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COMPREHENSIVE INVITED REVIEW

Nuclear Redox Signaling

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Abstract

Reactive oxygen species have been described to modulate proteins within the cell, a process called redox regulation. However, the importance of compartment-specific redox regulation has been neglected for a long time. In the early 1980s and 1990s, many *in vitro* studies introduced the possibility that nuclear redox signaling exists. However, the functional relevance for that has been greatly disregarded. Recently, it has become evident that nuclear redox signaling is indeed one important signaling mechanism regulating a variety of cellular functions. Transcription factors, and even kinases and phosphatases, have been described to be redox regulated in the nucleus. This review describes several of these proteins in closer detail and explains their functions resulting from nuclear localization and redox regulation. Moreover, the redox state of the nucleus and several important nuclear redox regulators [Thioredoxin-1 (Trx-1), Glutaredoxins (Grxs), Peroxiredoxins (Prxs), and APEX nuclease (multifunctional DNA-repair enzyme) 1 (APEX1)] are introduced more precisely, and their necessity for regulation of transcription factors is emphasized. *Antioxid. Redox Signal.* 12, 713–742.

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I. Introduction

Oxygen is one of the most relevant molecules for all aerobic organisms. For many years, it has been clear that aerobic organisms produce reactive oxygen species (ROS) from oxygen. The molecules, which belong to the ROS, are superoxide anion $O_2^{-\bullet}$, hydroxyl radical ($OH^\bullet$), and hydrogen peroxide (H_2O_2). Even under physiologic conditions, all of these molecules are produced within cells.

Several oxidative systems exist that generate $O_2^{-\bullet}$ from oxygen. Potential sources of this ROS production are, for example, the NADPH oxidases, the respiratory chain within the mitochondria, and the xanthine/xanthine oxidase system (Fig. 1A). Conversely, a pool of several antioxidative enzymes scavenge, reduce, or inactivate ROS (Fig. 1A). Specifically, $O_2^{-\bullet}$ is reduced by superoxide dismutases (SODs) to H_2O_2. H_2O_2 is then metabolized to H_2O by several enzymes, mainly by catalase, but also by glutathione peroxidase (GPx), which in turn results in oxidation of glutathione. The glutathione disulfide (GSSG) itself is then reduced to glutathione by the glutathione oxidoreductase (GR) to feed back into this cycle (Fig. 2). Another important antioxidative system, the thioredoxin/thioredoxin-reductase (Trx/TR) also has been described to metabolize H_2O_2 directly. However, this seems to be a rather rare event in cells, and the Trx/TR system mainly reduces oxidized proteins. In the course of this process, reduced Trx itself is oxidized. Reduced Trx is then regenerated by TR by using the cofactor NADPH to be further available for the reduction of oxidized proteins (Fig. 3).

Therefore, a controlled redox balance exists in cells. Perturbation of this balance either by increased production of ROS or by reduced antioxidative capacity will result in so-called oxidative stress (Fig. 1B). Increased ROS lead to modifications of biological molecules, including proteins, DNA, and lipids. In line with this, under conditions of oxidative stress, $O_2^{-\bullet}$ can directly react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), which readily crosses cell membranes. Although possessing a short half-life, $ONOO^-$ interacts with target molecules even in neighboring cells. Peroxynitrite is involved in protein nitration by nitrating tyrosine residues irreversibly to form 3-nitrotyrosine (95). The interplay of $ONOO^-$ with ROS to damage cells is then termed nitrosative stress. $ONOO^-$ itself is extremely toxic to cells because it is readily converted to two other radical species, $OH^\bullet$ and $NO_2^\bullet$, by hemolytic decomposition (92). Therefore, these species are often referred to as ROS/RNS (reactive nitrogen species). Oxidative and nitrosative stress–induced modifications of biological molecules have been implicated in a variety of diseases, such as cardiovascular diseases, neurologic disorders, and cancer. Over a long period, it was believed that the production of ROS is, in principle, bad for cells, and therefore, the term "redox signaling" was underestimated. However, several lines of evidence have established that redox signaling exists and is required for organisms to survive. Moreover, ROS have a wide range of action because of their ability to interact with almost all biological molecules. The mode of action of ROS is also dependent on the localization of their target molecules. Thus, the modifications induced by ROS can be separated depending on the cellular compartments they affect. Several studies investigated whether mitochondrial and nuclear redox signaling exist, in addition to the well-described cytosolic (previously termed cellular) redox-dependent events. Under physiologic conditions, ROS can induce changes in gene expression (203), whereas under conditions of oxidative and nitrosative stress, ROS and RNS can directly damage DNA in the nucleus, which can result in apoptosis or malignant transformation (140, 233).

This review focuses on nuclear redox signaling and oxidative stress–induced nuclear translocation of proteins and their resultant nuclear modes of actions. The antioxidative enzymes, Trx-1 and the APEX nuclease (multifunctional DNA repair enzyme) 1 (APEX1, also known as Ape/Ref-1), and their important role in nuclear redox signaling are highlighted. A number of proteins, which belong to the classes of transcription factors, chromatin-modifying enzymes, kinases, and phosphatases, are discussed in detail. All of the mentioned proteins have in common that they have a nuclear localization and function and are redox regulated or regulated by oxidative stress in higher eukaryotes. Although this review tries to be as comprehensive as possible, we are aware that we might not have discussed all of them.

II. Nuclear Redox Regulators

A. Redox state of the nucleus

The term redox state describes the balance between the oxidized and reduced forms of biologically relevant redox pairs, including $NADH/NAD^+$, $NADPH/NADP^+$, and GSH/GSSG. It is reflected in the balance of several sets of

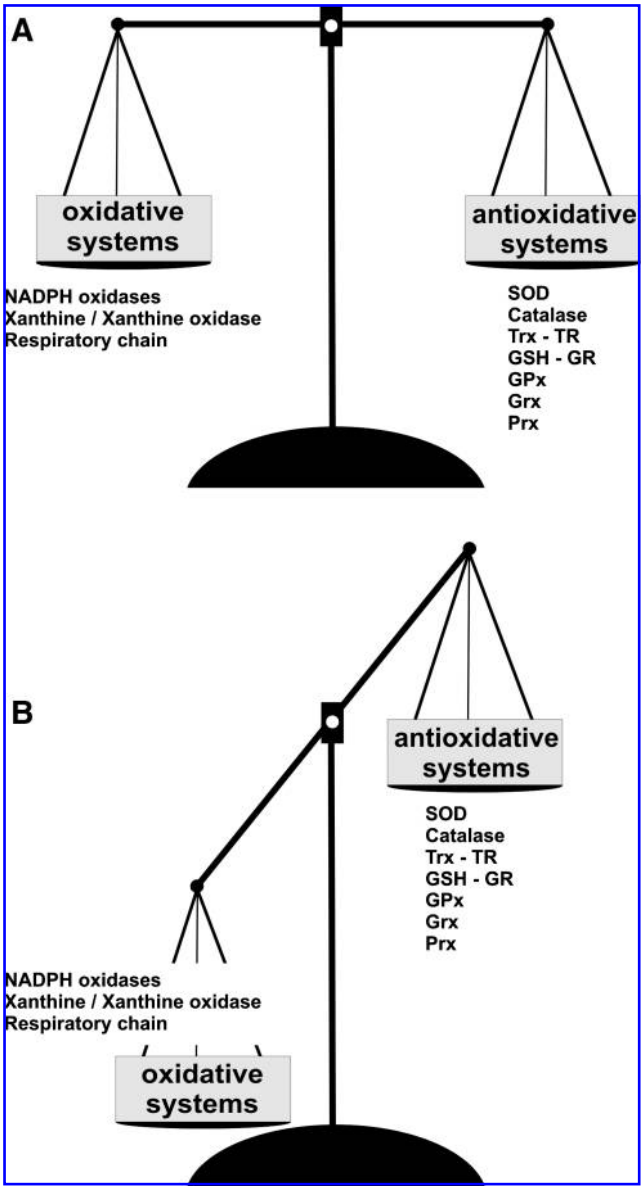

FIG. 1. Balance between oxidative and antioxidative systems. (**A**) The generation of ROS by oxidative systems (*e.g.*, NADPH oxidase, xanthine/xanthine oxidase, and the respiratory chain) is controlled by the activity of antioxidant systems, like superoxide dismutase (SOD), catalase, thioredoxin/thioredoxin reductase (Trx/TR), glutathione/glutathione reductase (GSH/GR), glutathione peroxidase (GPx), glutaredoxin (Grx), and peroxiredoxin (Prx). They scavenge ROS or reduce their levels and thus maintain the redox balance in healthy cells and tissues. (**B**) A state of oxidative stress occurs either by increased activity of the oxidative systems or by reduced antioxidative capacity of the cells.

metabolites (*e.g.*, lactate and pyruvate) whose interconversion is dependent on these ratios. An abnormal redox state can develop in a variety of deleterious situations, such as hypoxia, shock, and sepsis. Determination of the relative concentrations of the components of these redox pairs showed that the GSH/GSSG and NADPH/$NADP^+$ ratios are >1 (234, 236), whereas the NADH/NAD^+ ratio is <1 (221, 261). Generally, the nucleus provides a reductive environment.

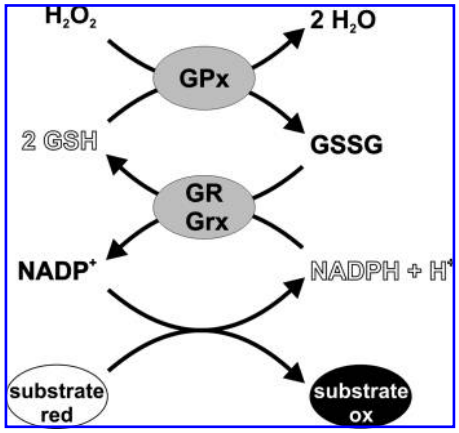

FIG. 2. The GSH/GR/GPx system: a general model of the GSH/GR/GPx system. Glutathione peroxidase (GPx) reduces H_2O_2 to H_2O and thereby oxidizes GSH to GSSG. GSSG itself is reduced by glutathione reductase (GR) to regenerate GSH. The reductant in this reaction is NADPH + H^+, created by metabolic substrate oxidation.

NAD^+/NADH is required as a coenzyme for metabolic processes. The high NAD^+/NADH ratio allows this coenzyme to act as both an oxidizing and a reducing agent. In contrast, the main function of $NADP^+$ is as a reducing agent in anabolism. Since NADPH is needed to drive redox reactions as a strong reducing agent, the NADPH/$NADP^+$ ratio is kept high. Because no barrier exists to diffusion of these dinucleotide coenzymes across the nuclear membrane (160, 261), it is assumed that the NAD^+/NADH and $NADP^+$/NADPH ratios are the same in the cytoplasm and the nucleus. The majority of these coenzymes are protein bound, and the concentration of free NADH in the nucleus has been calculated to be 130 n*M* (261). It is assumed that the NADPH/NADH ratio is ~4 (210); therefore, the concentration of free NADPH in the nucleus must be ~500 n*M*.

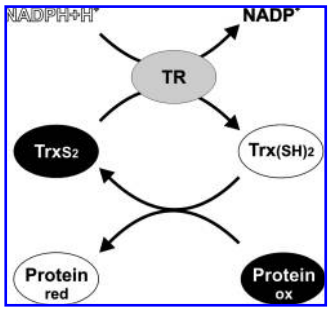

FIG. 3. The Trx/TR system: simplified model of the Trx/TR system and Trx function. The Trx/TR system consists of two oxidoreductase enzymes, thioredoxin (Trx) and thioredoxin reductase (TR). Reduced Trx ($Trx_{(SH)2}$) directly interacts with oxidized proteins by forming disulfide bridges. This involves formation of a mixed-disulfide intermediate in the thiol-disulfide exchange reaction (124). As a consequence of this two-step reaction, the protein is reduced, and Trx itself is oxidized (Trx_{S2}). The regeneration of reduced Trx from its oxidized form is catalyzed by TR by using NADPH + H^+.

Glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) is a ubiquitous thiol tripeptide and is the most abundant thiol present inside the cell. GSH has multiple direct and indirect functions in many critical cellular processes like synthesis of proteins and DNA, amino acid transport, enzyme activity, and metabolism (149). GSH also serves as a reductant to destroy free radicals, hydrogen peroxide, and other peroxides and as a storage form of cysteine. The redox state of the GSH/GSSG couple is often used as an indicator of the overall redox environment of the cell (200). GSH is found in a fairly high concentration of ~10 m*M* within cells. As it can freely diffuse (160, 172), the cytoplasmic and nuclear concentrations are similar (215, 236). Interestingly, the nuclear levels change during the cell cycle, with the highest levels found in the S and G_2/M phases (137). In line with these findings, depletion of GSH leads to reduced proliferation and apoptosis (138, 151). Taken together, one would hypothesize that during the G_2/M phase, a more reductive nuclear environment is required for cell proliferation to proceed. This was corroborated by a combined *in vitro*/bioinformatic investigation, which showed that 69 proteins containing redox-sensitive motifs have functions in central cell cycle processes like transcription, nucleotide metabolism, (de)phosphorylation, and (de)ubiquitinylation. The majority of these oxidant-sensitive proteins function during the G_2/M phase, indicating that oxidant-sensitive proteins may be temporally regulated by oscillation of the intracellular redox environment (39).

B. Antioxidative enzymes in the nucleus

As mentioned earlier, this review focuses on nuclear redox signaling. Therefore, it is important to introduce the antioxidative systems that have been described to be localized in the nucleus. Already in the late 1980s, Cu/Zn SOD and catalase were detected in the nuclear fractions of the developing rat cerebral cortex (43). In contrast, in adult Langerhans cells in catalase-overexpressing mice, catalase could not be detected in the nucleus (34). These findings point out that the presence of catalase in the nucleus seems to be dependent on cell type and perhaps also on age.

Several studies investigated the nuclear localization and activity of enzymes of the glutathione system, including GR, GPx, and glutathione *S*-transferases (GSTs). In rat liver cells, all proteins were found to be localized in the nucleus. Moreover, enzymatic activity could be measured in nuclear extracts (191). Recently, the exact localization of GSTα-a was determined to be at the nuclear membrane. Thus, the authors speculated that GSTα-a probably has a role as a defense barrier at the nuclear envelope (220). Several lines of evidence demonstrated that Trx-1 is localized in the nucleus, and its major nuclear function seems to be the binding to and reduction of transcription factors, thereby modulating their activities. Other thiol reductases, namely nucleoredoxin and glutaredoxin (Grx), which have similar, but also distinct functions from those of Trx-1, have been reported to be localized in the nucleus (80). Another important enzyme this context is APEX1. It is known that the DNA base excision-repair pathway is responsible for the repair of alkylation and oxidative DNA damage. A crucial step in the base excision-repair pathway involves the cleavage of an apurinic/apyrimidinic (AP) site in DNA by AP endonucleases (248). The major AP endonuclease in mammalian cells is APEX1, a multifunctional enzyme that acts not only as an AP endonuclease but also as a redox-modifying factor for a variety of transcription factors. Moreover, several studies demonstrated that APEX1 and Trx-1 act in concert in regulating transcription factors, which is discussed in more detail later in this review.

The variety of antioxidative enzymes reported to be localized in the nucleus and to be present in their active forms underscores the existence of a nuclear redox signaling network. Several nuclear proteins will now be introduced whose activity and functions depend on the redox balance and on nuclear redox signaling.

C. Thioredoxin-1 (Trx-1)

One major redox regulator in cells besides the glutathione system is the thioredoxin system. Thioredoxin was first discovered by Peter Reichard and co-workers (118) in 1964 as an electron donor for ribonucleotide reductase from *Escherichia coli*. In 1968 Holmgren and co-workers (85) determined the amino acid sequence of thioredoxin from *E. coli* after isolation of the pure protein and showed the classic active site -Cys-Gly-Pro-Cys-. The crystal structure of *E. coli* Trx in its oxidized form resulted in the definition of the thioredoxin fold: a central β-sheet surrounded by α-helices with the active site at the end of a β-strand and in the beginning of an α-helix (88). This structure defines a large superfamily of proteins (124). It is adopted by bacterial glutaredoxins (Grx) and appears in the other members of the family as a substructure or domain (9, 50, 139). Besides Trxs and Grxs, protein disulfide isomerases, GSTs, GPxs, peroxiredoxins, and chloride intracellular channels (CLICs) are members of the Trx superfamily (12, 139, 202). Mammalian cells contain two Trxs, Trx-1 and Trx-2 (exclusively localized in mitochondria) and three TRs: cytosolic TR1, mitochondrial TR2, and the testis-specific thioredoxin glutathione reductase (TGR). In 1985, Holmgren (87) introduced Trx-1 as a small, ubiquitous protein with two redox-active cysteine residues in an exposed active center, having the same amino acid sequence as *E. coli* Trx -Cys-Gly-Pro-Cys- (Cys 32 and Cys 35 within Trx-1), which is essential for its redox-regulatory function. The thioredoxin-1 system consists of Trx-1 and TR1. The regeneration of reduced Trx-1 from its oxidized form is catalyzed by TR1 by using NADPH (162). One important function of Trx-1 is the reduction of oxidized proteins, which depends on cysteine 32 and cysteine 35. The functions of Trx-1 are dependent on its cellular localization. Cytosolic Trx-1 interacts with its active-site cysteines with several proteins by forming disulfide bridges and thereby inhibits apoptosis induction. Trx-1 associates with ASK1 and the vitamin D_3–upregulated protein 1 (Txnip, also named VDUP-1) (194, 258). Thereby, reduced Trx-1 protects cells from apoptosis through an inhibitory binding to ASK1, whereas this binding is lost when Trx-1 is oxidized (194). Similarly, binding of Trx-1 to Txnip completely abrogates the antiproliferative function of Txnip (204). It has been demonstrated that, under certain conditions, Trx-1 accumulates in the nucleus and directly or indirectly interacts with different transcription factors (203). In the case of direct association, Trx-1 reduces oxidized transcription factors and thereby allows them to interact with DNA. The capacity of Trx-1 to interact with several transcription factors and thereby alter their functions is discussed in more detail later in this review. In case of an indirect action on transcription factors,

Trx-1 binds to APEX1, and APEX1 itself reduces the oxidized transcription factors. APEX1 is a complex protein with several functions, including endonuclease activity, redox factor, and redox chaperone properties. Because of its importance in nuclear redox signaling, APEX1 is introduced in more detail later in this review.

D. Glutaredoxins (Grxs) and peroxiredoxins (Prxs)

Glutaredoxins (Grxs) belong to the Trx superfamily of proteins. Grxs were first described as glutathione-dependent reductases three decades ago (86). They play an important role in cellular redox-dependent processes, mainly through deglutathionylation of proteins. In addition to the early-discovered dithiol Grxs, another group of Grxs has been identified. These monothiol Grxs lack the C-terminal active-site cysteine but contain all structural and functional elements to bind and use GSH. Therefore, two distinct mechanisms are known, the dithiol reaction in which the two cysteines in the active center take part in reducing both low-molecular-weight and protein disulfides and the monothiol mechanism in which only one cysteine in the active center is present (for review, see ref. 123). In humans, four Grx isoforms have been identified: the dithiol isoforms Grx1 and Grx2 and the monothiol isoforms Grx3 (PICOT/TXNL-2) and Grx5 (94, 131, 249, 250). Grx1 is considered mainly to be a cytosolic protein, although some studies show a nuclear localization (133, 219) and a presence in the intermembrane space of mitochondria (171). Several studies have shown that Grx1 is able to deglutathionylate a variety of proteins, like actin, protein tyrosine phosphatase-1B, glyceraldehyde-3-phosphate dehydrogenase, Ras, and caspase 3 (3, 19, 126, 173, 240) and thereby influences several important cellular functions, including actin polymerization, hypertrophy, and apoptosis (3, 173, 240, 242). Furthermore, Grx1 regulates transcription factor activity directly as well as indirectly, which is discussed later in this review in the respective transcription factor sections. Grx2 is known to exist in at least three different splicing variants, Grx2a, Grx2b, and the newly discovered Grx2c. Grx2a is considered a mitochondrial protein, and Grx2b was detected in nuclear fractions (62, 131). A recent screening of diverse tissues showed a ubiquitous expression of Grx2a, whereas Grx2b and Grx2c were found exclusively in testis and some cancer cell lines (129). Grx3, also termed PICOT (protein kinase C–interacting cousin of thioredoxin), was first identified in 2000 as an interaction partner of protein kinase C θ (PKCθ) and described to exist in the cytosol (250). Grx5 is a monothiol enzyme with a mitochondrial localization signal and therefore resides within the mitochondria (190). Thus, only Grx1 and Grx2b are localized in the nucleus, and regulation of transcription factor activity in the nucleus has been demonstrated only for Grx1.

Peroxiredoxins (Prxs) are members of a superfamily of Se-independent peroxidases. Six members have been identified and characterized in mammals. Prxs execute enzymatic degradation of H_2O_2 and organic hydroperoxides by using electrons donated by Trx-1 (Prx1 to Prx5), cyclophilin A (Prx1 to Prx4), or glutathione (Prx6). Prxs are divided into three classes: typical 2-Cys Prxs; atypical 2-Cys Prxs; and 1-Cys Prxs (for review, see refs. 102 and 187). These enzymes share the same basic catalytic mechanism, in which the single, redox-active cysteine is oxidized to a sulfenic acid by the peroxide substrate (38). The recycling of the sulfenic acid back to a thiol distinguishes the three enzyme classes: 2-Cys Prxs are reduced by thiols, particularly thioredoxin (188), whereas the 1-Cys enzymes are reduced by glutathione (136) and ascorbic acid (152). By using crystal structures, a detailed catalytic cycle has been derived for typical 2-Cys Prxs, including a model for the redox-regulated oligomeric state proposed to control enzyme activity (251). Prxs have a wide tissue distribution and specific subcellular localization. Nuclear localization has been demonstrated for Prx1, Prx2, Prx4, Prx5, and Prx6, at least in tumor cells (109). However, only for Prx5 has a nuclear function been demonstrated. Nuclear Prx5 significantly reduced nuclear DNA damage induced by H_2O_2 (15).

E. APEX nuclease (multifunctional DNA-repair enzyme) 1 (APEX1)

APEX1 is a multifunctional protein. Its first abbreviation, human apurinic/apyrimidinic (AP) endonuclease, or Ape1 (also called HAP1 or APEX) derived from one of its functions as an essential enzyme in the base-excision repair (BER) pathway. This pathway is responsible for repair of apurinic/apyrimidinic (AP) sites in DNA, which are a major end product of ROS damage. Unrepaired AP sites can halt mRNA and DNA synthesis or act as noncoding lesions resulting in the increased generation of DNA mutations (248). To initiate repair, APEX1 cleaves 5′ to the baseless site, which leads to generation of a 3′-hydroxyl group and an abasic deoxyribose-5-phosphate. Subsequently, DNA polymerase β and DNA ligase I are recruited to the abasic site to complete the repair process (223).

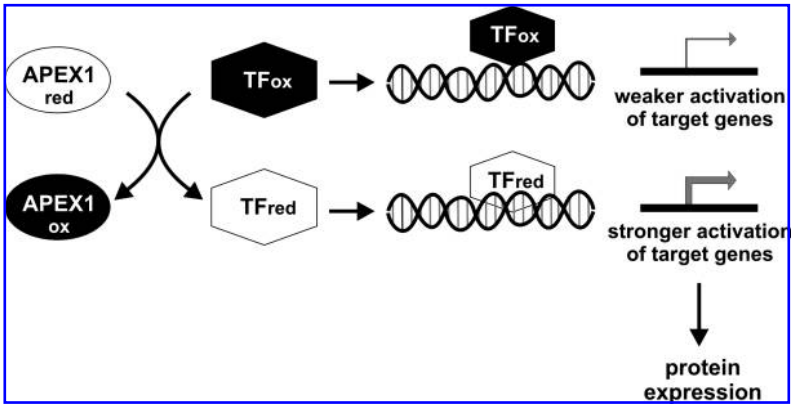

FIG. 4. APEX1. General model of APEX nuclease (multifunctional DNA-repair enzyme) 1 (APEX1) redox regulator function. APEX1 converts a transcription factor (TF) from an oxidized, less DNA binding competent state to its reduced state that can bind more avidly to the regulatory regions of a variety of genes. This leads to activation of these downstream targets.

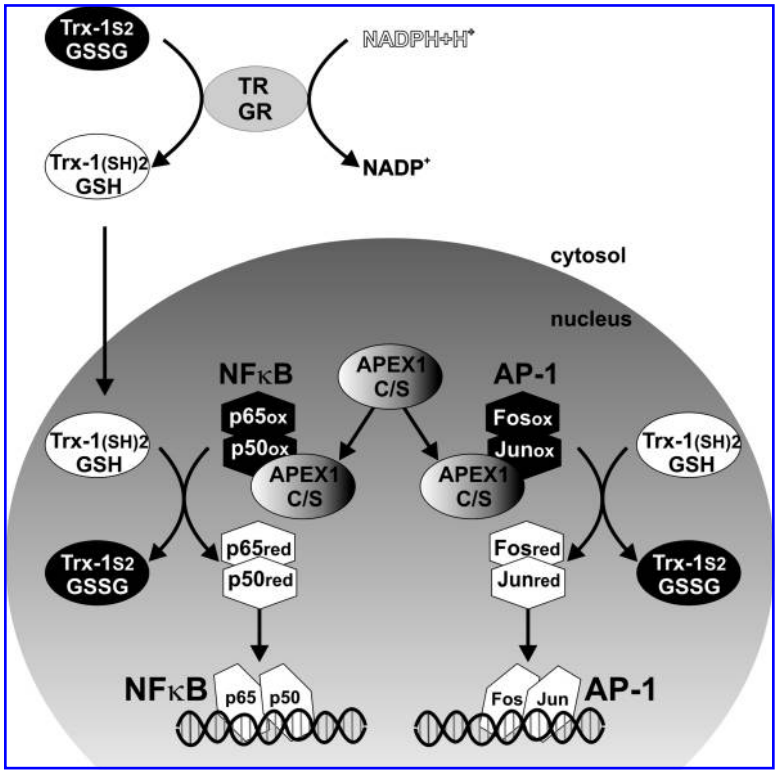

FIG. 5. APEX1 as a redox chaperone: model for the redox chaperone function of APEX1. A mutant of APEX nuclease (multifunctional DNA-repair enzyme) 1, in which all seven cysteine residues are replaced with serine (APEX C/S), can bind to the p50 and Jun subunits of the transcription factor complexes nuclear factor-kappa B (NF-κB) and activator protein 1 (AP-1), respectively. In this complex, APEX C/S can recruit thioredoxin-1 (Trx-1) or glutathione (GSH), which subsequently reduce both subunits of the heterodimeric transcription factors. Reduction enables them to bind their cognate response elements and activate transcription of their target genes.

The second abbreviation, Ref-1, stands for redox effector factor-1 and reflects its function as a redox regulator of transcription factors (Fig. 4). Through its redox function, APEX1 maintains transcription factors in an active, reduced state required for DNA binding and transcriptional activation. The two functions of APEX1, repair and redox regulation, are independent and located in separate domains of the protein, which was demonstrated by deletion analysis. The N-terminal portion of APEX1 that is not present in functionally related proteins from other organisms is required for the redox activity, whereas the DNA-repair activity requires conserved C-terminal sequences. Chemical alkylation or oxidation of cysteines inhibits the redox activity of APEX1 without affecting its DNA-repair activity (255). In addition, mutation of cysteine residue 65 (Cys 65), which is unique to mammalian APEX1, abrogates the redox function of the human protein. The wild-type zebrafish APEX has a threonine residue in the corresponding position (Thr 58) and is redox inactive, but can be converted to a redox enzyme by conversion of Thr 58 to cysteine (59). Conversely, when Cys 65 in human APEX1 is converted to alanine, the resulting protein is redox deficient (132).

Two reports uncovered a third function of APEX1, the stimulation of transcription factor reduction independent of its intrinsic redox activity. *In vivo* analysis of mouse APEX1 mutated at the cysteine previously identified as the redox catalytic site revealed a surprising result. Unlike APEX1-null mice, which die very early in embryonic development, homozygous APEX1(C64A) mice were viable with no overt phenotype. Although APEX1 is the major redox regulator of activator protein 1 (AP-1) in murine cells, AP-1 DNA binding activity and reduction of Fos and Jun were unaltered compared with wild-type mice, demonstrating that the redox activity of APEX1 is not required for redox regulation of AP-1 (169). A more-robust proof was obtained by the analysis of human APEX1, in which all seven cysteine residues were substituted to serine (APEX1 C/S; Fig. 5). It was demonstrated that APEX1 can directly reduce *in vitro* oxidized recombinant p50, a subunit of nuclear factor-kappa B (NF-κB), at relatively high concentrations (*i.e.*, at concentrations >50-fold higher than that of p50). However, when Trx-1 or GSH was included in the reaction, a much lower concentration of APEX1 was sufficient to stimulate p50 DNA binding activity, indicating that APEX1 might facilitate reduction of transcription factors by other reducing molecules such as Trx-1. Surprisingly, unlike the direct reduction of p50, this effect was not dependent on the redox activity of APEX1 because APEX1 C/S increased p50 DNA binding as efficiently as APEX1 wt in the presence of GSH or Trx-1. This activity of APEX1 was not restricted to the NF-κB transcription factor complex, as also the DNA binding activity of AP-1 was stimulated by APEX1 C/S. Interestingly, a physical interaction of APEX1 and the C/S mutant occurs only with one subunit of these heterodimeric transcription factors (with p50, p52, c-Rel, and c-Jun, but not with p65 and c-Fos) (8).

Thus, APEX1 binding to certain subunits of heteromeric transcription factors leads to reduction of the transcription factor subunits by other reductive systems. Therefore, APEX1 can function as a redox chaperone. This activity may be important for the modulation of the activity of a subset of transcription factors.

F. Trx-1/APEX1 interactions

A direct interaction between overexpressed APEX1 and Trx-1 has been demonstrated in the nucleus. Detailed analysis

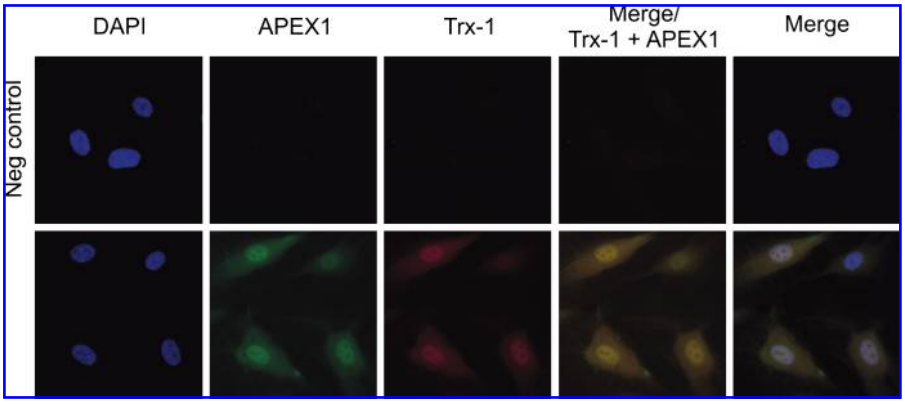

FIG. 6. Interaction of endogenous APEX1 and Trx-1 in human endothelial cells. Nuclear association of endogenous thioredoxin-1 (Trx-1) and APEX nuclease (multifunctional DNA-repair enzyme) 1 (APEX1). Representative immunostainings of human umbilical vein endothelial cells are shown. Nuclear staining with DAPI is shown in blue, APEX1 staining in green, and Trx-1 staining in red. The second panel from the right shows the merging of APEX1 and Trx-1 staining; the rightmost panel, the merging of DAPI, APEX1, and Trx-1. Cells were fixed and permeabilized. For coimmunostaining, cells were first incubated with an antibody against human Trx-1 (mouse, 1:50, overnight, 4°C; BD Pharmingen); as a secondary antibody, anti-mouse rhodamine RedX (1:100, 30 min, 37°C; Invitrogen) was used. Because both the Trx-1 and the APEX1 antibodies are from the same host species, a blocking step with an excess of mouse IgG was performed. After that, the cells were incubated with an antibody against human APEX1 (mouse, 1:200, overnight, 4°C; Novus Biologicals) followed by a secondary anti-mouse Alexa 488 (1:200, 30 min, 37°C; Invitrogen). Nuclei were stained with DAPI (0.2 μg/ml). Cells were visualized with fluorescence microscopy (Zeiss Axiovert 100, magnification 1:40, oil). The top row represents the negative control without primary antibodies.

of the cysteines in Trx-1 in a yeast two-hybrid system revealed that cysteines 32 and 35 are required for direct interaction with APEX1 (79). Conversely, cysteines in APEX1 were not mapped for Trx-1 interaction. We found that colocalization of the two endogenous proteins, APEX1 and Trx-1, is detectable mainly in the nucleus in human endothelial cells (Fig. 6). Mapping of the interaction domain within APEX1 is under further investigation. It must be noted that, under certain conditions, Trx-1 and APEX1 act in concert to regulate transcriptional activation (8, 244), whereas in other cases, APEX1 or Trx-1 alone is sufficient to control DNA binding of transcription factors. This is discussed in closer detail for the redox-regulated transcription factors in the following sections of this review.

Table 1. Redox-regulated Transcription Factors

Transcription factor	*Critical amino acid*	*Affected function*	*Compartment of modification*
AP-1	Cys 269 (c-Fos) Cys 154 (c-Jun)	DNA binding (1)	Nucleus (79)
BPV E2	Cys 340	DNA binding (146)	n.d.
CBP/PEBP2	Cys 115, Cys 124	DNA binding (5)	n.d.
c-Myb	Cys 130	DNA binding (67, 155)	Nucleus (67)
CREB	Cys 300, Cys 310	DNA binding (64)	n.d.
Egr-1	n.d	DNA binding (91)	Nucleus (91)
Estrogen receptor	n.d. (DBD)	DNA binding (122)	n.d.
Glucocorticoid receptor	n.d. (DBD) Cys 481	DNA binding (93, 229) Nuclear import (167)	Nucleus (135) Cytoplasm (167)
HIF-1α	Cys 800	CBP interaction (51)	n.d.
HLF	Cys 28 Cys 844	DNA binding (116) CBP interaction (51)	n.d.
HoxB5	Cys-232	Cooperative DNA binding (58)	n.d.
MyoD	Cys 135	DNA binding (218)	n.d.
NFI/CTF	Cys 3 Cys 427	DNA binding (13) Transcriptional activation (154)	n.d.
NF-κB	Cys 62 (p50) Tyr 66, Tyr 152 (p65)	DNA binding (81, 142) Stability, nuclear retention (176)	Nucleus n.d.
NF-Y	Cys 85, Cys 89	DNA binding (156)	n.d
Nrf-2	Cys 506	DNA binding (26)	Nucleus (76)
p53	Cys 173, Cys 235, Cys 239	DNA binding (73, 185)	n.d.
Pax-5	n.d.	DNA binding (226, 227)	Nucleus (226, 227)
Pax-8	n.d.	DNA binding (103, 226)	Nucleus (226)
Sp1	n.d.	DNA binding (6, 7)	Nucleus (36)
TTF-1	Cys 87	DNA binding (11, 103, 225)	n.d.

III. Redox-Regulated Transcription Factors and Chromatin Modifiers

Transcription factors regulate cellular functions through altering the gene expression profile. A number of transcription factors have been shown to be redox regulated through modulation of their DNA binding capacity. Additional layers of regulation are on the level of transcriptional activation by changing cofactor interactions, oligomerization, or subcellular localization. Thereby cells can modulate their transcriptome to adjust to physiologic and pathophysiologic changes in ROS levels and exogenous noxae. Table 1 provides an overview over the mammalian transcriptional regulatory proteins for which a redox regulation has been shown. In the following sections, we review the molecules for which a more-detailed knowledge of their redox regulation is available.

A. Activator protein-1 (AP-1)

Activator protein-1 (AP-1) is one of the first mammalian transcription factors that were identified (119). AP-1 is not a single protein, but represents various homo- or heterodimers formed between the proteins of the basic region-leucine zipper (bZIP) family. They belong to the Jun (c-Jun, JunB, JunD), Fos (c-Fos, FosB, Fra-1, and Fra-2), Maf (c-Maf, MafB, MafA, MafG/F/K, and Nrl), and ATF (ATF2, ATF3/LRF1, B-ATF, JDP1, JDP2) subfamilies of the bZIP proteins. Their complexes bind to a canonical AP-1 site, originally described as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) response element, or variants thereof. Recruitment of other transcription factors, coactivators, and chromatin-remodeling proteins generates a plethora of regulatory complexes with cell- and stimulus-specific transcriptional activities (for review, see ref. 35). Therefore, AP-1 is involved in a wide range of physiologic functions, including proliferation and survival, differentiation, growth, apoptosis, cell migration, transformation, and carcinogenesis. AP-1 itself is target of a variety of upstream kinases like c-Jun NH_2-terminal kinases (JNKs), extracellular regulated kinases (ERKs), and p38 mitogen-activated protein kinases (MAPKs) through phosphorylation of Jun and Fos proteins.

DNA binding of AP-1 is regulated by the redox state of a cysteine residue within the DNA binding domains of both proteins. Oxidation of Cys 154 in human Fos and Cys 269 in human c-Jun inhibits DNA binding of AP-1 *in vitro* (1). This was ascribed to an intermolecular disulfide bridge formed between the two cysteines. Oxidation of these residues could be achieved enzymatically, by using GPx, and DNA binding protected them from oxidation *in vitro* (16). In the viral homologue of *c-jun*, the transforming oncogene *v-jun*, the corresponding cysteine residue is replaced by a serine, which results in an insensitivity to oxidation, leading to constitutive DNA binding and aberrant transcriptional regulation. Similarly, Fos can escape regulation and is converted to a transforming protein when the critical cysteine is mutated (168). Shortly after uncovering the redox sensitivity of AP-1 DNA binding, a nuclear protein was identified that copurified with AP-1 and stimulated DNA binding of oxidized Fos-Jun heterodimers, Jun-Jun homodimers, and AP-1 complexes purified from HeLa cells (253, 254). This protein turned out to be APEX1. Besides a direct redox function, APEX1 can act as redox chaperone for AP-1, as discussed earlier.

B. Cyclic AMP response element–binding protein (CREB)

The transcription factor CREB binds the cAMP response element (CRE) and functions in glucose homeostasis, growth factor–dependent cell survival, and has been implicated in learning and memory. CREB is activated in response to, among other signals, cAMP. The accumulation of cAMP triggered by extracellular signals induces most cellular responses through protein kinase A (PKA). An increase in cellular cAMP levels liberates the catalytic from the regulatory subunits of PKA, which then translocate into the nucleus and induce cellular gene expression by phosphorylating CREB at serine residue 133. Together with the related activating transcription factor 1 (ATF1) and the CRE modulator (CREM), CREB comprises another family of bZIP transcription factors (for review of the CREB/ATF family, see refs. 145, 175, and 197). Originally, the Fos/Jun and ATF/CREB protein families were regarded as distinct sets of transcription factors that recognize closely related, but different DNA binding sites and form intrafamily dimers. However, it was shown that members of these two families can form selective cross-family heterodimers. These display DNA binding specificities distinguishable from each other and from their parental homodimers (21, 72). Analogously to AP-1, DNA binding of CREB is regulated by its oxidation status. The reduction of two cysteine residues (Cys 300 and Cys 310) located in the DNA binding domain, enhances the binding efficiency of CREB to DNA and regulates CRE-mediated gene expression. Substitution of these residues to serine renders CREB insensitive to reduction. These substitutions, which do not alter the secondary structure of the protein (189), enhance the binding of CREB to its cognate DNA sites under oxidative conditions and CREB dependent gene expression during normoxia (64). Interestingly, this redox regulation is bypassed by the Tax protein of the human T-cell leukemia virus type 1 (HTLV1), which recruits CREB independent of phosphorylation and redox status to the HTLV1 promoter (61, 64) to promote viral transcription.

C. Specificity protein 1 (Sp1)

Specificity protein 1 (Sp1) is one of the best-characterized zinc-finger transcription factors. It is a member of an extended family of DNA binding proteins, harboring three Cys_2-His_2 zinc-finger motifs, which bind to GC-rich DNA recognition elements. By regulating the expression of a large number of genes that have GC-rich promoters, Sp1-like transcription factors are involved in the regulation of many cellular processes, including proliferation, apoptosis, differentiation, and neoplastic transformation. Individual members of the Sp1 family can function as activators or repressors, depending on which promoter they bind and the co-regulators with which they interact (for review of the Sp1 family, see refs. 100 and 193).

A first hint that DNA binding by Sp1 is regulated by the cellular redox status came from the analysis of nuclear extracts from 30-month-old rat tissues, in which the DNA binding efficiency of Sp1 was greatly decreased, although the protein was present in levels comparable to those in younger tissues (6). This was attributed to increased levels of reactive oxygen intermediates in the aged animals because high concentrations of DTT, added to the aged tissue extracts, fully restored Sp1 DNA binding. Conversely, H_2O_2 treatment of extracts from young tissues strongly decreased the Sp1 DNA

binding activity, which again could be restored with DTT. The same results were obtained with purified Sp1, clearly demonstrating that oxidation directly affected Sp1 and not a cofactor (7). A similar phenomenon was observed during the transition of thymocytes from the resting to the proliferating state, in which production of ROS upon priming with phorbol 12-myristate 13-acetate (PMA) is nearly abolished. In the proliferating state, Sp1 DNA binding activity increased and could be compromised by the addition of H_2O_2 to extracts from these cells, whereas the binding activity in nuclear extracts from resting cells could be fully restored with DTT (199). This susceptibility to oxidation is conferred by thiol groups, depends on zinc coordination, and is prevented by DNA binding, suggesting that the DNA binding domain is the target for oxidation and is protected when in contact with DNA. This was corroborated *in vivo* by findings that arsenic treatment of promyelocytic leukemia cells prevented DNA binding of Sp1 to specific promoters. Of note, the nuclear levels of Sp1 did not change with arsenic treatment, suggesting that the oxidation occurs in the nucleus (36). In addition, Sp1 DNA binding was inversely correlated with the GSSG/GSH ratio added to nuclear extracts *in vitro* (110). Moreover, Trx-1 alone or in conjunction with the full thioredoxin system (Trx-1/TR and NADPH) was able to increase the DNA binding activity of recombinant Sp1 produced in *Escherichia coli* and of the protein from a mammalian cell line (27). Interestingly, not only DNA binding of Sp1 might be affected by oxidation, but also its transactivation properties; however, this phenomenon has not been investigated in detail (153).

D. Nuclear receptors

Nuclear receptors constitute to a large superfamily of ligand-activated transcription factors (for review, see ref. 147). These intracellular receptors are activated by lipophilic ligands and play crucial roles in development, differentiation, metabolic homeostasis, and reproduction. The prototypic glucocorticoid and estrogen receptor (ER) consist of an N-terminal transactivation domain (TAD), a central DNA binding domain (DBD) containing zinc-finger motifs, and a C-terminal ligand-binding (LBD) domain. In the cytosol, they are complexed with various chaperones of the heat-shock protein family. After steroid binding, glucocorticoid and estrogen receptors are released from these cytosolic complexes and translocate to the nucleus, where they interact as homodimers with their cognate DNA binding sites, the glucocorticoid response element (GRE), and estrogen response element (ERE), respectively, from where they activate transcription.

Early studies with biochemically purified glucocorticoid receptor showed that it changes its conformation under oxidizing conditions, when it seemingly can form intra- and intermolecular disulfide bonds. This conformational change was completely reversible with DTT, and only the reduced form of the receptor was capable of binding DNA (93, 212, 229), which was later confirmed in intact cells (52). A role for Trx-1 in this process was first suggested by experiments that showed that suppression of Trx-1 expression decreases glucocorticoid-inducible gene expression (134). Mammalian two-hybrid and pull-down assays finally demonstrated a direct interaction between the two proteins involving the DNA binding domain of the glucocorticoid receptor. Analysis of the subcellular localization demonstrated that this interaction most likely takes place in the nucleus under oxidative conditions (135). Besides DNA binding, the nuclear import of the glucocorticoid receptor also is under redox control, which affects a cysteine residue in its nuclear localization signal (167). Similar observations were made for the estrogen receptor, whose DNA binding activity is also sensitive to oxidation, which alters the conformation of the DNA binding domain (122). As for the glucocorticoid receptor, the transcription of endogenous and transfected synthetic ER target genes was shown to depend on Trx-1 when cells were placed under oxidative stress (78).

E. Nuclear factor-kappa B (NF-κB)

NF-κB is a collective name for inducible dimeric transcription factors composed of members of the Rel family of DNA binding proteins that recognize a common sequence motif, the κB site. NF-κB is found in essentially all cell types and is involved in activation of an exceptionally large number of genes in response to infections, inflammation, and other stressful situations requiring rapid reprogramming of gene expression. NF-κB was originally identified as a nuclear protein binding to the immunoglobulin kappa light-chain enhancer (208). Shortly thereafter, it was demonstrated by the same investigators that its DNA binding activity in pre-B cells can be induced by bacterial lipopolysaccharide (LPS) with a superinduction upon cycloheximide treatment (207), which then provided the first evidence that the activity of transcription factors can be regulated posttranslationally. Later it was shown that NF-κB represents a protein complex composed of hetero- or homodimeric combinations of five different members of the NF-κB/Rel family: NF-κB1 encoding p50 and p105, NF-κB2 encoding the p52 precursor p100, RelA or p65, RelB, and c-Rel. All subunits contain a conserved Rel-homology domain important for nuclear localization, dimerization, and DNA binding (for review see ref. 161). However, only the three Rel proteins contain transactivation domains required for the transcriptional activation of target genes. Therefore, and because each subunit has distinct biologic activities, different dimer combinations regulate specific sets of genes (for review, see ref. 157). This combinatorial mode of action results in diverse effects on cell fate and function.

In unstimulated cells, NF-κB is sequestered in an inactive form in the cytosol. It can be released from these cytosolic pools by two main pathways (for review, see ref. 201), resulting in nuclear translocation of NF-κB complexes. The canonic pathway, which is triggered by several proinflammatory cytokines, pathogen-associated molecules, and antigen receptors, depends on phosphorylation of the inhibitor of NF-κB (IκB) by an IκB kinase (IKK) complex consisting of the catalytic subunits IKKα, IKKβ, and a regulatory IKKγ subunit. Phosphorylated IκB is then subject to proteasomal degradation. This pathway leads primarily to the activation of p50/RelA and p50/c-Rel dimers. The noncanonic pathway engaged by various members of the tumor necrosis factor (TNF)-receptor family selectively requires IKKα activated by the upstream kinase NF-κB–inducing kinase (NIK). IKKα in turn phosphorylates p100, leading to proteasomal degradation of its C-terminus to generate p52, which then forms heterodimers predominantly with RelB. Besides these two major pathways, other mechanisms can release cytosolically

sequestered NF-κB components [*e.g.*, the phosphorylation of p105 by TPL-2, accelerating proteasome-mediated removal of the carboxy terminus yielding p50 (20)].

The observation that increased levels of the oxidoreductase Trx-1 are found in lymphocytes under conditions in which the transcription factor NF-κB is active [*e.g.*, after lymphocyte activation or in EBV- or HTLV-1–infected cells (238)], led to the suspicion that NF-κB might be under redox control. It could be shown that *in vitro* DNA binding activity of NF-κB is inhibited by agents modifying free sulfhydryls (141, 142, 230). Mutagenesis of conserved cysteine residues in the p50 subunit revealed that Cys 62 is critical for high-affinity binding to the κB motif. DNA binding activity of wild-type p50, but not a mutant, in which Cys 62 was exchanged to serine (C62S), was stimulated by Trx-1. Detection of disulfide cross-linked dimers in p50 wild type but not C62S suggested that Trx-1 stimulates DNA binding by reduction of a disulfide bond involving Cys 62 of the NF-κB subunit p50. Cotransfection of a plasmid expressing human Trx-1 and an NF-κB–dependent reporter construct demonstrated that Trx-1 also can regulate DNA binding and transcriptional activation by NF-κB in living cells (142). Interestingly, depending on its subcellular localization, Trx-1 can have opposing effects on NF-κB. In the cytosol, it interferes with signals to IKKs and thereby blocks the degradation of IκB, resulting in cytosolic retention of NF-κB, which prevents the activation of target genes. In contrast, nuclear Trx-1 has a positive effect on NF-κB transcriptional activity by enhancing its ability to bind to DNA (81). Molecular-modeling studies finally provided a clue to this dual role of Trx-1. Oxidized, disulfide-bridged NF-κB is more compact than the reduced form, which might facilitate its nuclear translocation. However, the inter-subunit disulfide blocks DNA from entering the active site of the oxidized dimer, explaining why reduction in the nucleus is essential for DNA binding and transcriptional activation to occur (32). Similar to Trx-1, Prx1 has the same dual role in the regulation of NF-κB activity (75). Besides disulfide cross-linked dimer formation, another oxidation product of NF-κB subunits has been detected. Both p50 and p65 can be glutathionylated, which leads to reduced NF-κB DNA binding and transactivation (181, 182). In the case of p65, glutathionylation was detected under hypoxic conditions, when the intracellular GSH levels were increased. The modification of p65 required Grx1, and the authors speculated that p65-SSG formation takes place in the cytosol and that modified p65 is still transported to the nucleus (182). However, as Grx1 can be detected in the nucleus, it also is possible that glutathionylation of this transcription factor subunit takes place there.

In addition to cysteine oxidation, the modification of specific tyrosine residues in p65 has been reported. Peroxynitrite inhibited NF-κB activity through nitration of p65 at Tyr 66 and Tyr 152, leading to p65 destabilization and nuclear export (176).

An interesting observation was made concerning the role of NF-κB redox regulation in the action of thalidomide, which causes severe malformations, especially of the extremities, in children when taken by the mothers during pregnancy. Thalidomide increases the production of free radicals and elicits oxidative stress. Oxidative stress, as marked by GSH depletion, occurs preferentially in limbs of thalidomide-sensitive rabbits. Activation of an NF-κB–dependent reporter gene is attenuated in limb bud cells of treated rabbits and can be restored by addition of *N*-acetylcysteine and a free radical spin-trapping agent (74). NF-κB is a key factor in limb development, because it regulates expression of *twist* and fibroblast growth factor 10 (*fgf10*), two genes necessary for proper limb outgrowth. Therefore, its oxidative inactivation triggered by thalidomide might explain the malformations occurring in children.

F. p53

The protein p53 is one of the best-characterized tumor suppressors and is constitutively expressed in nearly all cells and tissues. It functions as a tetrameric transcription factor found at very low levels in normal cells. Several genome-wide surveys have been undertaken to map genomic binding sites of p53 and thus to identify its target genes (83, 104, 213), yielding a plethora of genes potentially regulated by p53. Various kinds of cellular stress that alter normal cell cycle progression or induce mutations in the genome lead to stabilization and thus accumulation of the protein. Depending on the cell type and tissue and the extent of damage, p53 now either leads to cell cycle arrest to repair the lesions or forces the cell into apoptosis. Therefore, p53 has been termed "guardian of the genome" (117). Somatic mutations in the *p53* gene are critical events in a wide variety of malignancies, and *p53* is the gene most frequently mutated in human cancers. The majority of the mutations are missense mutations, and a hotspot is the region of the gene coding for the DNA binding domain (for review of *p53* and its mutations, see refs. 28, 84, 217, and 239).

Because of its prominent role in human cancers, the regulation of *p53* has been at the focus of intensive studies. With respect to redox regulation, it has to be noted that all 10 cysteine residues in p53 are within its DNA binding domain. This prompted very early studies on redox regulation. It was shown that oxidation of *in vitro* translated and recombinant, baculovirus-produced p53 disrupted its DNA binding, which was attributed to a change in conformation that could be reversed by reduction (44, 73). Interestingly, the redox state of p53 seemingly regulates only sequence-specific DNA binding and activation of p53-dependent genes. In contrast, no difference is found in the binding of oxidized p53 and reduced p53 to double-stranded nonspecific DNA (177).

That APEX1 can modulate the redox-dependent properties of p53 was demonstrated by the stimulation of sequence-specific DNA binding of oxidized p53 *in vitro* (97). The expression of reporter genes driven by p53-responsive promoters and endogenous p53 target genes, like *p21* and cyclin G, was equally stimulated on overexpression of APEX1 (57, 97). Importantly, downregulation of APEX1 caused a marked reduction in p53-dependent induction of p21 and diminished the transcriptional activation of *p21* and *Bax* by p53. In addition, the same authors demonstrated a physical interaction between APEX1 and p53, although only a small portion of both proteins was present in the same complex (57). Interestingly, APEX1 modulates p53 DNA binding, not only as a redox regulator, but also to facilitate formation of p53 tetramers, the most active form in terms of DNA binding (245), independent of its redox activity (77). This is substantiated by the fact that the cysteine residues, which are exclusively found in the DNA binding domain of p53 and are the targets for oxidation/reduction, have no influence on tetramerization

(185). Collectively, these data suggest that APEX1 stimulates p53 by both redox-dependent and -independent means and imply a key role for it in p53 regulation.

G. Nuclear factor I/CAAT transcription factor (NFI/CTF)

NFI/CTF was originally described as being required for the replication of adenovirus DNA (for review, see ref. 42). Later it was shown that NFI can regulate the transcription of a large number of cellular and viral genes. NFI represents a family of four genes in vertebrates (*NFI-A*, *NFI-B*, *NFI-C*, and *NFI-X*), which are expressed in overlapping patterns. The transcripts of all four genes can be spliced differentially, yielding distinct proteins. NFI proteins have been associated with changes in the growth state of cells and a number of malignancies (for review of NFI proteins, see ref. 65).

As for other transcription factors, it has been shown for NFI that its DNA binding activity is redox sensitive (164). A single cysteine residue (Cys 3) in the DNA binding domain of the NFI-family proteins, which is conserved from *Caenorrhabditis elegans* to humans is the target for this regulation, as was shown by site-directed mutagenesis (13). Oxidized, inactive NFI can be reduced to a DNA binding form by Grx1 *in vitro*. This requires the GSH/GR system to regenerate reduced Grx1. The *in vivo* relevance has been shown by treatment of HeLa cells with buthionine sulfoximine, an agent that inhibits GSH synthesis. This GSH depletion potentiated the inactivation of NFI by the oxidizing agent diamide. Similarly, a stronger restoration of NFI activity after oxidation with diamide was observed, when the cells were treated with *N*-acetylcysteine, an agent that can replenish intracellular GSH (14).

In the case of NFI, not only DNA binding is subject to redox control. By using fusions with a heterologous DNA binding domain from the Gal4 protein, it was demonstrated that transcriptional activation by NFI also is regulated through oxidation of a cysteine residue (Cys 427) in the transcription-activation domain (TAD) (153) (Fig. 7). These findings were corroborated in living cells subjected to various stress conditions that induce cellular ROS formation, including inflammatory cytokine treatment, GSH depletion, heat and osmotic shocks, and chemical stress. In all cases, suppression of a reporter gene was specific for the NFI TAD, as no effects were observed with the transactivation domains of activator protein 2 (AP-2) and octamer transcription factor 2 (Oct-2). A common target for all these stressors was again Cys 427, leading to the suggestion that the NFI TAD might be a negative sensor of cellular stress (154). Based on the concentrations of exogenously applied H_2O_2 required to block transcriptional activation and DNA binding by NFI, it was calculated that a 100-fold difference in sensitivity to oxidation exists between the DNA binding domain of NFI and its TAD (153). During this investigation, TADs from other transcription factors (Sp1 and Oct-2) were analyzed to show that redox regulation of TADs is not a general effect. Whereas the Oct-2 TAD was not sensitive to oxidative stress, the analogous domain of Sp1 showed some responsiveness, indicating that other transcription factors could be subject to similar control mechanisms, which might have been overlooked during the characterization of the redox dependency of their DNA binding capacity.

It is not clear whether the thiol moiety of Cys 427 of NFI undergoes an oxidation with a gain of oxygen atoms or if it forms an intra- or intermolecular disulfide bridge, although mutations of a cysteine in the neighborhood (Cys 405) indicate that the formation of an intramolecular disulfide bridge within the TAD is not the mechanism most likely to trigger the effect. The oxidation of Cys 427 could affect the conformation of the TAD, which is the interface for interactions with the TATA-box–binding protein TBP, the coactivator CBP/p300, and histones H1 and H3.

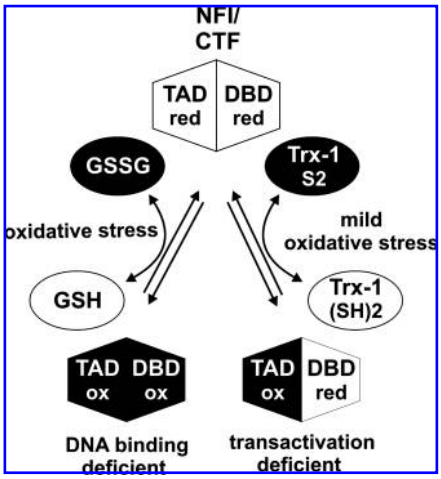

FIG. 7. Redox regulation of NFI/CTF: dual regulation of NFI/CTF transcriptional activity. Nuclear factor I/CAAT transcription factor (NFI/CTF) must be reduced to activate its target genes. Oxidative stress leads to oxidation of its transactivation domain (TAD) and DNA binding domain (DBD), which prevents DNA binding. Much lower concentrations of ROS only oxidize the TAD in a thioredoxin-1 (Trx-1)-dependent manner. This partially oxidized molecule can still bind to DNA, but is incapable of activating transcription.

H. Hypoxia-inducible factor 1 (HIF-1)

HIF-1 is the most prominent regulator of genes induced by hypoxia. It is a transcription factor that binds to the hypoxia-responsive element (HRE) in the promoters and enhancers of various hypoxia-inducible genes. HIF-1 is a heterodimer composed of HIF-1α and HIF-1β, which is identical to the aryl hydrocarbon-receptor nuclear translocator (ARNT). Both proteins contain a basic helix–loop–helix (bHLH) and a PAS domain at their N-terminus, the latter being an acronym for Per, ARNT, and Sim, the first three members of the protein family characterized. Whereas the basic domain is essential for DNA binding, the HLH domain and the N-terminal half of the PAS domain are required for heterodimerization and DNA binding. HIF-1β contains a single C-terminal transactivation domain, whereas in HIF-1α, two such domains are found, termed NAD and CAD, according to their location closer to the N- or C-terminus. The NAD is embedded in a region controlling protein stability, the oxygen-dependent degradation domain (ODD). Both subunits of HIF-1 are expressed constitutively, but the α-subunit has an extremely short half-life (<5 min) under normoxic conditions, because of continuous proteolysis by the ubiquitin-proteasome pathway targeting the ODD. As both subunits are required for the activation of HIF-1 target

genes, these are not or only weakly expressed at normal oxygen tension. During hypoxia, HIF-1α is instantaneously stabilized, resulting in a functional heterodimer and expression of hypoxia-inducible genes. HIF-1α is hydroxylated at proline 564 (Pro 564) in the ODD in an oxygen-dependent fashion. This hydroxylated HIF-1α is bound by the von Hippel–Lindau (VHL) protein, which acts as an E3-ubiquitin ligase, tagging HIF-1α for proteasomal degradation. The critical molecules for the rapid turnover of HIF-1α under normoxia are members of a family of prolyl-4-hydroxylases (PHs). These enzymes require oxygen and 2-oxoglutarate as cosubstrates and contain iron liganded by two histidine and one aspartic acid residues. Oxygen binding requires the vitamin C–dependent maintenance of iron in its ferrous state. When oxygen is low, Pro 564 in HIF-1α remains unmodified, resulting in stabilization of the protein, which is no longer bound by VHL. Similarly, iron chelation or replacement by transition metal ions (Co^{2+}, Ni^{2+}, Mn^{2+}) can mimic hypoxia-inducible stabilization, explaining the apparent "upregulation" of HIF-1α by these metals (for review of HIF-1, see refs. 205, 206, 246, and 247).

In addition to HIF-1α, two other family members are known, HIF-2α/HLF (HIF-1α–like factor) and HIF-3α. They show a more-restricted expression pattern, but contain domains similar to HIF-1α and have comparable biochemical properties.

In 1996, it was shown that pretreatment of cells with hydrogen peroxide has an inhibitory effect on transcriptional activation by HIF-1, which suggested an additional redox control mechanism. Sulfhydryl alkylation or oxidation, the latter being reversible, indicated an involvement of cysteine residues in this process. These findings were supported by the fact that purified, oxidized Trx-1 in combination with DTT could stimulate HIF-1 DNA binding in hypoxic extracts. In addition, overexpression of Trx-1 or APEX1 enhanced the hypoxic induction of a HIF-1–dependent reporter gene (90). Interestingly, DNA binding of HLF-, but not HIF-1α–containing complexes was redox dependent. This is explained by the fact that the critical cysteine residue in the basic domain of HLF (Cys 28), which seems to be a target for APEX1, is replaced by a serine residue at the corresponding position (Ser 25) in HIF-1α. However, APEX1 also is important for transcriptional activation by HIF-1α (116). The last finding is in agreement with a previous report that the CADs of HIF-1α and HLF interact with the transcriptional coactivator CBP/p300 and that APEX1 and Trx-1 further enhanced the activity of a luciferase reporter activated by a fusion protein between the Gal4 DNA binding domain and CAD under hypoxic conditions. This potentiation required the catalytic activity of Trx-1, suggesting that a redox reaction is involved. Interestingly, a single cysteine residue is conserved in the 49-amino-acid CAD between HLF (Cys 844) and HIF-1α (Cys 800). Mutation of this cysteine abolished the hypoxia-inducible transcriptional activation by Gal4-CAD and interaction with CBP. The importance of this cysteine residue was confirmed by mutation in the context of full-length HIF-1α and HLF, which markedly reduced the transcription-enhancing activity (51).

I. Nuclear factor erythroid 2–related factor 2/ NF-E2 related factor 2 (Nrf-2)

The NF-E2–related factor 2 (Nrf-2), not to be confused with the GA-binding protein nuclear respiratory factor 2, which is also called Nrf-2, is a transcription factor implicated in the cellular responses to oxidative stress and to chemical compounds that are metabolically transformed to reactive or electrophilic intermediates. *Nrf-2*–deficient mice show a lower expression of xenobiotic enzymes and are predisposed to tumors induced by carcinogens (186). Nrf-2 is a member of the so-called cap 'n' collar basic region leucine zipper (CNC-bZIP) family of transcription factors, the bZIP region of which is distinct from other bZIP families, such as the Jun/Fos family. Nrf-2 heterodimerizes with other bZIP transcription factors, including the small Maf (sMaf) proteins. These heterodimers bind to antioxidant-response elements (AREs) and thereby upregulate numerous genes coding for detoxification enzymes, antioxidants, and the enzymes required for *de novo* GSH synthesis (148). However, Nrf-2 controls not only inducible, but also low-level gene expression under nonstressed conditions, suggesting that its activity is tightly controlled. It has been shown that Nrf-2 is tethered by the Kelch-like ECH-associated protein 1 (Keap1), and in this complex, is not available as a transcriptional activator. Keap1 serves not only simply to sequester Nrf-2, but also functions as an adaptor for a Cul3-dependent E3 ubiquitin ligase modifying Nrf-2 for proteasomal degradation, which explains the short half-life of Nrf-2 (~15 min). It is still a matter of debate whether Keap1 transiently enters the nucleus and targets Nrf-2 for ubiquitinylation there or is capable of engaging in a nucleocytosolic shuttling of Nrf-2 dependent on CRM-1 (235). Nevertheless, the generally accepted scenario involves a cytosolic retention and degradation of Nrf-2 by Keap1.

Keap1 contains an N-terminal BTB/POZ domain (for broad-complex, Tramtrack and Bric-a-brac; also known as a Poxvirus and zinc finger domain) potentially serving as an interface for homomeric or heteromeric interactions. The C-terminus comprises six Kelch repeats, each of which forms a four-stranded β-sheet resulting in a propeller-like structure binding Nrf-2. Keap1 contains 25 cysteine residues, the most reactive of which are found in the intervening region between the BTB/POZ and Kelch repeat domains (47). Inducers of ARE-dependent genes disrupt the Keap1/Nrf-2 interactions by modifying two of these residues (Cys 273 and Cys 288). Transfection of *Keap*1- and *Nrf*-2–deficient mouse embryonic fibroblasts with constructs expressing cysteine-to-alanine mutants of these two amino acids in Keap1 demonstrated that release of Nrf-2 is the consequence of the formation of an intermolecular, disulfide-linked Keap1 dimer. In this dimer, the disulfide bridges most likely are formed crosswise between Cys 273 and Cys 288 (237). After release from Keap1, Nrf-2 escapes degradation and can bind to AREs in a heteromeric complex with an sMaf to activate gene expression. Several kinases have been shown to phosphorylate Nrf-2, but the molecular consequences of these phosphorylation events have not been elaborated (for review of Nrf-2 and its regulation by Keap1, see refs. 107, 158, and 159).

Besides the cytosolic retention of Nrf-2 by Keap1 and its release upon formation of an intermolecular Keap1 dimer, a second layer of Nrf-2 activity regulation exists. Like other transcription factors, Nrf-2 must be in a reduced state for efficient DNA binding. The critical residue is Cys 506, whose oxidation reduced its affinity for the ARE, leading to decreased expression and antioxidant induction of NAD(P)H/quinine oxidoreductase 1 (*NQO1*). However, mutation of this residue to serine did not affect the retention of Nrf-2 by Keap1

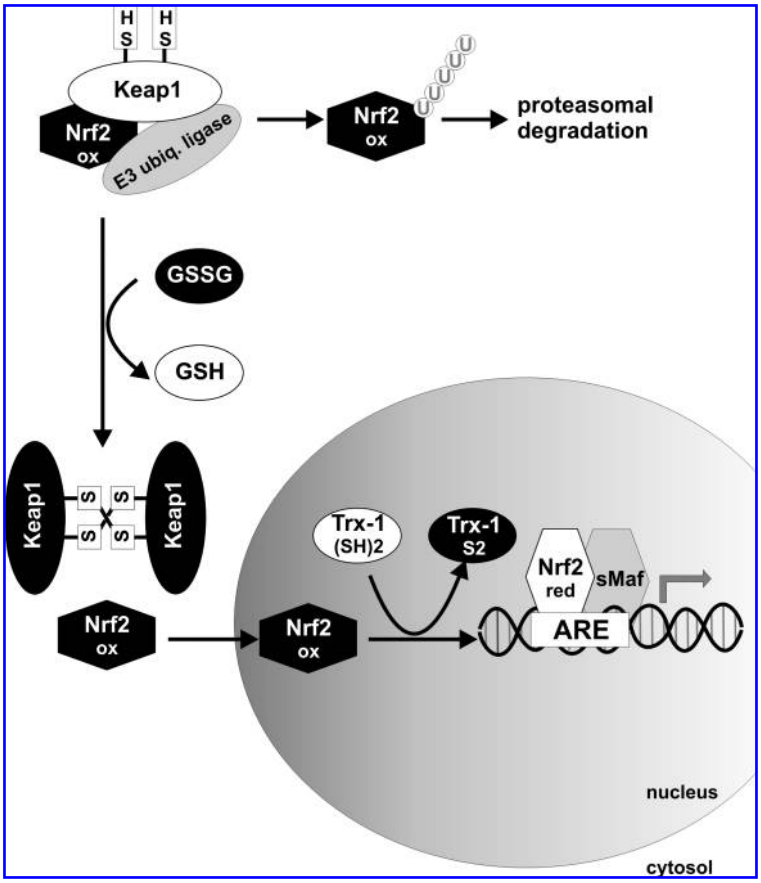

FIG. 8. Activation of Nrf-2: general model of gene induction by the Keap1/Nrf-2 pathway. Nuclear factor erythroid 2–related factor 2/NF-E2–related factor 2 (Nrf-2) is sequestered in the cytosol by Kelch-like ECH associated protein 1 (Keap1). In addition to binding Nrf-2, Keap1 functions as an adaptor for an E3 ubiquitin ligase, which ubiquitinates Nrf-2, thereby tagging it for proteasomal degradation. After induction, Keap1 is oxidized to an intermolecular, disulfide-linked dimer involving reciprocal cysteine residues of both monomers. This oxidation requires GSSG. Released Nrf-2 translocates to the nucleus, where it is reduced by thioredoxin-1 (Trx-1). Nrf-2 must be in a reduced state for efficient DNA binding as a heterodimer with a small Maf protein (sMaf). These heterodimers induce transcription of genes, whose promoters contain antioxidant responsive elements (AREs).

in the cytosol or its release in response to antioxidants (26). It has been shown that this two-layered, compartmentalized regulation of Nrf-2–dependent gene expression involves two cellular redox systems. Whereas cytosolic retention is controlled by GSH/GSSG ratios, the Nrf-2/DNA interactions depend on nuclear Trx-1 (76) (Fig. 8).

J. Homeobox B5 (HoxB5)

To our knowledge, only one case exists in which oxidation of a cysteine residue in a transcription factor can enhance DNA binding, homeobox B5 (HoxB5). It is a homeodomain (HD) protein of the antennapedia family and functions as a sequence-specific transcription factor that is involved in lung and gut development. In the cardiovascular system, HoxB5 is an upstream transcriptional switch for differentiation of the vascular endothelium from precursor cells (252). It was shown *in vitro* that the cooperative stabilization of HoxB5 DNA binding, but not sequence-specific DNA binding, is under redox regulation. Cooperative binding and redox regulation were found to require the presence of a cysteine residue (Cys 232) in the turn between homeodomain helices 2 and 3 and that oxidation of this cysteine is necessary for cooperative binding of the protein to tandem binding sites. This was shown by DTT treatment of purified, recombinant HoxB5, which resulted in loss of cooperativity (58). Multiple clustered HD protein-binding sites are found in the promoters of *Drosophila* and vertebrate genes whose expression is regulated by antennapedia-type HD proteins. Thus, cooperative interactions could have a large influence on the DNA binding of HD proteins to these sites.

K. Other redox-regulated transcription factors

Besides the transcription factors discussed in detail, DNA binding of several others is inhibited by oxidation: CBP/PEBP2 (5, 101), c-Myb (67, 155), Egr-1 (91), MyoD (218), NF-Y (156), Pax-5 (226, 227), Pax-8 (103, 224), TTF-1 (11, 103, 225), and bovine papilloma virus E2 (BPV E2) (146). In some of these cases, oxidation sensitivity has been mapped to the DNA binding domain; in others, it has been shown that Trx-1 or APEX1 can restore the DNA binding capacity after oxidation.

L. Histone deacetylase 2 (HDAC2)

Interestingly, not only transcription factors as *bona fide* DNA binding proteins are affected by modification of cysteine residues. Recently, it was shown that the chromatin modifier histone deacetylase 2 (HDAC 2) becomes nitrosylated after treatment of rat cortical neurons with neurotrophins. This modification occurs on two cysteine residues (Cys 262 and Cys 274) and is dependent on neuronal nitric oxide synthase (nNOS), as shown in neurons from nNOS-deficient mice and nonneuronal cells expressing nNOS. Intriguingly, nitrosylation of the two cysteines did not change the enzymatic activity of HDAC 2 but rather induced its release from chromatin. This dissociation of HDAC 2 leads to acetylation of histones H3 and H4, activation of BDNF target genes, and dendritic

Table 2. Nuclear Kinases and Phosphatases

	Critical amino acid	*Molecular consequence*	*Cellular consequence*	*Compartment of modification*	*Ref.*
Nuclear kinase					
PKCδ	Tyr 512	Kinase activation	Apoptosis	Cytosol	46, 222
PKA	n.d	Kinase activation	Cell survival	Nucleus	17
JNKs	n.d	Kinase activation	Apoptosis	Nucleus	24
Akt	Ser 473	Kinase activation	Apoptosis inhibition, delayed	Nucleus	68, 70
ERK2	Thr 183, Tyr 185	Kinase activation	*De novo* GSH synthesis	Nucleus	108, 263
Src	Tyr 416	Kinase activation	Senescence, apoptosis	Cytosol	68, 69
	Cys 277	Kinase inactivation	n.d.	Cytosol	106
Yes	Tyr 426	Kinase activation	Senescence, apoptosis	Cytosol	68, 69, 96
	n.d. (Cys)	Kinase inactivation	n.d.	Cytosol	106
Nuclear phosphatase					
Shp-2	Cys 459	Reduced phosphatase activity	ROS induction, apoptosis	n.d.	37, 96
	Cys 331, Cys 367	Backdoor cysteines, protection of Cys 459	n.d.	n.d.	33
TC-PTP	n.d.	Dephosphorylation of transcription factors	n.d.	Nucleus	228, 257
Cdc25C	Cys 330	Reduced phosphatase activity	Cell cycle progression	Nucleus	179, 198
	Cys 377	Backdoor cysteine, protection of Cys 330	Cell cycle progression		

growth (163). This mechanistic study could explain why HDAC 2 can negatively regulate memory formation and synaptic plasticity (66).

IV. Redox-Regulated Nuclear Kinases and Phosphatases

A. Nuclear-localized kinases

Protein kinases are involved in many different cellular signaling pathways. Therefore, a strict regulation of these kinases is necessary for the survival of the cell. The most important regulatory mechanism is their activation by phosphorylation (for review, see refs. 98 and 99). Kinases are phosphorylated under physiologic and pathophysiologic conditions. Recently a direct oxidation of Src and the fibroblast growth factor type 1 was demonstrated, leading to their inactivation. This mechanism seemingly only works for kinases containing a cysteine in the Gly loop capable of forming disulfide homodimers and therefore applies only to a small number of human protein tyrosine kinases (106).

In the context of this review, we restrict the detailed discussion to kinases, for which a nuclear localization has been demonstrated, even if the redox regulation takes place outside the nucleus. Nuclear localization has been shown for protein kinase C δ (PKCδ), PKA, JNKs, Akt, ERKs, and some members of the Src kinase family. The following paragraphs discuss the kinases mentioned earlier in more detail; a brief overview is given in Table 2.

1. Protein kinase Cδ (PKCδ). The protein kinase C (PKC) family of serine/threonine kinases is involved in many cellular signaling pathways such as growth, proliferation, and cell death (for review, see ref. 259). This family consists of three groups: (a) the conventional PKCs (cPKCs: α, βI, βII γ); (b) the novel PKCs (nPKCs: $\delta, \varepsilon, \theta, \mu$); and (c) the atypical PKCs (aPKCs: ζ, λ). PKCδ belongs to the novel PKCs and is redox regulated in several cell types. One well-described role for PKCδ is in mitochondria-dependent apoptosis induction. Overexpression of PKCδ in keratinocytes leads to translocation of PKCδ to mitochondria, alterations in mitochondrial functions, and induction of cell death (121). However, other studies also show translocation of PKCδ into the nucleus in various cell types. In response to cellular stresses, like oxidative stress, PKCδ is activated by tyrosine phosphorylation, and nuclear translocation occurs. PKCδ possesses its own nuclear localization sequence (NLS) (46) and has several tyrosine phosphorylation sites, which regulate its kinase activity. One of these sites, tyrosine 512, is phosphorylated by the proapoptotic tyrosine kinase c-Abl in response to oxidative stress. Phosphorylation results in activation of PKCδ followed by its nuclear translocation (222, 260). After nuclear translocation, activated PKCδ initiates a sequence of events that activates caspase 3, which in turn cleaves PKCδ (25, 46). This cleavage results in a 40 kDa catalytically active fragment and a 38 kDa regulatory fragment of PKCδ. The catalytically active fragment induces apoptosis by phosphorylation of the apoptosis-related protein DNA-dependent protein kinase (DNA-PK) (22, 60). DNA-PK is essential for repair of DNA double-strand breaks (214). Phosphorylation by PKCδ and also its catalytically active fragment induces the dissociation of DNA-PK from DNA, resulting in an inhibition of DNA repair and enhanced DNA fragmentation (22) DeVries *et al.* (46) showed that caspase 3–dependent cleavage of PKCδ increases the rate of nuclear translocation of the 40 kDa PKCδ cleavage fragment, which results in an amplification of the apoptotic signal (46) (Fig. 9).

PKCδ also interacts with and activates IKKα in response to oxidative stress. Active IKKα translocates into the nucleus

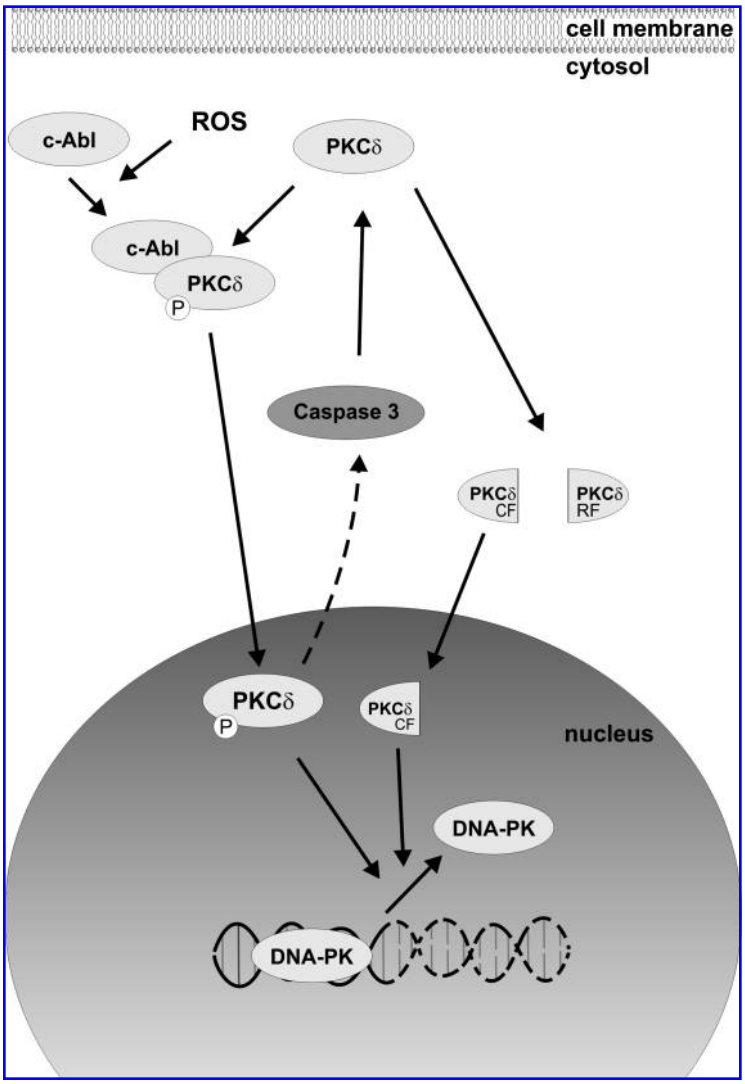

FIG. 9. Role of nuclear PKC*δ*. ROS induce association of protein kinase C *δ* (PKC*δ*) and c-Abl. This leads to phosphorylation, activation, and nuclear import of PKC*δ*. In a direct or indirect way, phosphorylated PKC*δ* activates caspase 3, which in turn leads to cleavage of PKC*δ* into a catalytically active (PKC*δ* CF) and a regulatory fragment (PKC*δ* RF). PKC*δ* CF translocates into the nucleus and, like PKC*δ*, induces the dissociation of DNA protein kinase (DNA-PK) from the DNA, which leads to fragmentation of DNA.

and regulates the transcriptional activity of the tumor-suppressor p53 by phosphorylation at serine 20 (256). This is a recently described mechanism for ROS-induced p53 activation.

2. Protein kinase A (PKA). Cyclic AMP formed by adenylyl cyclases binds the regulatory subunits (R) of the tetrameric PKA holoenzyme and promotes dissociation of the catalytic subunits (C-PKA). A fraction of C-PKA translocates to the nucleus and stimulates cAMP-dependent gene expression in different cell types (174). The best-characterized target of nuclear C-PKA is CREB, which is phosphorylated at serine 133 by C-PKA (71). H_2O_2 has been shown to initiate an increase in CREB phosphorylation at serine 133 in a non-transformed murine alveolar type II epithelial cell line (18), leading to transcriptional activation (145). The increased phosphorylation of CREB was due to an H_2O_2-induced increase in nuclear accumulation of C-PKA (17). This was confirmed by pharmacologic inhibition of PKA with H89, which reduced H_2O_2-mediated phosphorylation of CREB. Because the downregulation of CREB by siRNA increased the sensitivity of cells to H_2O_2-induced apoptosis and reduced transcription of the antiapoptotic gene B-cell lymphoma protein 2 (*Bcl*-2), CREB seems to play a role in cell survival in response to oxidative stress (17).

3. c-Jun NH_2-terminal kinases (JNKs). JNKs are involved in the regulation of cell proliferation and apoptosis. The activation of these pathways is dependent on the actual stress stimulus and cell type (125, 128). Sustained activation of JNKs leads to apoptosis, whereas the acute and transient activation induces survival pathways and cell proliferation (196). The sustained stress-induced activation of JNKs by phosphorylation through upstream MAP-kinases, ASK1, and MAP kinase kinase (MKK) 4/7 leads to an induction of apoptosis (for review, see ref. 127). JNKs translocate into the nucleus and phosphorylate c-Jun and activating transcription factor 2 (ATF2) (for review, see ref. 41), leading to the formation of an AP-1 complex and to the transcription of genes coding for proapoptotic proteins [*e.g.*, tumor necrosis factor *α* (TNF-*α*), Fas-L, and Bak] (53). However, JNKs also regulate physiologic and homeostatic processes. One attractive explanation for these differences is the existence of three isoforms of JNK and the subcellular pools of the JNKs. Only combined siRNA knockdown of all JNKs 1, 2, and 3 provides substantial protection from cell death. In contrast, knockdown or knockout

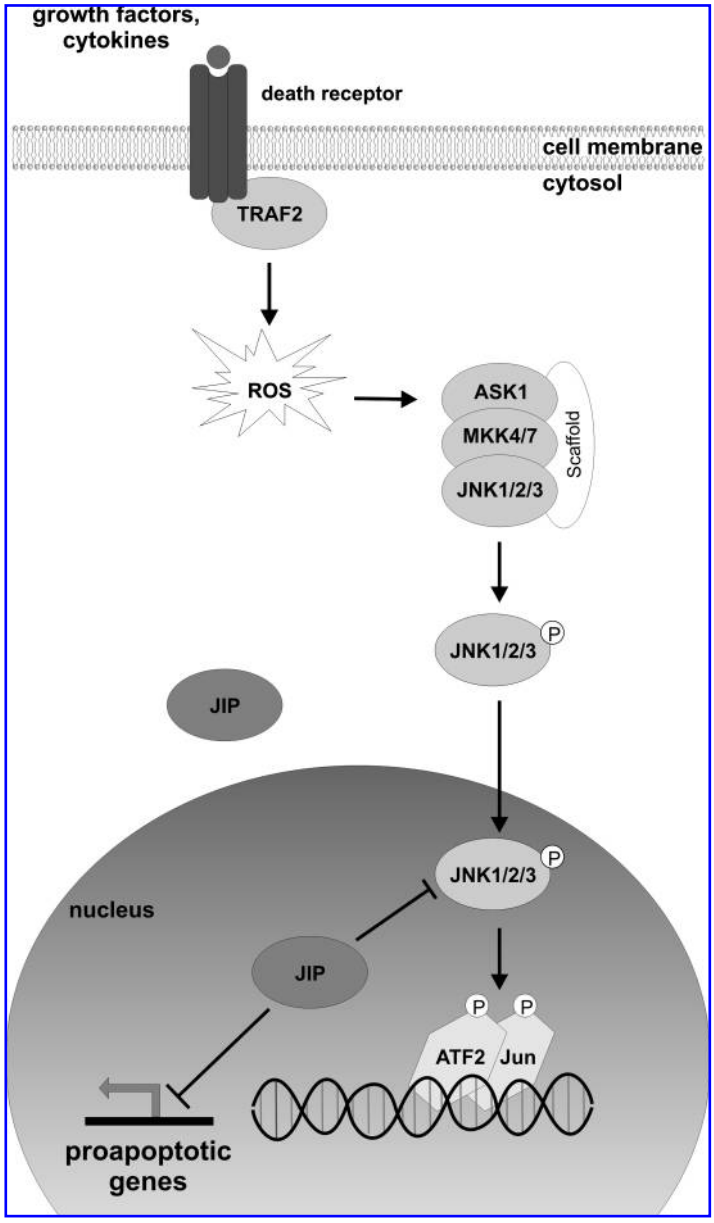

FIG. 10. Nuclear targeting and signaling of JNKs in the regulation of apoptosis. Growth factors and cytokines elevate ROS levels mediated by death receptor–coupled Traf2. This induces the activation of c-Jun NH_2-terminal kinases (JNKs) through upstream kinases apoptosis-signaling kinase 1 (ASK1) and MAP kinase kinase (MKK) 4/7. Activated JNK1, 2, and 3 translocate into the nucleus and phosphorylate c-Jun and activating transcription factor 2 (ATF2), which leads to formation of an activator protein 1 (AP-1) complex and to transcription of proapoptotic genes. Inhibition of nuclear active JNK1, 2, and 3 by JNK inhibitor protein (JIP) results in complete apoptosis inhibition.

of individual JNKs or two JNKs together does not protect. Moreover, to determine whether cytosolic or nuclear JNKs are responsible for JNK-dependent cell death, compartment-specific inhibitors for JNKs were generated. Therefore, a nuclear-exclusion sequence (NES) from MKK1 or three NLSs from SV40 large-T antigen were fused in tandem upstream of the JNK inhibitor protein JIP. These targeted proteins localized to cytosolic and nuclear compartments, respectively (23). Overexpression of these compartment-specific JNK inhibitors revealed that cell death requires nuclear but not cytosolic JNK activity, as the nuclear dominant-negative inhibitor of JNK protected against apoptosis, whereas the cytosolic form only blocked physiologic JNK function (24). Thus, any one of the three JNKs is capable of mediating apoptosis, and this is dependent on their nuclear localization (Fig. 10).

4. Protein kinase B (Akt). The activity of the serine/threonine kinase Akt is redox regulated by Grx. By keeping Akt in its reduced form, Grx enables the activation and stimulation of the endothelial NO-synthase/NO signaling pathway (241). However, it is unclear whether this has to be attributed only to cytosolic Akt or also to the nuclear Akt.

We previously reported that nuclear Akt is required for the maintenance of telomerase activity and inhibition of apoptosis in human umbilical vein endothelial cells (70). Mechanistically, after the first assembly of the telomerase holoenzyme, which requires Hsp90 and p23 (89), Akt is recruited into this complex in the nucleus. Upon its recruitment, Akt phosphorylates TERT at serine 823, which increases nuclear telomerase activity (29, 70). Furthermore, the complex formation of TERT with HSP90 and Akt protects Akt from being dephosphorylated by the protein phosphatase 2A and thereby keeps Akt and TERT in their active forms (70, 105). Independent of its telomere-elongation function, nuclear TERT also has antiapoptotic effects. Inhibition of telomerase activity or overexpression of nuclear TERT induces or inhibits

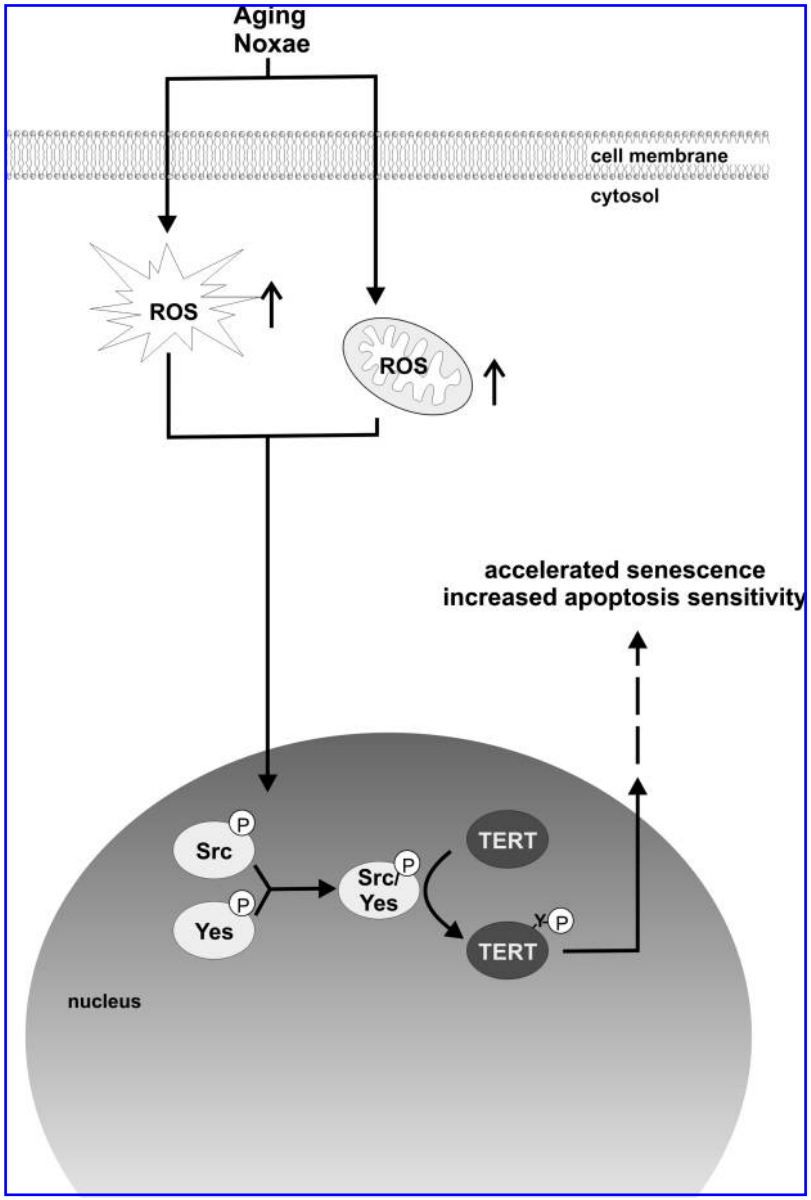

FIG. 11. Nuclear Src and Yes induce TERT export under conditions of oxidative stress. Cytosolic and mitochondrial ROS induce increased activity of nuclear Src and Yes. This leads to tyrosine phosphorylation of telomerase reverse transcriptase (TERT) and its nuclear export. The functional consequences are increased apoptosis sensitivity and accelerated senescence.

apoptosis, respectively (68, 166, 192, 262). Thus, maintaining TERT in its active state in the nucleus by Akt protects cells against apoptosis.

It has been demonstrated that, upon its activation, myocardial Akt accumulates in the nucleus (30). Therefore, important studies investigated the specific function of nuclear Akt in cardiomyocytes by using an adenovirus expressing Akt with nuclear localization signals. Nuclear-targeted Akt inhibited cardiomyocyte hypertrophy and apoptosis (211, 232). Moreover, transgenic mice with cardiac-specific expression of nuclear-targeted Akt were protected from ischemia/reperfusion injury (211). Given the fact that constitutive active Akt causes hypertrophy in cardiomyocytes, specifically, nuclear-targeted Akt provides a new opportunity for therapeutic applications.

5. Extracellular regulated kinases 1/2 (ERK1/2). ERK1/2s are a subfamily of the MAP kinases and are involved in many important cellular processes like cell proliferation, survival, apoptosis, and metabolism (165). Their stimulation is triggered by growth factors and different environmental and oxidative stresses. The cytokine epidermal growth factor (EGF) is one of the activators of ERK1/2 through phosphorylation of the EGF receptor (EGFR) (31), whereas H_2O_2 activates ERK1/2 by EGFR-dependent and -independent pathways (63, 243). The phosphorylation state of ERK2 is more important for its nuclear retention than is the activity of ERK2. This was confirmed with catalytically inactive ERK2, in which lysine 52 was mutated to arginine [ERK2(K52R)]. Like the wild-type protein, microinjected ERK2(K52R) transiently translocated to the nucleus after stimulation. To reduce the susceptibility to dephosphorylation, the two activating phosphorylation sites were thiophosphorylated *in vitro*. The stably phosphorylated wt and K52R mutant directly moved into and remained in the nucleus, demonstrating that ERK2 activity is not necessary for nuclear accumulation (108).

Besides phosphorylation of ERK2, homodimerization is necessary for its nuclear accumulation (108). The dimerization

of ERK2 induces a change of conformation and exposes a binding site for a NLS-containing protein. One of the potential candidates for such a NLS protein is growth factor–receptor bound protein 2-associated protein 1 (Gab1), which associates with ERK2. It was suggested that binding of ERK2 and Gab1 regulates the nuclear import of phosphorylated ERK2 (170). Another important role for ERK1 and ERK2 is their involvement in the nuclear localization of Nrf-2, which, among others, activates genes coding for enzymes required for *de novo* GSH synthesis (263). Thus, ERK1/2 or mainly ERK2 plays a role in ensuring an appropriate GSH supply for the cell.

6. Src kinase family. The Src kinase family consist of at least nine members (178). The most prominent members are the kinases Src, Fyn, and Yes. In the nucleus, only the kinases Src and Yes can be found, but not Fyn (96). In response to oxidative stress or growth factors, Src is phosphorylated at tyrosine 416, and Yes at tyrosine 426, which increases their enzymatic activity. Under conditions of oxidative stress or during the process of aging, these nuclear kinases are involved in tyrosine phosphorylation of TERT, which then leads to nuclear export of TERT. This has been demonstrated by mutation of tyrosine 707 within TERT, resulting in nuclear retention of the enzyme and by pharmacologic inhibition of Src kinase family activation, which inhibits tyrosine phosphorylation of TERT and its subsequent export from the nucleus (70, 96) (Fig. 11). The underlying export mechanism is mediated by a complex formation between TERT, the well-described export receptor CRM-1, and the nuclear GTPase Ran (70). Functional consequences of this Src kinase family–dependent nuclear tyrosine phosphorylation of TERT are increased apoptosis sensitivity and accelerated senescence (68, 69). This is in agreement with other studies, demonstrating that nuclear TERT acts as an inhibitor of apoptosis in several cell types (56, 144, 166). Therefore, an imbalance in the redox status seems to enhance active nuclear Src and Yes, which importantly contribute to apoptosis and accelerated senescence.

Recently a new mechanism for inactivation of the Src kinase was discovered. Src is active only in its reduced form, and oxidation results in complete inactivation. This inactivation is caused by a specific, reversible oxidation of Cys 277, located in the catalytic domain of Src, which results in homodimerization of Src linked by a disulfide bridge. This cysteine residue is conserved only in three of the Src family members, Src, Yes, and Egr, and could therefore be a specific mechanism for regulating a specific subset of Src kinases (106). Interestingly, only Src and Yes could be found in the nucleus, leading to the speculation that the formation of disulfide homodimers might be a prerequisite for nuclear import.

B. Redox-regulated phosphatases

Protein phosphatases can be divided into serine/threonine phosphatases and tyrosine phosphatases (PTPs). Both are important regulators in the activation and inactivation of cell-signaling pathways. Serine/threonine phosphatases are generally regulated by association with regulatory subunits and their own phosphorylation (49, 209). In the last few years, the role of oxidative stress in the regulation of PTPs has received more attention. PTPs have been shown to be redox sensitive and to be inhibited reversibly or irreversibly, depending on the degree and mechanism of oxidation (120, 150, 180, 231). Oxidation of the essential cysteine in the active site by H_2O_2 inactivates phosphatase activity (45). The reversible oxidation of this cysteine residue to sulfenic acid (Cys-SO^-) has been identified as a key mechanism for the regulation of many pathways. Higher oxidation to sulfinic (Cys-SO_2^-) or sulfonic (Cys-SO_3^-) acid leads to an irreversible inactivation of the phosphatase (Fig. 12A). Many PTPs prevent a higher oxidation by formation of intracellular disulfide bonds between the active-site cysteine and nearby so-called backdoor cysteines [for example, Cdc25 phosphatases (216) or the phosphatase and tensin homologue (PTEN) (113)]. These intramolecular disulfides can then rapidly and effectively be reduced by

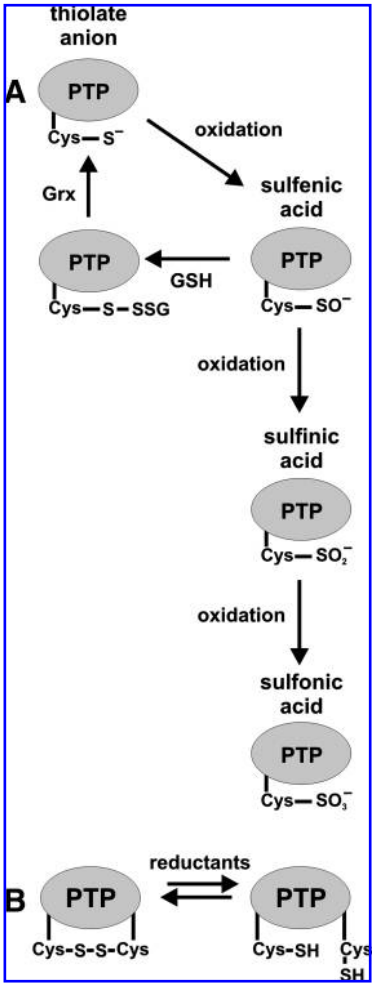

FIG. 12. Model for the regulation of PTP activity. (**A**) Under physiologic conditions, the catalytic cysteine of active protein tyrosine phosphatases (PTPs) is in the thiolate anion form. Oxidation leads to reversible sulfenic acid formation. Further oxidation is irreversible and leads to sulfinic and sulfonic acid formation. To prevent this further oxidation, GSH can form a mixed disulfide with the sulfenic acid, which is then reduced by glutaredoxin (Grx). (**B**) To prevent PTPs from being irreversibly oxidized, the reversible inactive state is stabilized by formation of an intramolecular disulfide bond between the cysteine in the catalytic center and a backdoor cysteine. This intramolecular disulfide bond can be rapidly and effectively reduced by several reductants.

reductants like dithiothreitol (DTT), GSH, Trx-1, or a combination of these (113, 216) (Fig. 12B). The oxidation of PTPs is important for the regulation of many cellular signaling pathways in response to oxidative stress.

C. Nuclear-localized phosphatases

Recently a new concept emerged, which emphasizes an important role for nuclear tyrosine phosphatases and their signaling pathways in response to oxidative stress. We concentrate on the detailed discussion of PTPs for which a nuclear localization has been shown, even if the redox regulation takes place outside the nucleus: Shp-2, T-cell protein tyrosine phosphatase (TC-PTP), and Cdc25C (an overview is given in Table 2).

1. Protein tyrosine phosphatase Shp-2. The Src homology 2 (SH2) domain containing protein tyrosine phosphatase Shp-2 is involved in many signal-transduction processes induced by cytokines and growth factors (2, 4, 54, 55). Further findings indicated a regulation of Shp-2 by ROS (150). Stimulation of Rat-1 cells with platelet-derived growth factor (PDGF) induced production of intracellular ROS, which leads to oxidation and inactivation of Shp-2. This oxidation requires complex formation between Shp-2 and the activated PDGF receptor (PDGFR). In its basal state, the active site of Shp-2 is covered by the N-terminal SH2 domain (N-SH2). The binding of a specific phosphotyrosyl ligand opens the active site of Shp-2 (82). Meng *et al.* (150) speculated that binding of PDGFR to Shp-2 promotes an open, active conformation of Shp-2, and that not only substrates but also ROS can interact with this site.

Like other PTPs, Shp-2 is oxidized by ROS. Recently, two backdoor cysteines were found to be involved in the redox regulation of Shp-2. Instead of forming a disulfide bond between the active-site cysteine and a backdoor cysteine to prevent the phosphatase from further oxidation, the reduced catalytic cysteine in Shp-2 (and also in Shp-1) is shielded by a disulfide bond between two backdoor cysteines (33).

In 2002, Chughtai *et al.* (37) reported a nuclear localization of Shp-2 in connection with an association of the signal transducer and activator of transcription 5 (STAT5) and Shp-2. This nuclear translocation of Shp-2 in a complex with Stat5 is induced by the stimulation with prolactin in mammary cells. Formation of this complex requires the carboxy-terminal SH2 domain and the catalytic activity of Shp-2 and correlates with the tyrosine phosphorylation of STAT5 by Janus kinase 2 (JAK2) on the tyrosine residue 694 (Fig. 13). The authors speculated that the nuclear Shp-2/STAT5 complex binds to DNA and regulates transcription of milk-protein genes (37). In endothelial cells, Shp-2 is localized in nuclear and cytosolic fractions under basal conditions (96). Our findings identified nuclear Shp-2 acting as a counterplayer for the nuclear export of TERT. Under conditions of oxidative stress, the nuclear export of TERT is blocked by Shp-2 overexpression. This is dependent on the activity of Shp-2 because the dominant-negative Shp-2(C459S) reduces nuclear TERT protein and telomerase activity. Ablation of endogenous Shp-2 leads to an increased tyrosine phosphorylation of TERT. Tyrosine 707 within TERT (which has previously been shown, once phosphorylated, to be essential for nuclear export of TERT) seems to be the target tyrosine for Shp-2. Thus, Shp-2 inhibits the nuclear export of TERT by regulating the tyrosine 707 phosphorylation (Fig. 14). This implies a new role for nuclear Shp-2 in protecting nuclear TERT, and therefore, nuclear Shp-2 may delay cellular senescence.

2. T-cell protein tyrosine phosphatase (TC-PTP). T-cell protein tyrosine phosphatase (TC-PTP) is a ubiquitously expressed PTP. As a result of alternative splicing, two isoforms of TC-PTP are generated, a 45 kDa isoform located in the nucleus and a 48 kDa isoform targeted to the endoplasmic reticulum (130). Specific cellular stresses cause a reversible cytosolic accumulation of the 45 kDa isoform of TC-PTP (114). For example, oxidative stress or hyperosmolarity induces the nuclear export of TC-PTP, whereas other stresses like heat shock have no effect on the localization of TC-PTP. This change in

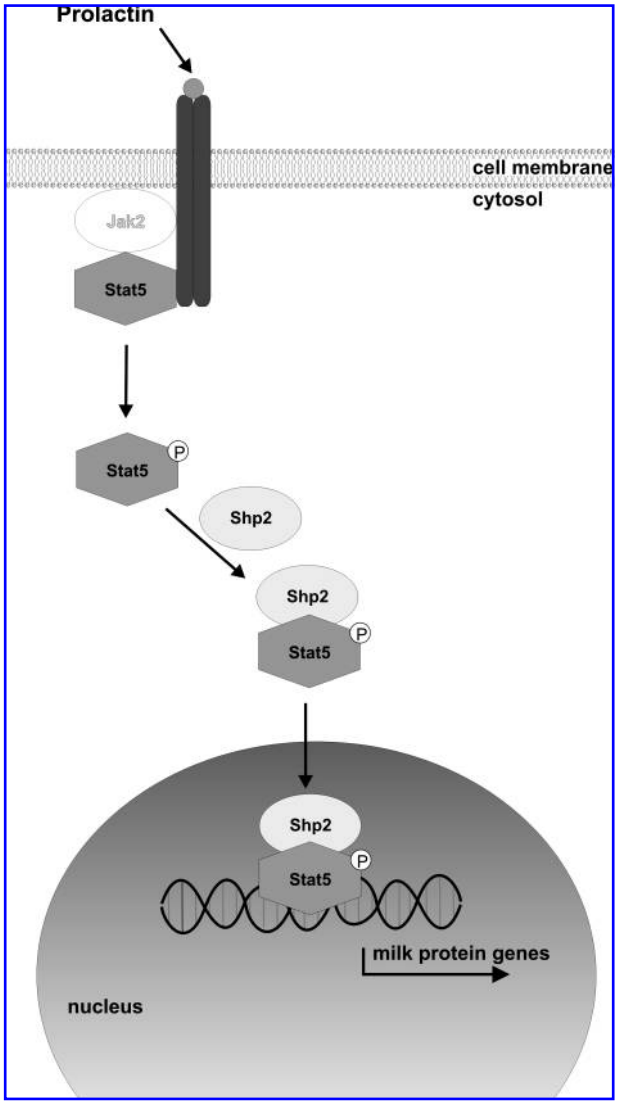

FIG. 13. Nuclear translocation of Shp-2 induces STAT5-dependent gene transcription. Prolactin induces tyrosine phosphorylation of STAT5 at tyrosine 694 by the Janus kinase 2 (JAK2), which leads to complex formation of Shp-2 and STAT5. This complex translocates into the nucleus, binds to DNA *via* STAT5, and induces milk-protein gene transcription.

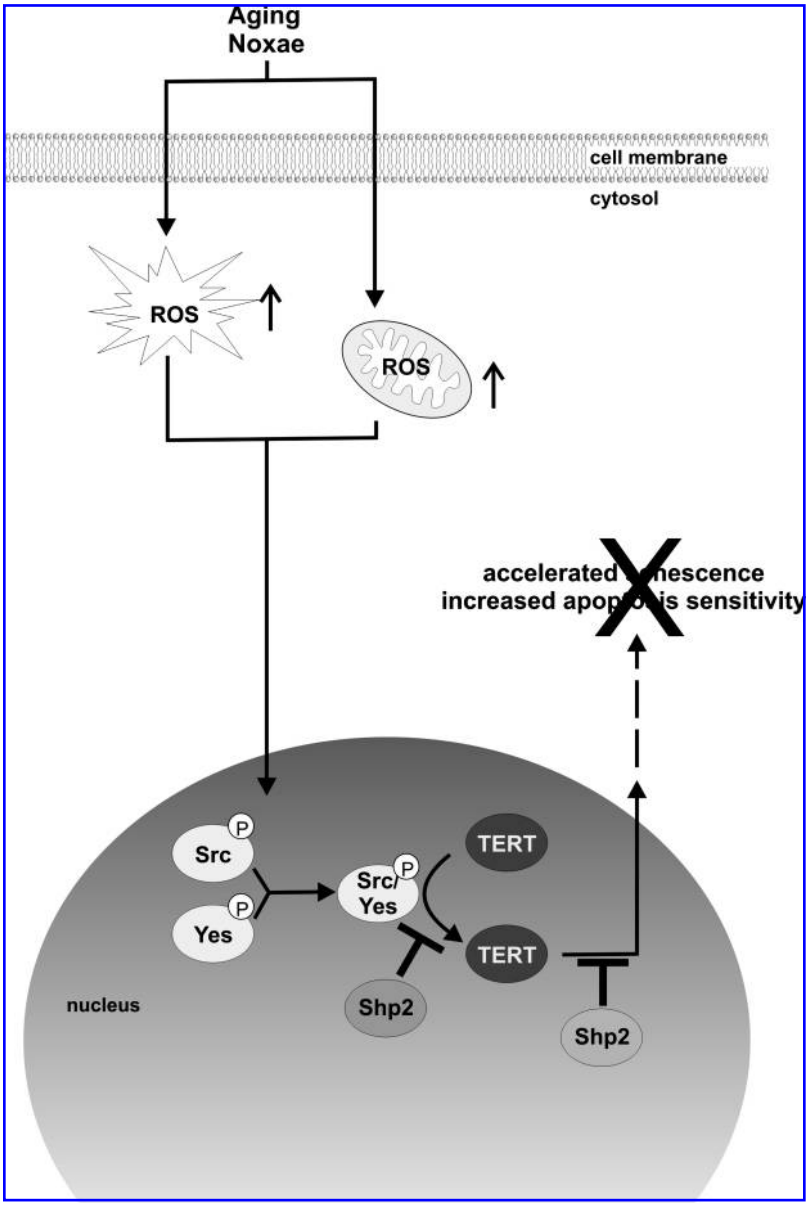

FIG. 14. Shp-2 inhibits TERT nuclear export induced by oxidative stress. ROS induce increased activity of nuclear Src and Yes. This leads to tyrosine phosphorylation of telomerase reverse transcriptase (TERT) and to its nuclear export. Nuclear Shp-2 prevents this export.

localization could be observed in different cell types. Nonnuclear TC-PTP is involved in the regulation of cell growth and cell differentiation in endothelial cells (143). There, TC-PTP binds to vascular endothelial growth factor receptor 2 (VEGFR2) and dephosphorylates specific tyrosine residues. Dephosphorylation of tyrosines 1045 and 1059 delays VEGF-induced VEGFR2 internalization and thus reduces sprouting angiogenesis.

Another target tyrosine of TC-PTP is tyrosine 1214, which has been implicated in VEGF-induced actin remodeling and thereby plays a role in endothelial cell migration (115). Together, these data suggest an important role for cytosolic TC-PTP in endothelial cell growth and differentiation.

Several other groups have investigated the functions of nuclear TC-PTP. The 45 kDa form of TC-PTP was first identified as a nuclear STAT1 tyrosine phosphatase. Upon stimulation, STAT1 becomes tyrosine phosphorylated by the family of JAKs and translocates into the nucleus. There it binds to DNA and activates transcription (40). To terminate this transcription, STAT1 must be dephosphorylated to dissociate from DNA. Ten Hoeve *et al.* (228) were the first to identify the 45 kDa form of TC-PTP to be the tyrosine phosphatase of STAT1. They also investigated the dephosphorylation of other STATs (STAT3, STAT5, and STAT6) and found that, in *TC-PTP*–deficient mouse embryonic fibroblasts, only the dephosphorylation of STAT1 and STAT3 is affected on interferon stimulation, but not that of STAT5 and STAT6 (228). This is in agreement with findings of Yamamoto *et al.* (257), who demonstrated dephosphorylated STAT3 after interleukin 6 treatment and showed a direct interaction between STAT3 and nuclear TC-PTP. Similarly, Aoki and Matsuda (10) found that in epithelial cells, stably expressing mouse *TC-PTP* STAT5a and STAT5b are dephosphorylated after prolactin stimulation (10). TC-PTP has different regulatory roles in diverse pathways, dependent on its localization. Because nuclear export of TC-PTP occurs under specific stresses, one

can imagine stress-specific functions of TC-PTP. This opens up an interesting field for further studies to understand why specific stress inducers like heat shock do not induce the nuclear export of TC-PTP.

3. Cdc25C. In mammalian cells, cell cycle progression is tightly regulated by the cyclin-dependent protein kinases (CDKs). CDK1 (also named Cdc2) is the key component of the checkpoint pathway, which delays mitotic entry after DNA damage or stalled replication. CDK1 forms complexes with cyclin B1 that, in their phosphorylated, inactive form, are retained in the cytosol. During prophase, CDK1/cyclin B1 complexes accumulate in the nucleus and are activated through the phosphatase Cdc25C by dephosphorylation of threonine 14 and tyrosine 15 of CDK1 (48, 112). As mentioned earlier, phosphatases can protect themselves from irreversible oxidation by forming a disulfide bond between their catalytic cysteine and a backdoor cysteine. This protection has also been demonstrated for Cdc25C. Its catalytic cysteine is cysteine 330, and the backdoor is cysteine 377. Mutation of cysteine 377 in Cdc25C leads to irreversible oxidation of cysteine 330, which results in degradation of Cdc25C (198). During interphase of the cell cycle, a formation of the disulfide bond between cysteine 330 and 377 in Cdc25C is induced, and Cdc25C is phosphorylated at serine 216. This phosphorylation leads to binding of Cdc25C to 14-3-3 proteins and results in the nuclear export of Cdc25C (179). Mutation of serine 216 in Cdc25C perturbs mitotic timing and allows cells to escape the G_2-checkpoint arrest (179, 195).

For the fate of the cytosolic Cdc25C, different hypotheses exist. One possible mechanism is the degradation of Cdc25C, according to the destruction hypothesis of Savitsky and Finkel (198). It has been demonstrated that vitamin C induces formation of ROS, which leads to decreased Cdc25C levels. Conversely, the formation of cytosolic Cdc25C/14-3-3 complexes occurs without reduction in the Cdc25C levels. Thus, one may speculate that, dependent on the levels of ROS, cells decide to induce cell death or G_2-checkpoint arrest. High ROS levels would lead to irreversible oxidation of Cdc25C, its degradation, and finally to cell death. Minor damage, conversely, could induce formation of the disulfide bond in Cdc25C, its phosphorylation, and cytosolic sequestration by 14-3-3 proteins. Thus, Cdc25C would be immediately available for cell cycle progression after damage repair.

V. Conclusions and Outlook

Redox regulation plays an important role in intracellular signal transduction. Numerous proteins have been described to be redox regulated. However, it must be noted that, for the nuclear import and export receptors, only initial studies in yeast have shown that these receptors change their localization from the nucleus to the cytosol and can be oxidized on oxidative stress, which could importantly contribute to gene regulation (111, 183, 184). Thus, further studies in higher eukaryotes are required to investigate the redox regulation of the nuclear import and export machinery. Furthermore, many studies do not distinguish between the intracellular compartments in which the redox modification of the protein of interest takes place. This is of special importance, because many antioxidative systems exist in different cellular compartments like the cytosol, the mitochondria, and the nucleus. However, not all antioxidative systems are equally distributed throughout the cell. The distribution strongly depends on the cell type and on the stimulus used. Therefore, it is noteworthy that cellular functions regulated by redox modifications of proteins are intimately associated with their cellular localization. Unfortunately, many previous investigations did not accurately discriminate between the compartments where redox regulation occurs and the corresponding cellular function. Without this differentiation, pharmacologic interventions may exhibit undesirable and unanticipated side effects because the compartment-specific effects of the proteins have been disregarded in the past. Therefore, compartment-specific investigations will help us to determine protein functions in more detail and to uncover misunderstood protein functions.

Finally, compartment-specific delivery of inhibitors and activators will open a new field of drug design to regulate protein actions more precisely and to reduce unwanted side effects.

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Abbreviations Used

AP-1 = activator protein 1
APEX1 = APEX nuclease (multifunctional DNA-repair enzyme) 1
ATF = activating transcription factor
bZIP = basic region-leucine zipper
cAMP = cyclic adenosine monophosphate
CDK = cyclin dependent kinase
CRE = cAMP response element
CREB = cAMP response element–binding protein
CREM = CRE modulator
DBD = DNA binding domain
DNA-PK = DNA protein kinase
DTT = dithiothreitol
Egr-1 = early growth-response factor 1
ER = estrogen receptor
ERE = estrogen response element
ERK = extracellular regulated kinase
GPx = glutathione peroxidase
GR = glutathione reductase
GRE = glucocorticoid response element
Grx = glutaredoxin
GSH = glutathione
GST = glutathione *S*-transferase
HIF = hypoxia-inducible factor
HLF = HIF-1α–like factor
HRE = hypoxia-response element
Hsp = heat-shock protein
HTLV = human T-lymphotropic virus
IκB = inhibitor of nuclear factor-κB
IKK = IκB kinase
JAK = Janus kinase
JIP = JNK-inhibitor protein
JNK = c-Jun NH_2-terminal kinase
Keap1 = Kelch-like ECH-associated protein 1
MAP kinase = mitogen-activated protein kinase
MKK = MAP kinase kinase
NFI/CTF = nuclear factor I/CAAT transcription factor
NF-κB = nuclear factor-κB
NIK = NF-κB–inducing kinase
NLS = nuclear localization signal
Nrf-2 = nuclear factor erythroid 2–related factor 2/NF-E2 related factor 2
ODD = oxygen-dependent degradation domain
PKA = protein kinase A
PKC = protein kinase C
Prx = peroxiredoxin
PTP = protein tyrosine phosphatase
ROS = reactive oxygen species
SOD = superoxide dismutase
Sp1 = specificity protein 1
STAT = signal transducer and activator of transcription
TAD = transcription-activation domain
TC-PTP = T-cell protein tyrosine phosphatase
TERT = telomerase transcriptase
TR1 = thioredoxin-1 reductase
Trx-1 = thioredoxin-1

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