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Role of redox potential and reactive oxygen species in stress signaling

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Stress-activated signaling cascades are affected by altered redox potential. Key contributors to altered redox potential are reactive oxygen species (ROS) which are formed, in most cases, by exogenous genotoxic agents including irradiation, inflammatory cytokines and chemical carcinogens. ROS and altered redox potential can be considered as the primary intracellular changes which regulate protein kinases, thereby serving as an important cellular component linking external stimuli with signal transduction in stress response. The mechanisms, which underlie the ROS-mediated response, involve direct alteration of kinases and transcription factors, and indirect modulation of cysteine-rich redox-sensitive proteins exemplified by thioredoxin and glutathione S-transferase. This review summarizes the current understanding of the mechanisms contributing to ROS-related changes in key stress activated signaling cascades.

Keywords: stress kinases; oxygen radicals; ROS; GST; thioredoxin; NF-κB

Introduction

Homeostatic control of reactive oxygen species (ROS) is one of the key determinants in maintaining cell growth pathways which can incorporate proliferation (Biguet *et al*., 1994) apoptosis (reviewed by McConkey and Orrenius, 1996; Wang *et al*., 1998a; Tan *et al*., 1998) and senescence (reviewed in Powis *et al*., 1995). ROS include superoxide, singlet O_2, H_2O_2, and the highly reactive hydroxyl radical. Intercellular levels of ROS are influenced by a number of endogenous and exogenous processes, and controlled by several radical scavenging enzymes. Variability or inductive changes in the expression of these enzymes can significantly influence cellular redox potential. Such changes have been shown to occur during differentiation, aging and senescence (Chen *et al*., 1995). Exogenous agents which induce ROS formation include chemical and physical carcinogens and various cytokines. Common to all, is their ability to produce directly or indirectly electrophilic metabolites capable of generating reactive oxygen (reviewed by Primiano *et al*., 1997). Such ROS can alter signal transduction cascades (reviewed by Chakraborti and Chakraborti, 1998) as well as induce changes in transcription factors that mediate immediate cellular stress responses (reviewed by Sen and Packer, 1996; Monterio and Stern, 1996; Lander *et al*., 1996; Piette *et al*., 1997). Almost every gene that has been implicated in response to stress has also been shown to be affected by altered redox or ROS levels. Table 1 lists the kinases and respective transcription factor substrates that have been found to be ROS-responsive. The data provided in Table 1 represent a fraction of the abundant information accumulated which suggest that ROS-mediated changes are found in different tissue types and cell systems, illustrating the preponderance of ROS effects.

While the mechanisms underlying ROS induced alterations of kinase and transcription factor activities are not completely understood, progress over the past couple of years may provide new insight into the regulation of stress responsive proteins by ROS. The nature of ROS elicited cellular changes can be divided into two major categories: (i) the direct effect of ROS on the kinase or transcription factor, which can alter conformation and activity. (ii) the effect of cysteine rich, redox-sensitive proteins, which have been shown to play important roles in the regulation of stress responsive proteins. Oxidative conditions produce conformational changes and generation of dimers/multimers of these redox responsive proteins. In most cases, their association with, and regulatory effect on respective signaling molecules is neutralized. These redox responsive proteins include thioredoxin (reviewed by Russel, 1995) and glutathione S-transferase (GST; Tew 1994; Hayes and Pulford, 1995). This review summarizes the current knowledge of ROS effects on stress responsive proteins and highlights present understanding of possible underlying mechanisms.

ROS-induced modification of redox sensitive proteins which regulate stress kinases

Cysteine-rich molecules have been implicated in the response to altered redox potential and generation of ROS. In all cases, ROS causes the formation of intra- or inter-molecular disulfide bond between respective cysteines. Disulfide bridges within the molecule (intra-) alter protein conformation; disulfide bonds between cysteine rich molecules (inter-) create dimers and multimers potentially influencing association with other cellular proteins. Such changes have been shown to occur in redox-sensitive proteins such as thioredoxin and GST. In most cases the formation of dimers results in the dissociation of the redox sensitive molecule from its associated protein. There appears to be a reasonably linear relationship between the degree of oxidative stress, the formation of disulfide species and the induction of stress responsive proteins. Thioredoxin has been the best characterized cysteine-rich redox-responsive protein implicated in the regulation of several stress responsive proteins via direct or intermediate interactions (reviewed in Schulze-Osthoff *et al*., 1995; Sen and Packer, 1996; Flohe *et al*., 1997;

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Table 1 ROS effects on Protein kinases and transcription factors

Source of ROS	*Cellular changes*	*Cell system*	*Biol. implication*	*Reference*
H_2O_2	↑adenyl cyclase	A10, murine vascular smooth muscle cells	Vasodilation	Tan *et al*., 1995
H_2O_2	↑MAPK	Neonatal rat ventricular myeocytes	↑Na^+, H^+ exchange	Taher *et al*., 1998; Sabri *et al*., 1998
H_2O_2	↑BMK1/ERKs ↑c-*src*	Mouse fibroblasts		Abe *et al*., 1997
H_2O_2	↑MAPK	Vascular smooth muscle cells		Hayashi *et al*., 1998
Hypoxia/re-Oxygenation	↑JNK	Cardiac myeocytes		Laderoute and Webster, 1997
H_2O_2	↑ERK1/2, ↑BMK1	Rat vascular smooth muscle cells		Abe *et al*., 1996 Warner *et al*., 1996
H_2O_2 Doxorubicin	α actin, treponin I creatine kinase	Cardiocytes	Cardiotoxicity	Torti *et al*., 1998
TNFα/H_2O_2	↑MnSOD, ↑NF-κB	Human pulmonary adenocarcinoma cells H441		
Aziridinylebenzoquinones or H_2O_2	↑$p21^{waf}$	HCT 116 colon carcinomas cells and K562 human chronic myelogenous leukemia cells	Apoptosis	Qui *et al*., 1996
H_2O_2	↑MAPK, ↑p21ras	Jurkat T cells		Lander *et al*., 1995
H_2O_2	↑ERK, ↑JNK, ↑p38	HeLa cells	Apoptosis	Wang *et al*., 1998b
HIV-1		Jurkat and H9 Human T cells (Cd4)	Apoptosis	Bank *et al*., 1998
ROS	↓SP1	Rat hepatoma	Regulating glycolytic enzymes in resting vs proliferating cells	Hamm-Kunzelmann *et al*., 1997
ROS	↓Phospho-tyrosine, P105; p81	Human sperm	Sperm capacitation	Leclerc *et al*., 1997
Sulfhydryl	↑c-Src		Mouse NIH3T3 fibroblasts	Pu *et al*., 1996
Oxygen	Dimerized S100B	Glial cells	DNA synthesis	Scotto *et al*., 1998
H_2O_2, NO	↑MAPK p42/p44	Rat hippocampal		Kanterewicz *et al*., 1998
Oxidative stress	↑ras/MAPK/ERK2, ND	*In vitro*		Muller *et al*., 1997
Oxygen	↑cAMP-dependent kinase I, II, ND	T lymphocytes	Lymphocyte activation	Dimon-Gadal *et al*., 1997
Mitogenic stimulation	↑MAPK/ERK2, NF-κB/AP1	T lymphocytes		Goldstone and Hunt, 1997
N-acetylcysteine	↑JNK, ↑Jun ↑fos	Jurkat T lymphocytes		Gomez del Arco *et al*., 1996
NO	↑MAPK ↑$p21^{ras}$ ↑NF-κB ↑ERK, ↑p38, ↑JNK ($\times 100$)	Jurkat T cells		Lander *et al*., 1996
NO	PI3K (p110β/110γ) recruitment to $p21^{ras}$	Jurkat T cells		Deora *et al*., 1998
NO	↓Angiotensin II activation of MAPK	Cardiac fibroblasts	Hormonal activation of mitogenic steps are ROS dependent	Wang *et al*., 1998
UV	↓GST-p (24 h) ↑c-Jun (1–4 h) ↑c-*fos*	Rat keratinocytes		Nakano *et al*., 1997
Paraquat	↑PKC ↑ODC	WI-38 human lung cells		Kuo *et al*., 1995
Arsenite	MAPK, JNK, p38 AP1	CHO-k1	Apoptosis	Cavigelli *et al*., 1996;
BPV-E5	↑NF-κB			Ludwig *et al*., 1998
Asbestos	↑PKC, PLC PTK	Alveolar macrophages	Transformation	Kilk *et al*., 1996
NO, thiol redox	↓JAK2/3	Ba/F3 cells		Lim *et al*., 1997
Sodium orthovanadate	↑Syk, ERK2, ↑NF-κB		Proliferation	Duhe *et al*., 1998
Oxidized dopamine	↑JNK ↓Bcl_2	PC12	Apoptosis	Krejsa *et al*., 1997 Kang *et al*., 1998
Thiuramdisulfides	↓cdk2, ↓Bcl2, ↑p53 ↓NF-κB ↓AP1	Hep G2 human hepatoma	Apoptosis	Lui *et al*., 1998
Dopamine	↑JNK, Jun	293 primary neonatal cells	Apoptosis	Luo *et al*., 1998
Oxidized LDL	↓PKC, PTK, ↑Fas ↓Bcl_2	Human coronary endothelial cells	Apoptosis	Li *et al*., 1998
Cytoxic xenobiotics	↑MKK4, JNK, p38, ↑c-Jun, ↑c-*fos*			Wilhelm *et al*., 1997
Angiotensin II	↑p38 ↑p42/44	Vascular smooth muscle cells	Vascular hypertrophy	Ushio-Fakai *et al*., 1998
Diethyl-maleate	↑MAPK, ↑$p21^{waf}$	Neural cell lines PC12 GT10–7		Esposito *et al*., 1997
BCL2	↑glutathione reduced ratio of oxidized glutathione, ↑superoxide dismutase ↑catalase			Ellerby *et al*., 1996
TGFβ	↓Reduced glutathione	Primary fetal rat hepatocytes	↑Apoptosis blocked by cyclohexamide	Sanchez *et al*., 1997
$p21^{rasV12}$	↓MAPK ↑Rac1 (no effect on JNK)	Mouse 3T3 fibroblasts	Transformation	Irani *et al*., 1997
Hypoxia	↓MAPK, ↓c-*fos* ↓Elk1	HeLa		Muller *et al*., 1997
Hypoxia	↓NF-κB not MAPK dependent	A549 human alveolar epithelial cells	Protection from apoptosis	Li *et al*., 1997

Piette *et al*., 1997). The recent identification of thioredoxin as an inhibitor of stress kinase ASK1 (Saitoh *et al*., 1998), has shed new light on the modes of redox regulatable stress response. Similarly, the identification of GSTP1-1 as an inhibitor of JNK signaling (Adler *et al*., 1999) creates an additional paradigm for the regulation of stress kinases. In both cases, the inhibition of the stress kinases is mediated via the association with the respective redox sensitive molecule, which takes place under non-stressed growth conditions.

Thioredoxin-ASK1

Screening for ASK1 associated proteins using the yeast two-hybrid system, led to the identification of thioredoxin (Saitoh *et al*., 1998). Thioredoxin association with ASK1 was found in non-stressed cells and required the amino-terminal domain of ASK1. Thioredoxin association with ASK1 inhibits its activity as a kinase. Deletion of specific amino-terminal residues renders ASK1 constitutively active, and no longer influenced by thioredoxin. Thioredoxin inhibition of ASK1 is subject to attenuation by ROS, which induce dimerization of thioredoxin. ROS-dependent dissociation of thioredoxin from ASK1 is abolished upon treatment of cells with free radical scavengers. Further support for the role of thioredoxin in inhibition of ASK1 comes from the use of antisense to thioredoxin, which has also been shown to attenuate ASK1 inhibition. Of interest is the finding that ASK1 can undergo multimerization in response to ROS and thus has been associated with the active form of the kinase (Gotoh and Cooper, 1998). This suggests that thioredoxin association with ASK1 may serve to prevent its multimerization. The finding that signaling to apoptosis via DAXX is also mediated via the amino-terminal domain of ASK1 (Chang *et al*., 1998), suggests that dissociation of thioredoxin from ASK1 may also be accomplished upon the association of ASK1 with other cellular proteins. Alternatively, since ROS is normally expected to accumulate in response to either TNF or Fas signaling, ASK1 activation could occur via displacement of the inhibitory protein thioredoxin with an activating one, such as DAXX.

Thioredoxin-glucocorticoid receptor

Glucocorticoid receptors (GR) belong to a group of transcription factors that are redox responsive. A mammalian two hybrid assay revealed direct association between thioredoxin and GR. Such an association is mediated via the DNA binding domain, which is highly conserved among other nuclear receptors, thus suggesting that similar association and regulation may also exist for other members of this receptor family. Interaction between thioredoxin and GR was found to take place in the nuclei under oxidative conditions (Makino *et al*., 1999).

GSTp-JNK

ASK1 has been implicated in the activation of MKK3/6, MKK4/MKK7 and subsequently, p38 or JNK (Ichijo *et al*., 1997), resulting in the phosphorylation of respective p38 and JNK substrates, including ATF2, c-Jun, (reviewed by Ip and Davis, 1998) and p53 (Adler *et al*., 1997; Fuchs *et al*., 1998a). The key role of these substrates in the cellular protection from stress and damage suggests that their kinases should be tightly regulated. One indicator for such regulation is the low basal activity of JNK in cells, even if maintained under high concentration of growth factors, which were shown to serve as potent inducers of JNK (Minden *et al*., 1994). This suggests that JNK activity is inhibited in non-stressed cells. Indeed, extracts prepared from normal growing cells contain an inhibitory component which was purified and identified through microsequencing as GSTp (Adler *et al*., 1999). GSTp inhibition of JNK activity requires its association, which, in turn, limits the degree of Jun phosphorylation under non stressed growth conditions. GSTp-JNK association was primarily found in non-stressed cells. Upon treatment with H_2O_2 or UV, GSTp dissociated from JNK and formed dimers/multimers. Under physiological conditions GSTp inhibition was limited to JNK and did not involve the upstream JNK kinase MKK4. Furthermore, in the cells over-expressing GSTp, constitutively active MEKK1 efficiently phosphorylated both MKK4 and JNK, but did not elicit Jun phosphorylation due to GSTp-mediated inhibition. These observations suggest that GSTp-mediated inhibition of JNK is maintained upon the association between JNK and GSTp, regardless of the activation of JNK *per se*. Free radical scavengers maintain the GST-JNK association and JNK inhibition by GSTp. GSTp inhibitors as well as a GSTp-derived peptides were able to alter the degree of JNK activity prior to and after UV-irradiation, further defining the nature of this inhibition. Cells derived from GSTp null cells were found to have high basal JNK activity, which was reduced upon forced expression of GSTp cDNA (Adler *et al*., 1999). Being a key regulator of glutathione and, in turn, of redox potential, the identification of GSTp as a JNK regulator provides an important link between cellular redox potential and the regulation of stress kinase activities.

PAG-cAbl

PAG, a 23 kDa protein, was found to interact with c-Abl through the SH3 domain of c-Abl. This association mediated the inhibition of c-Abl tyrosine kinase activity and rescued cytostatic and cytotoxic effects mediated by c-Abl. Dissociation of Pag and c-Abl is thought to be caused by the elevation of ROS level in cells (Wen and Van Etten, 1997).

Direct effects of ROS on protein kinases

ROS-induced dimerization of ASK1

TNFα or H_2O_2 treatments caused ASK1-dependent apoptosis and concomitantly revealed changes from monomeric to dimeric or higher order oligomeric forms of ASK1. Pretreatment with free radical scavengers decreased these dimeric/multimeric forms and reduced ASK1 activity and the extent of apoptosis. Synthetic dimerization of an ASK1-gyrase B fusion protein by coumermycin resulted in substantial activation of

ASK1, suggesting that dimerization of ASK1 is sufficient for its activation (Gotoh and Cooper, 1998). Respectively, it is possible that ROS activates ASK1 via induction of ASK1 oligomerization.

Redox effects on p21^{ras}, Rac1 and PI3K

p21^{ras} has been shown to serve as a redox responsive signaling molecule (Lander *et al*., 1995, 1996). Moreover, cells expressing the mutant ras (H-ras-V12) were shown to produce a large amount of ROS (Irani *et al*., 1997), resulting in the activation of MAPK, ERK, ELK1 and c-fos (Muller *et al*., 1997a,b). Molecular interactions between nitric oxide (NO) and p21^{ras} activates p21-mediated signaling. Such an interaction results in S-nitrosylation of p21^{ras} on cysteine 118. Mutant forms of p21^{ras} at cysteine 118 no longer mediate guanine nucleotide exchange and further downstream signaling (i.e. activation of MAPK cascade) by NO (Lander *et al*., 1997). ROS were also reported to mediate recruitment of PI3K to p21^{ras}, an association that contributed to activation of PKB/Akt and MAPK, which are downstream targets for these kinases, respectively (Deora *et al*., 1998). Of interest is the independent role implicated for p85, the regulatory subunit of PI3K, in p53-dependent apoptotic response to oxidative stress (Yin *et al*., 1998).

Another GTP binding protein, Rac1, has been associated with the generation of ROS and activation of NF-κB (Sundaresan *et al*., 1996; Sulciner *et al*., 1996). ROS activation of Rac1 has also been implicated in the alteration of cell shape via transactivation of interleukin1α and of collagenase-1, both of which affect cellular cytoskeleton reorganization associated with wound healing, inflammation and malignancy (Kheradmand *et al*., 1998).

Redox modification of PKC

As a phorbol ester and oxidant responsive kinase, PKC has unique redox active cysteine-rich regions. Selenocompounds have been shown to affect PKC activity, in a concentration-dependent manner. Such alterations are mediated via the formation of disulfide bridges contingent upon the dose administered. At low dose, two disulfide bridges were identified between four cysteines, whereas at high concentrations, up to four disulfide bridges were formed (Gopalakrishna *et al*., 1997). These observations suggest that the cysteine residues present within the catalytic domain of the different PKC isoenzymes, although apart in the sequence, may be clustered in the tertiary structure to react with selenite, as well as being in a close proximity to some of the cysteines in the regulatory domain.

Redox modifications of substrates

Neurogranin

Neurogranin (Ng) is a neuron specific protein kinase C selective substrate that binds calmodulin (CaM) in the dephosphorylated form (Sheu *et al*., 1996). Ng contains active cysteine residues that are readily oxidized by several nitric oxide donors as well as by other oxidants to form intracellular disulfides. The cysteine residues involved in NO-mediated intramolecular disulfide bridge formation are at positions 3, 4, 9 and 51. While mutation at cysteine 51 abrogated disulfide bridge formation, it was necessary to mutate the other three sites (3, 4 and 9) to achieve the same effect. The intramolecular disulfide bridge between Ng proteins displayed dramatically attenuated CaM binding affinity and 2–3-fold weaker phosphorylation by PKC (Mahoney *et al*., 1996).

Rieske iron-sulphur protein – example of redox effect on RNA synthesis

The observation that ROS mediated redox changes decreased RNA synthesis in potato mitochondria led to identification of the Rieske iron sulphur protein as a key determinant in reducing the rate of UTP incorporation upon oxidative burst. Furthermore, broad-spectrum protein phosphatase inhibitors also decreased (up to 50%) UTP incorporation (Wilson *et al*., 1996). The latter observation implies that redox effects on RNA synthesis may be mediated in part via protein phosphorylation.

Redox effect on mRNA stability

Changes in the stability of mRNA of p21$^{waf1/cip1}$ and interleukin's 2 and 3 were recorded upon treatment with ROS producing agents, or via activation of ROS responsive kinases. The elevated expression of p21$^{waf1/cip1}$ in response to a variety of DNA damaging and ROS generating agents was also shown to be p53-independent. Increased levels of mRNA were found to be due to the ROS-dependent increased stability since exposure to free radical scavengers attenuated this increase (Esposito *et al*., 1997). Of interest is a possible link between elevated ROS-mediated p21$^{WAF1/cip1}$ expression and a reported role of ROS in senescence (Chen *et al*., 1995).

Activation of JNK by various forms of stress forming ROS results in the stabilization of mRNA for interleukins 2 and 3. This phenomenon is mediated through specific elements in the 5′ and 3′ UTRs for IL2 and 3′ UTR for IL3 (Chen *et al*., 1998; Ming *et al*., 1998). While the nature of JNK role in this stabilization is to be determined, the JNK-dependency points to a clear relationship between ROS and the stabilization of these mRNAs. Furthermore, altered cellular redox has been shown to prolong the half-lives of both the transcript and protein for GSTP1-1 (Shen *et al*., 1995). This observation could have implications to the overall model of redox reactive thiol control proposed in this review.

Redox effect on protein stability

Iron has been shown to be an important cellular cofactor involved in the maintenance of balanced ROS level. IRP2, a protein that is responsible for iron uptake and distribution is degraded by the proteasome in iron-replete cells. Iron dependent oxidation converts IRP2 into a substrate for ubiquitination *in vitro*. Thus, excess iron is 'sensed' by its ability to catalyze site specific oxidations in IRP2. Oxidized IRP2 is efficiently ubiquitinated and degraded (Iwai *et al*., 1998). A link between ROS and protein stability was also demon-

strated via GST inhibitory effects on JNK, which targets its non-phosphorylated substrates for ubiquitination and degradation (Fuchs *et al*., 1998b). In this setting, GST inhibition of JNK results in a lower level of c-Jun phosphorylation and respective increase in Jun ubiquitination (Adler *et al*., 1999).

Thioredoxin effects on transcription factors

Earlier studies have demonstrated the effects of thioredoxin on stress activated transcription factors, primarily from the AP1 and NF-κB families, (reviewed in Schulze-Osthoff *et al*., 1995; Sen and Packer, 1996; Okamoto *et al*., 1997; Flohe *et al*., 1997 and Mercurio and Manning, this issue). In this respect the opposing effects of thioredoxin on AP1 and NF-κB are interesting. While inhibiting the transcriptional activities of NF-κB, thioredoxin was shown to activate AP1-mediated transcription (Meyer *et al*., 1993; Schenk *et al*., 1994). The latter may provide an explanation for scenarios where activation of NF-κB (upon hyperoxia) provides protection from apoptosis (Li *et al*., 1997).

Among the possible mechanisms that could underlie the effect of thioredoxin on these transcription factors are (i) changes to upstream kinases, as demonstrated for ASK1 (ii), its direct effects on the transcription factor as shown for GR and (iii) its effect on a mediator protein, as shown for Ref1. Ref1 is a dual functional protein that plays an important role in the repair of a-basic sites via its apurinic/apyrimidinic endonuclease activity, and also elicits a redox-responsive regulation of c-Jun /NF-κB transcription. While phosphorylation by CKII has been thought to play a regulatory role in its DNA repair activity (Yacoub *et al*., 1997), altered ROS determines the association and nature of the Ref1 effect on the transcription factor's activity. Thioredoxin activation of AP1 is explained by its association with Ref1, which binds to AP1 and renders it transcriptionally active. Mutations on specific cysteines on thioredoxin abolished its association with Ref1 and consequently reduced Ref1's ability to mediate AP1 transactivation. Respective cysteines on c-Jun and c-fos were also mapped (Nikitovic *et al*., 1998).

Similar to thioredoxin's effect on AP1, a relationship between thioredoxin and the transcriptional activity of p53 was demonstrated in *Saccharomyces cerevisiae*, as deletion of the thioredoxin gene led to inhibition of p53-mediated transcription (Pearson and Merril, 1998). The effect of thioredoxin on p53 may occur in a fashion similar to the thioredoxin effects on AP1, i.e. via Ref1. This is proposed based on the established role of Ref1 in regulation of p53 transcriptional activities depending on the redox status and oxidized state of p53 (Jayaraman *et al*., 1997). Thus, a Ref1-thioredoxin complex may also participate in the regulation of p53 by ROS. The role of ROS in p53-mediated apoptosis has been demonstrated (Johnson *et al*., 1996). Furthermore, an increased tumor cells capacity for undergoing apoptosis in response to ROS was also shown to be p53-dependent (Graeber *et al*., 1996). This is consistent with the increased radio sensitivity that has been associated with oxidative signaling in repair deficient syndromes, such as ataxia telangiectasia (Lavin, 1998).

Runt domain proteins are transcriptional regulators that specify cells' fate for processes extending from pattern formation in insects to leukemogenesis in humans. The transcription factor PEBP2/CBF consists of a DNA binding subunit which has an evolutionary conserved 128-amino acid region termed 'Runt domain', responsible for DNA binding and heterodimerization with beta subunit. The Runt domain contains two conserved cysteins (aa 115, 124) which are responsible for redox regulation. Furthermore, two thiol-reactive proteins, thioredoxin and Ref1 play a role in the positive activation of the Runt domain (Akamatsu *et al*., 1997). Runt domain is dynamically regulated by altered potential.

Viral infection and ROS

Several studies have pointed to the ability of DNA and RNA viruses including influenza, paramyxovirus, hepatitis B virus and HIV to lead to the formation of ROS (reviewed in Schwarz, 1996). In most cases, this has been a consequence of the activation of monocytes and polymorphonuclear leukocytes. The relationship between viral infection and ROS production is better elucidated for HIV. HIV-induced apoptosis is thought to underlie the efficient depletion of T cells. T cell apoptosis by HIV is preceded by an exponential increase in reactive oxygen intermediates produced in the mitochondria, leading to caspase 3 activation, phosphatidylserine externalization and GSH depletion. Trans-aldolase is a key enzyme in the pentose phosphate pathway, which govern the supply of NADPH to mitochondria, the latter being a major determinant of ROS levels (Banki *et al*., 1998).

Overexpression of trans-aldolase increased ROS formation and apoptosis induced by HIV, whereas down regulation of trans-aldolase via the use of antisense expressing trans-aldolase constructs had the opposite effect (Banki *et al*., 1998). Thus, manipulation of GSH production and ROS generation in the mitochondria of HIV infected cells may control the degree of apoptosis induced by this virus. Another example of a viral protein, which has been associated with generation of ROS, is bovine papilloma virus E5 that was shown to induce NF-κB via superoxide radicals (Kilk *et al*., 1996).

The feedback loop of redox responsive proteins

While normal growing cells frequently express the monomeric form of cysteine rich redox sensitive molecules, such as thioredoxin and GSTp, exposure to ROS generating stress results in their dimerization and inactivates their normal maintenance functions. While the fate of the multimer forms of thioredoxin and GSTp has yet to be investigated, it is assumed that they will be subjected to accelerated degradation. At the same time, stress treatments mediate new synthesis of these redox responsive proteins. For example, heat shock was shown to increase transcriptional activation of thioredoxin (Leppa *et al*., 1997), thereby providing a new pool of monomeric thioredoxin that is expected to re-establish inhibition of ASK1 and its effectors. Similarly, GST and related family members are frequently identified as p53 inducible proteins (Polyak

et al., 1997; Komarova *et al*., 1998). Another JNK substrate, c-Jun was also shown to increase GSTp expression (Ainbinder *et al*., 1997). Therefore, similar to the case of thioredoxin, synthesis of new monomeric forms of GST may take place hours after altered redox potential, which could then assure new production of monomeric GST in the recovering cells resuming inhibition of JNK activity. In addition to its effects on JNK, GST was found to play an important role in coordinated regulation of ERK, p38 and NF-κB (Yin *et al*., unpublished observations). Of interest is the finding that protein synthesis is also required for the production of ROS and glutathione depletion, as shown in cellular response to TGFβ in fetal rat hepatocytes (Sanchez *et al*., 1997). This suggests that other cellular components, possibly other redox regulating enzymes, may also determine cellular fate through altering levels of oxygen radicals, thereby representing an escalating cellular loop that is expected to lead to an enhanced rate of apoptosis.

Discussion

Examples given in this review clearly suggest that ROS effects on cellular stress response involve almost all cellular compartments and regulatory levels (Figure 1). From its direct or cis-effects on redox responsive proteins which regulate key stress kinases and transcription factors, to the direct effect on signaling components such as $p21^{ras}$, and further to transcription factors at the level of RNA translation, RNA and protein stability. Our current knowledge points to the existence of ROS-related feedback loops, which ensure that the changes elicited upon ROS formation will be homeostatic and cyclical. Given the complexity of ROS regulating enzyme/systems, one could envision additional regulatory circuits that could increase the degree of sensitivity and responsiveness. For example, glutathione depletion has been implicated in the regulation of stress kinases JNK and p38 (Wilhelm *et al*., 1997), inactivation of PKC (Ward *et al*., 1998) and attenuated Bcl_2 inhibition of apoptotic protease (AP24) which induces DNA fragmentation (Wright *et al*., 1998), although the nature of these changes is not completely understood. The current state of knowledge also permits the design of peptides and pharmacomimetic reagents that selectively block stress responsive pathways, as a possible means to alter cellular responses to ROS. Since tumor cells are often found to express elevated levels of ROS responsive proteins and consequently, different drug resistance and apoptotic capacity, it is suggested that altering ROS control may sensitize tumor cells for a better response to selective treatments.

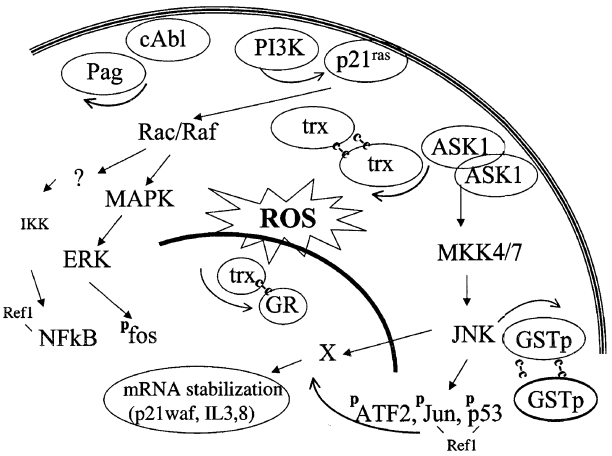

Figure 1 Changes elicited to stress responsive kinases and transcription factors following production of ROS are shown. Arrows point to either dissociation or association of corresponding molecules in response to the generation of ROS, represented here by H_2O_2. Shown is PAG dissociation from c-Abl which maintains inhibition of c-Abl under non stressed conditions. Dimerization of thioredoxin (trx) results in its dissociation from ASK1, enabling ASK1 dimerization and activation. Similarly, dimerization of GSTp relieves JNK from GSTp-mediated inhibition thus allowing JNK phosphorylation of its substrates, Jun, ATF2 and p53. ROS recruits PI3K to p21ras, leading to the activation of the MAPK and PKB pathways. While the dimerized form of thioredoxin dissociates from respective kinases, within the nuclei it has been shown to associate with the glucocorticoid receptor (GR), which increases GR transcriptional activities. Effect of ROS on mRNA stabilization of $p21^{waf}$ and interleukins is also indicated in the figure

Abbreviations

ASK, apoptosis signal-regulating kinase; ERK, extracellular signal regulated kinase; GR, glucocorticoid receptor NO -nitric oxide; GST, glutathione S-transferase; IRP, iron responsive protein; JNK, Jun NH2 terminal kinase; MAPK, mitogen activated protein kinase; Ng, neurogranin; PI3K, phosphoinositol 3 kinase; PKC, protein kinase C; ROS, reactive oxygen species; TNF, tumor necrosis factor

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