) *Biochemistry 28*, 10054–10061.

Thomas, G., Thorne, B. A., Thomas, L., Allen, R. G., Hruby, D. E., Fuller, R., & Thorner, J. (1988) *Science 241*, 226–230.

Titani, K., Takio, K., Handa, M., & Ruggeri, Z. M. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5610–5614.

Tollefsen, S. E., Lajara, R., McCusker, R. H., Clemmons, D. R., & Rotwein, P. (1989) *J. Biol. Chem. 264*, 13810–13817.

Trivedi, B., & Daughaday, W. H. (1988) *Endocrinology 123*, 2201–2206.

Turner, A. J., & Hooper, N. M. (1989) *Biochem. Soc. Trans. 17*, 864–866.

Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle,

N., Waterfield, M. D., & Seeburg, P. H. (1984) *Nature 309*, 418–425.
von Heijne, G., & Gavel, Y. (1988) *Eur. J. Biochem. 174*, 671–678.
Wall, R., & Kuehl, M. (1983) *Annu. Rev. Immunol. 1*, 393–422.
Watson, S. R., Fennie, C., & Lasky, L. A. (1991) *Nature 349*, 164–167.
Weber, W., Gill, G. N., & Spiess, J. (1984) *Science 224*, 294–297.
Wei, L., Alhenc-Gelas, F., Soubrier, F., Michaud, A., Corvol, P., & Clauser, E. (1991) *J. Biol. Chem. 266*, 5540–5546.
Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J. M., Masters, C. L., & Beyreuther, K. (1989) *Cell 57*, 115–126.
Weinstock, J. V. (1986) *Sarcoidosis 3*, 19–26.
Weinstock, J. V., & Boros, D. L. (1982) *Gastroenterology 82*, 106–110.
Weitzhandler, M., Streeter, H. B., Henzel, W. J. & Bernfield, M. (1988) *J. Biol. Chem. 263*, 6949–6952.
Wise, R. J., Barr, P. J., Wong, P. A., Kiefer, M. C., Brake, A. J., & Kaufman, R. J. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 9378–9382.
Yokoyama, M., Takada, Y., Iwata, H., Ochi, K., Takeuchi, M., Hiwada, K., & Kokubu, T. (1982) *J. Urol. 127*, 368–370.
Yonezawa, S., Takahashi, T., Wang, X.-J., Wong, R. N. S., Hartsuck, J. A., & Tang, J. (1988) *J. Biol. Chem. 263*, 16504–16511.
Yost, C. S., Lopez, C. D., Prusiner, S. B., Myers, R. M., & Lingappa, V. R. (1990) *Nature 343*, 669–672.
Zheng, Q.-X., Tease, L. A., Shupert, W. L., & Chan, W.-Y. (1990) *Biochemistry 29*, 2845–2852.
Zupan, A. A., Osborne, P. A., Smith, C. E., Siegel, N. R., Leimgruber R. M., & Johnson, E. M., Jr. (1989) *J. Biol. Chem. 264*, 11714–11720.

Accelerated Publications

Structure–Function Relationships in Human Lecithin:Cholesterol Acyltransferase. Site-Directed Mutagenesis at Serine Residues 181 and 216†

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ABSTRACT: The functions of serine residues at positions 181 and 216 of human plasma lecithin:cholesterol acyltransferase have been studied by site-directed mutagenesis. The serine residue at either site was replaced by alanine, glycine, or threonine in LCAT secreted from stably transfected CHO cells. All substitutions at position 181 gave rise to an enzyme product that was normally secreted but had no detectable catalytic activity. On the other hand, all substitutions at position 216 gave active products, whose activity was fully inhibitable by the serine esterase inhibitor diisopropyl fluorophosphate (DFP). A secondary (although not direct) role for serine-216 was indicated by a 14-fold increase in catalytic rate when this residue was substituted by alanine. Sequence comparison with other lipases suggests that serine-216 may be at or near the hinge of a helical flap displaced following substrate binding. These data strengthen the structural–functional relationship between LCAT and other lipases.

Lecithin:cholesterol acyltransferase (LCAT;[1] phosphatidylcholine–sterol *O*-acyltransferase, EC 2.3.1.43) catalyzes the synthesis of cholesteryl esters from free cholesterol originating in cell membranes or plasma lipoproteins. LCAT drives the "reverse transport" of cholesterol from cell membranes to plasma and may play an important role in regulating cellular cholesterol accumulation (Fielding & Fielding, 1981; Davis et al., 1982). In the absence of cholesterol, LCAT acts as a phospholipase, generating free fatty acids in place of cholesteryl esters (Aron et al., 1978). The enzyme has been cloned and sequenced (McLean et al., 1986) and shares with other lipases several regions of local sequence similarity.

Both acyltransferase and phospholipase activities of LCAT are blocked by classical inhibitors of serine esterases such as diisopropyl fluorophosphate (DFP) and diethyl *p*-nitrophenyl phosphate (E-600) (Glomset, 1968; Aron et al., 1978; Jauhiainen & Dolphin, 1986). However, as in the case of other lipases, attempts to assign the active-site serine in the primary sequence by chemical modification have given inconclusive results. In pancreatic lipase, E-600 inhibited lipase activity with long-chain glycerides but not esterase activity, while DFP reacted with a tyrosine rather than a serine residue (Maylie et al., 1972). These data were interpreted to indicate the

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[1] Abbreviations: LCAT, lecithin:cholesterol acyltransferase; DFP, diisopropyl fluorophosphate. Amino acid sequences are described using the single-letter code. Subscripts refer to amino acid position within the primary sequence of the mature protein.

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