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Antioxidants, DNA Damage and Gene Expression

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Reactive oxygen species (ROS) are generated from incomplete reduction in the respiratory chain. On one hand they pose a serious threat of deleterious effects on important macromolecules, among which DNA is considered most important since it carries the genetic information and changes will be carried on to future generations, or will fundamentally change the behaviour of the cells. On the other hand, it is becoming evident that there are important changes in the cells in response to redox changes. This review summarises the genes, the intracellular signalling elements and molecules that presently are known to be regulated by oxidative stress. It is now clear that both oxidants and antioxidants can regulate a multitude of different cellular functions, signal transduction pathways and gene expression. However, the quantitative importance is unknown and as of yet there are no examples of regulation exclusively by oxidative stress. Also the response to oxidative stress is variable, can be up-regulation as well as down-regulation, and different responses to dose or magnitude of the oxidative stress can be demonstrated. The effect from supplementation with an antioxidant is difficult to predict, and ultimately must be assessed in clinical trials.

Keywords: Oxidative stress, DNA oxidation, gene expression, signal transduction

INTRODUCTION

Oxygen is the basis for mammalian life, as we know it. By four-electron transfer oxygen is reduced to water and the generated chemical energy is stored in a transportable form. Yet reduction of the ubiquitous oxygen can be incomplete and reactive oxygen intermediate species, ROS, are generated. Likewise, reactive nitrogen species, RNS, can be generated.[1] ROS/RNS such as singlet oxygen, superoxide anion, hydrogen peroxide, the hydroxyl radical, peroxynitrite and hypochlorite all have dual actions from a biological point of view. On one hand, they pose a serious threat of deleterious effects by oxidising important structures and macromolecules in the cells and on the other hand they also function as part of defence and signalling mechanisms. Often this is referred to as the double edge sword nature of free radical chemistry. Pro- as well as eucaryotes have developed a complicated defence network system to control ROS/RNS. This network function includes enzymatic and non-enzymatic antioxidants, various repair

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mechanisms and control mechanisms for elimination of severely but not mortally injured cells, i.e. apoptosis. It should be noted that in a chemical sense the term antioxidant is poorly defined, in chemistry there are oxidants and reductants. Therefore, the term antioxidant relates to a biological system and relates not to simple chemical reductants but to substances that are capable of performing a reduction–oxidation cycle and thereby transport electrons from very reactive molecules to less reactive molecules.

Recent developments in the construction of transgenic and knockout animals have brought more knowledge indicating the pathogenetic relevance of oxygen radicals. A transgenic mouse model for Huntington's disease shows increased levels of malondialdehyde, 8-oxodG, 3-nitrotyrosine and heme oxygenase in areas of brain degeneration.[2] Transgenic rats expressing glutathione S-transferase placental form have lower 8-oxodG levels in the liver[3] and overexpression of Cu/Zn SOD[4] or catalase[5] also seem to protect against oxidative DNA damage. Furthermore, animals homozygous for specific glycosylase defects in OGG1[6] have high levels of 8-oxydG, however, only with a moderately elevated spontaneous mutation rate. A heterozygous maganese SOD knockout mouse with 50% decreased mitochondrial SOD activity showed increased oxidative damage.[7] Collectively these data point to oxygen radicals as important pathogenic mechanisms.

OXIDATIVE STRESS AND ANTI-OXIDANTS

Antioxidants have attracted interest, particularly those that are dependent on dietary intake. An imbalance between oxidants and antioxidants, called oxidative stress, will lead to deleterious effects that are pathogenically important for development of diseases. Regarding arteriosclerosis the event is considered oxidation of LDL,[8,9] regarding cancer and ageing one important event is believed to be oxidative modification of DNA.[10,11]

These ideas led to huge intervention trials with antioxidants such as β-carotene and α-tocopherol. The first one showed a reduction in cancer incidence after supplementation with a combination of antioxidants.[12] However, three large and long running trials were negative,[13–15] and one even indicated increased lung cancer from β-carotene supplementation.[14]

These trials have been disappointing in showing no effect, yet there is clear biochemical evidence of the effect of such antioxidants and of oxidants on the cellular signalling system and gene expression. Recently, this disappointment seems to be overcome. A combination of vitamins C and E proved efficient in reducing preeclampsia in women[16,17] and an unpublished trial has demonstrated reduced arteriosclerotic progression from a combination of vitamins E and C supplementation.[18] The present review will focus on gene expression and cellular signalling. Oxidative DNA damage has been reviewed extensively by us before.[19–26]

SIGNAL TRANSDUCTION AND GENE EXPRESSION

During evolution aerobic organisms have evolved with an absolute dependence on oxygen. The restriction to live in an oxygen rich environment has provided no general need for oxygen sensors, except for special physiological functions, e.g. the carotid body. In contrast facultative and anaerobic organisms have general oxygen sensors in order to cope with shift from an anoxic environment to an oxygen rich environment. Mammalian cells require oxygen for most of their energy needs, for survival and for reproduction. The oxidative state, if sufficiently high, can be viewed as a sort of cellular beneficial state that could signal or allow critical biological events, i.e. mitogenesis. It appears that a variety of cellular signals can be elicited by both oxidants and mitogens.[27,28] However, in contrast to specific mitogenic stimuli, oxidants, besides

stimulating mitosis, also have considerable toxicological implications.

From a review of the literature it is evident that in eukaryotes there are no transcription factors that exclusively are activated by reactive oxygen species, and it can be hypothesised that such transcription factors do not exist. Detailed review of the molecular biology of redox regulation of cellular signalling and gene expression can be found elsewhere.[29,30]

The cellular response to a redox change appears to be highly variable. Upregulation and downregulation can be seen in different systems and the same system can react differently depending on the magnitude of the redox change. This is rather the rule than the exception, as can be seen from Table I. As clearly indicated for instance by protein kinase C and the caspases, the same enzyme system may react differently to different levels of oxidative stress/antioxidant status. In Table II, some selected responsive elements are tabulated similarly and they also show a highly varying response from oxygen and antioxidants. In Table III, the list of signalling molecules also shows a highly variable effect of oxidants.

It can thus be concluded that although antioxidants can have a direct effect in reducing oxidative deleterious effects, i.e. act as antioxidants, they have pronounced secondary effects that come from a change in redox status. Presumably, the functional changes that come from changes in signal transduction and gene expression will need some time to become established. In the search for antioxidant effects *in vivo* it is therefore important not only to look at immediate effects but also at the long-term effects. The redox status can be modulated from changes in antioxidant levels, however, also changes in oxygen consumption, e.g. from exercise, might be relevant, as reviewed earlier.[21] The effects of the increased oxygen consumption from exercise are highly variable, also with time.

A prime example of the unpredictable effects of modulation of response to changes in

TABLE II Oxidative stress and intracellular signalling

Elements	Effect of oxidants (O) and antioxidants (A)	References
NF-κb	Activation (O) blocking (A) controversial	[28,67–74]
AP-1	Activated (O,A) blocking (O,A)	[75–80]
Ca^{2+}	Intracellular increase (O)	[81,81,82]
AP-1	Activated (O)	[80,83]
p53	Complex regulation	[84–86]

TABLE I Oxidative stress and gene regulation/activity

Gene/enzyme/proteins	Regulation by oxidants	Regulation by antioxidants	References
JUN kinase (JNK)	Induced	Inhibited (GSH)	[37–40]
c-FOS	Increased transcription	Inhibited (phenolics)	[39,41,42]
MAP kinase	Stimulation		[43]
Tyrosine phosphatase	Inhibition		[43]
KAM-1, IL-1alfa, IL6, IL8, heme oxygenase	Induced		[44]
Metal binding proteins (MT-genes)	Induced		[45–51]
Heme oxygenase-1	Induced		[52]
Caspases	Induced		[53–56]
Caspases	Inhibited by severe oxidative stress		[57]
PKC	Induced		[43,58–60]
PKC	Inactivated		[61,62]
Ras	Activated		[40,63,64]
Phospholipase A and D	Activated		[65,66]

TABLE III Signalling molecules regulated by redox state in the cells

Signalling molecule	Effects of oxidants
Protein tyrosine kinase EGF receptor, insulin receptor, PDGF receptor, Src, Lck, Fyn, ZAP-70, Syk, Lyn, Fgr, Hck, Btk, Ltk	Activation
Protein tyrosine phosphatase The reduced cysteine residues are essential	Inactivation
Protein serine/threonine kinase MAP kinase, JNK, p38, BMK1, Akt, S6 kinase PKC	Activation (activation/ inactivation)
Protein serine/threonine phosphatase PP1, PP2A, calcineurin	Inactivation
Small G protein Ras	Activation
Lipid signalling PLC, PLD, PLA_2, PI 3-kinase	Activation
Ca^{2+} signal Ins $(1,4,5)P_3$ receptor, ryanodine receptor Ca^{2+}-ATPase, Ca^{2+}/Na^{+} exchanger	Activation
Transcription factors AP-1 (c-FOS, c-JUN), nfKb (p50), Rel, USF, ATTF-1, GR, BPV1E2, NFI, Myb, Nu-Y, p53, PEBP2/AML, Oct-2, Egr-1, BZLF1, Ets, GABP, Ah receptor, ATF, CREB, TTF-1, Ku	Inactivation

Adapted from Ref. [29].

oxidants/antioxidants stems from the ATBC study with long time intervention with high doses of β-carotene.[14] Unexpectedly, this trial demonstrated an increase in lung cancer risk from β-carotene intervention particularly related to smoking individuals. Following that observation animal studies looking at β-carotene supplementation and exposure to cigarette smoke revealed that expression of genes for retinoic acid receptors and activator protein-1, the latter encoded by the c-JUN and c-FOS genes, indicated suppression of RABβ gene expression and overexpression of activator protein-1, which combined with a strong proliferative response in lung tissue and squamous metaplasia could indicate a mechanism for enhanced lung tumourigenesis.[31] These trials clearly indicate that modulation of oxidative stress by tobacco smoke – a substantial oxidative stress inducer in humans – combined with an antioxidant, have a highly differential effect on gene expression.[31]

Since a major target of oxidative insult is DNA, it is of particular interest whether increased oxidative stress has any effect on DNA repair enzymes. A variety of specific repair enzymes for oxidative DNA modification has been described.[32] In general there is a lack of quantitative methods for estimation of DNA repair, particularly *in vivo* and most of the data on repair of DNA oxidative products *in vivo* are indirect. In Alzheimer's disease decreased repair has been indicated.[33] High vegetable consumption has been shown to reduce the genetic damage, and this has been attributed to enhancement of cytosolic glutathione transferase and DNA repair proteins by substances in tomato and carrot juices.[34] Indirectly, it is also suggested that cigarette smoking increases repair of 8-hydroxyguanine.[35] In general it is believed that most of the DNA repair enzymes are housekeeping genes that are not subjected to regulation.[32] However, it should be recognised that presently it cannot be ruled out that regulation of repair enzymes can occur.

Besides effects on DNA repair mechanisms, effects on a variety of antioxidant enzymes could be of interest, but so far there is no clear intervention study to demonstrate stimulation of these enzymes *in vivo*. However, it appears possible to develop gene therapy for oxidant induced diseases. In rats instillation of an adenovirus vector encoding human superoxide dismutase or catalase c-DNA led to expression in the lungs of human catalase and CuZn-SOD that lasted for at least 3 days. Overexpression of SOD led to increased ischaemia–reperfusion injury whereas concomitant overexpression of catalase prevented this effect.[36] Again this demonstrates the importance of multiple regulation by oxidants/antioxidants and the difficulties in predicting the outcome of intervention.

In conclusion there is clear evidence that antioxidants/oxidants are important for a multitude of different cellular functions and that there is a regulation of signal transduction pathway and gene expressions by the redox status for some cellular functions. The influence of, e.g. supplementation with an antioxidant is difficult to predict and ultimately controlled trials are needed to assess the overall effect.

References

[1] H. Wiseman and B. Halliwell (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochemical Journal*, **313**, 17–29.

[2] S.E. Browne, R.J. Ferrante and M.F. Beal (1999) Oxidative stress in Huntington's disease. *Brain Pathology*, **9**, 147–163.

[3] D. Nakae, A. Denda, Y. Kobayashi, H. Akai, H. Kishida, T. Tsujiuchi, Y. Konishi, T. Suzuki and M. Muramatsu (1998) Inhibition of early-phase exogenous and endogenous liver carcinogenesis in transgenic rats harboring a rat glutathione S-transferase placental form gene. *Japanese Journal of Cancer Research*, **89**, 1118–1125.

[4] Z. Radak, T. Kaneko, S. Tahara, H. Nakamoto, H. Ohno, M. Sasvari, C. Nyakas and S. Goto (1999) The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. *Free Radicals in Biology and Medicine*, **27**, 69–74.

[5] V. Nilakantan, B.T. Spear and H.P. Glauert (1998) Effect of the peroxisome proliferator ciprofibrate on lipid peroxidation and 8-hydroxydeoxyguanosine formation in transgenic mice with elevated hepatic catalase activity. *Free Radicals in Biology and Medicine*, **24**, 1430–1436.

[6] A. Klungland, I. Rosewell, S. Hollenbach, E. Larsen, G. Daly, B. Epe, E. Seeberg, T. Lindahl and D.E. Barnes (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proceedings of the National Academy of Science USA*, **96**, 13 300–13 305.

[7] M. D. Williams, H. Van Remmen, C. C. Conrad, T.T. Huang, C.J. Epstein and A. Richardson (1998) Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *Journal of Biological Chemistry*, **273**, 28 510–28 515.

[8] H. Esterbauer, J. Gebicki, H. Puhl and J. Gnther (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radicals in Biology and Medicine*, **13**, 341–390.

[9] J.L. Witztum (1994) The oxidation hypothesis of atherosclerosis. *Lancet*, **344**, 793–795.

[10] B.N. Ames and L.S. Gold (1991) Endogenous mutagens and the causes of aging and cancer. *Mutation Research*, **250**, 3–16.

[11] B.N. Ames, L.S. Gold and W.C. Willett (1995) The causes and prevention of cancer. *Proceedings of the National Academy of Science USA*, **92**, 5258–5265.

[12] W.J. Blot, J.Y. Li, P.R. Taylor, W. Guo, S. Dawsey, G.Q. Wang, C.S. Yang, S.F. Zheng, M. Gail and G.Y. Li (1993) Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *Journal of the National Cancer Institute*, **85**, 1483–1492.

[13] C.H. Hennekens, J.E. Buring, J.E. Manson, M. Stampfer, B. Rosner, N.R. Cook, C. Belanger, F. LaMotte, M. Gaziano, P.M. Ridker, W. Willett and R. Peto (1996) Lack of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine*, **334**, 1145–1149.

[14] The Alpha-Tocopherol and Beta-Carotene Cancer Prevention Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New England Journal of Medicine*, **330**, 1029–1035.

[15] G.S. Omenn, G.E. Goodman, M.D. Thornquist, J. Balmes, M.R. Cullen, A. Glass, J.P. Keogh, F.L. Meyskens, B. Valanis, J.H. Williams, S. Barnhart and S. Hammar (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease (see comments). *New England Journal of Medicine*, **334**, 1150–1155.

[16] L.C. Chappell, P.T. Seed, A.L. Briley, F.J. Kelley, R. Lee, B.J. Hunt, K. Parmar, S.J. Bewley, A.H. Shennan, P.J. Steer and L. Poston (1999) Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. *Lancet*, **354**, 810–816.

[17] J.M. Roberts and C.A. Hubel (1999) Is oxidative stress the link in the two-stage model of pre-eclampsia? *Lancet*, **354**, 788–789.

[18] J. Salonen, K. Nyyssonen, R. Salonen, H.M. Lakka, J. Kaikkonen, E. Porkkala-Sarataho, S. Voutilainen, T.A. Lakka, A. Rissanen, L. Leskinen, T.P. Tuomainen and H.E. Poulsen (1999) The Effect of Vitamin E and Vitamin C on Carotid Atherosclerotic Progression: The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study. *Circulation*, Suppl. 100, 1–238.

[19] H.E. Poulsen, H. Prieme and S. Loft (1998) Role of oxidative DNA damage in cancer initiation and promotion. *European Journal of Cancer Prevention*, **7**, 9–16.

[20] S. Loft and H.E. Poulsen (1999) Markers of oxidative damage to DNA: antioxidants and molecular damage. *Methods in Enzymology*, **300**, 166–184.

[21] H.E. Poulsen, A. Weimann and S. Loft (1999) Methods to detect DNA damage by free radicals: relation to exercise. *Proceeding of the Nutritional Society*, **58**, 1007–1014.

[22] H.E. Poulsen and S. Loft (1998) Interpretation of oxidative DNA modification: Relation between tissue levels, excretion of urinary repair products and sigle cell gel electrophoresis (comet assay). In: *DNA & Free Radicals: Techniques, Mechanisms and Applications*, pp. 262–270, OICA International, London.

[23] H.E. Poulsen, S. Loft, H. Prieme, K. Vistisen, J. Lykkesfeldt, K. Nyyssonen and J.T. Salonen (1998) Oxidative DNA damage *in vivo*: Relationship to age, plasma antioxidants, drug metabolism, glutathione-S-transferase activity and urinary creatinine excretion. *Free Radical Research*, **29**, 565–571.

[24] H.E. Poulsen and S. Loft (1995) Early biochemical markers of effects: enzyme induction, oncogene activation and markers of oxidative damage. *Toxicology*, **101**, 55–64.

[25] S. Loft, A. Fischer-Nielsen, I.B. Jeding, K. Vistisen and H.E. Poulsen (1993) 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. *Journal of Toxicology and Environmental Health*, **40**, 391–404.

[26] S. Loft and H.E. Poulsen (1996) Cancer risk and oxidative DNA damage in man (published erratum appears in *J. Mol. Med.* 1997 January; **75**(1): 67–8). *Journal of Molecular Medicine*, **74**, 297–312.

[27] Y.M. Janssen, B. Van Houten, P.J. Borm and B.T. Mossman (1993) Cell and tissue responses to oxidative damage. *Laboratory Investigation*, **69**, 261–274.

[28] K. Schulze-Osthoff, M.K. Bauer, M. Vogt and S. Wesselborg (1997) Oxidative stress and signal transduction. *Internation Journal for Vitamin and Nutrition Research*, **67**, 336–342.

[29] H. Kamata and H. Hirata (1999) Redox regulation of cellular signalling. *Cell Signal*, **11**, 1–14.

[30] T.P. Dalton, H.G. Shertzer and A. Puga (1999) Regulation of gene expression by reactive oxygen. *Annual Review of Pharmacology and Toxicology*, **39**, 67–101.

[31] X.D. Wang, C. Liu, R.T. Bronson, D.E. Smith, N.I. Krinsky and M. Russell (1999) Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke (see comments). *Journal of the National Cancer Institute*, **91**, 60–66.

[32] S. Boiteux and J.P. Radicella (1999) Base excision repair of 8-hydroxyguanine protects DNA from endogenous oxidative stress. *Biochimie*, **81**, 59–67.

[33] M.A. Lovell, S.P. Gabbita and W.R. Markesbery (1999) Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. *Journal of Neurochemistry*, **72**, 771–776.

[34] V.J. McKelvey-Martin, M.H. Green, P. Schmezer, B.L. Pool-Zobel, M.P. De Meo and A. Collins (1993) The single cell gel electrophoresis assay (comet assay): a European review. *Mutation Research*, **288**, 47–63.

[35] S. Asami, T. Hirano, R. Yamaguchi, Y. Tomioka, H. Itoh and H. Kasai (1996) Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. *Cancer Research*, **56**, 2546–2549.

[36] C. Danel, S.C. Erzurum, P. Prayssac, N.T. Eissa, R.G. Crystal, P. Herve, B. Baudet, M. Mazmanian and P. Lemarchand (1998) Gene therapy for oxidant injury-related diseases: adenovirus-mediated transfer of superoxide dismutase and catalase cDNAs protects against hyperoxia but not against ischemia-reperfusion lung injury. *Human Gene Therapy*, **9**, 1487–1496.

[37] M. Karin (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *Journal of Biological Chemistry*, **270**, 16 483–16 486.

[38] M. Karin and T. Smeal (1992) Control of transcription factors by signal transduction pathways: the beginning of the end. *Trends in Biochemical Sciences*, **17**, 418–422.

[39] H.S. Choi and D.D. Moore (1993) Induction of c-fos and c-jun gene expression by phenolic antioxidants. *Molecular Endocrinology*, **7**, 1596–1602.

[40] H.M. Lander, J.S. Ogiste, K.K. Teng and A. Novogrodsky (1995) p21ras as a common signaling target of reactive free radicals and cellular redox stress. *Journal of Biological Chemistry*, **270**, 21 195–21 198.

[41] B. Stein, H.J. Rahmsdorf, A. Steffen, M. Litfin and P. Herrlich (1989) UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-fos, and metallothionein. *Molecular and Cellular Biology*, **9**, 5169–5181.

[42] C. Sachsenmaier, A. Radler-Pohl, A. Muller, P. Herrlich and H.J. Rahmsdorf (1994) Damage to DNA by UV light and activation of transcription factors. *Biochemical Pharmacology*, **41**, 129–136.

[43] R.L. Whisler, M.A. Goyette, I.S. Grants and Y.G. Newhouse (1995) Sublethal levels of oxidant stress stimulate multiple serine/threonine kinases and suppress protein phosphatases in Jurkat T cells. *Archives of Biochemistry and Biophysics*, **319**, 23–35.

[44] S. Grether-Beck, R. Buettner and J. Krutmann (1997) Ultraviolet A radiation-induced expression of human genes: molecular and photobiological mechanisms. *Biological Chemistry*, **378**, 1231–1236.

[45] S.K. De, M.T. McMaster and G.K. Andrews (1990) Endotoxin induction of murine metallothionein gene expression. *Journal of Biological Chemistry*, **265**, 15 267–15 274.

[46] J.W. Bauman, J.M. McKim Jr., J. Liu and C.D. Klaassen (1992) Induction of metallothionein by diethyl maleate. *Toxicology and Applied Pharmacology*, **114**, 188–196.

[47] J.W. Bauman, C. Madhu, J.M. McKim Jr., Y. Liu and C.D. Klaassen (1992) Induction of hepatic metallothionein by paraquat. *Toxicology and Applied Pharmacology*, **117**, 233–241.

[48] J.W. Bauman, J. Liu, Y.P. Liu and C.D. Klaassen (1991) Increase in metallothionein produced by chemicals that induce oxidative stress. *Toxicology and Applied Pharmacology*, **110**, 347–354.

[49] K.S. Min, Y. Terano, S. Onosaka and K. Tanaka (1991) Induction of hepatic metallothionein by nonmetallic compounds associated with acute-phase response in inflammation. *Toxicology and Applied Pharmacology*, **111**, 152–162.

[50] T. Dalton, R.D. Palmiter and G.K. Andrews (1994) Transcriptional induction of the mouse metallothionein-I gene in hydrogen peroxide-treated Hepa cells involves a composite major late transcription factor/antioxidant response element and metal response promoter elements. *Nucleic Acids Research*, **22**, 5016–5023.

[51] T.P. Dalton, Q. Li, D. Bittel, L. Liang and G.K. Andrews (1996) Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy *in vivo* of metal response elements in the metallothionein-I gene promoter. *Journal of Biological Chemistry*, **271**, 26 233–26 241.

[52] N.M. Inamdar, Y.I. Ahn and J. Alam (1996) The heme-responsive element of the mouse heme oxygenase-1 gene is an extended AP-1 binding site that resembles the recognition sequences for MAF and NF-E2 transcription factors. *Biochemical and Biophysical Research Communications*, **221**, 570–576.

[53] J. Yang, X. Liu, K. Bhalla, C.N. Kim, A.M. Ibrado, J. Cai, T.I. Peng, D.P. Jones and X. Wang (1997) Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked [see comments]. *Science*, **275**, 1129–1132.

[54] R.M. Kluck, E. Bossy-Wetzel, D.R. Green and D.D. Newmeyer (1997) The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science*, **275**, 1132–1136.

[55] N. Zamzami, P. Marchetti, M. Castedo, C. Zanin, J.L. Vayssiere, P.X. Petit and G. Kroemer (1995) Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death *in vivo*. *Journal of Experimental Medicine*, **181**, 1661–1672.

[56] J.M. Dypbukt, M. Ankarcrona, M. Burkitt, A. Sjoholm, K. Strom, S. Orrenius and P. Nicotera (1994) Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin-secreting RINm5F cells.

The role of intracellular polyamines. *Journal of Biological Chemistry*, **269**, 30 553–30 560.

[57] Y.M. Kim, R.V. Talanian and T.R. Billiar (1997) Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. *Journal of Biological Chemistry*, **272**, 31 138–31 148.

[58] R. Larsson and P. Cerutti (1989) Translocation and enhancement of phosphotransferase activity of protein kinase C following exposure in mouse epidermal cells to oxidants (published erratum appears in *Cancer Res.* 1990 January 1; **50**(1): 212). *Cancer Research*, **49**, 5627–5632.

[59] M.K. Brawn, W.J. Chiou and K.L. Leach (1995) Oxidant-induced activation of protein kinase C in UC11MG cells. *Free Radical Research*, **22**, 23–37.

[60] G.E. Kass, S.K. Duddy and S. Orrenius (1989) Activation of hepatocyte protein kinase C by redox-cycling quinones. *Biochemical Journal*, **260**, 499–507.

[61] R. Gopalakrishna and W.B. Anderson (1987) Susceptibility of protein kinase C to oxidative inactivation: loss of both phosphotransferase activity and phorbol diester binding. *FEBS Letters*, **225**, 233–237.

[62] R. Gopalakrishna and W.B. Anderson (1991) Reversible oxidative activation and inactivation of protein kinase C by the mitogen/tumor promoter periodate. *Archives of Biochemistry and Biophysics*, **285**, 382–387.

[63] H.M. Lander, A.J. Milbank, J.M. Tauras, D.P. Hajjar, B.L. Hempstead, G.D. Schwartz, R.T. Kraemer, U.A. Mirza, B.T. Chait, S.C. Burk and L.A. Quilliam (1996) Redox regulation of cell signalling (letter). *Nature*, **381**, 380–381.

[64] H.M. Lander, D.P. Hajjar, B.L. Hempstead, U.A. Mirza, B.T. Chait, S. Campbell and L.A. Quilliam (1997) A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. *Journal of Biological Chemistry*, **272**, 4323–4326.

[65] Y. Ito, S. Nakashima and Y. Nozawa (1997) Hydrogen peroxide-induced phospholipase D activation in rat pheochromocytoma PC12 cells: possible involvement of Ca^{2+}-dependent protein tyrosine kinase. *Journal of Neurochemistry*, **69**, 729–736.

[66] V. Natarajan, S. Vepa, R.S. Verma and W.M. Scribner (1996) Role of protein tyrosine phosphorylation in H_2O_2-induced activation of endothelial cell phospholipase D. *American Journal of Physiology*, **271**, L400–L408.

[67] M.B. Toledano and W.J. Leonard (1991) Modulation of transcription factor NF-kappa B binding activity by oxidation–reduction *in vitro*. *Proceedings of the National Academy of Science USA*, **88**, 4328–4332.

[68] W. Droge, K. Schulze-Osthoff, S. Mihm, D. Galter, H. Schenk, H.P. Eck, S. Roth and H. Gmunder (1994) Functions of glutathione and glutathione disulfide in immunology and immunopathology. *FASEB Journal*, **8**, 1131–1138.

[69] D. Galter, S. Mihm and W. Droge (1994) Distinct effects of glutathione disulphide on the nuclear transcription factor kappa B and the activator protein-1. *European Journal of Biochemistry*, **221**, 639–648.

[70] K. Schulze-Osthoff, M. Los and P.A. Baeuerle (1995) Redox signalling by transcription factors NF-kappa B and AP-1 in lymphocytes. *Biochemical Pharmacology*, **50**, 735–741.

[71] Y. Devary, C. Rosette, J.A. DiDonato and M. Karin (1993) NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. *Science*, **261**, 1442–1445.

[72] N. Mohan and M.L. Meltz (1994) Induction of nuclear factor kappa B after low-dose ionizing radiation involves a reactive oxygen intermediate signaling pathway. *Radiation Research*, **140**, 97–104.

[73] K. Schulze-Osthoff, R. Beyaert, V. Vandevoorde, G. Haegeman and W. Fiers (1993) Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. *EMBO Journal*, **12**, 3095–3104.

[74] F.J. Staal, M. Roederer, L.A. Herzenberg and L.A. Herzenberg (1990) Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. *Proceedings of the National Academy of Science USA*, **87**, 9943–9947.

[75] T. Hunter and M. Karin (1992) The regulation of transcription by phosphorylation. *Cell*, **70**, 375–387.

[76] M. Karin (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *Journal of Biological Chemistry*, **270**, 16 483–16 486.

[77] H.F. Yang-Yen, R. Chiu and M. Karin (1990) Elevation of AP1 activity during F9 cell differentiation is due to increased c-jun transcription. *New Biologist*, **2**, 351–361.

[78] C. Abate, D. Luk and T. Curran (1991) Transcriptional regulation by Fos and Jun *in vitro*: interaction among multiple activator and regulatory domains. *Molecular and Cellular Biology*, **11**, 3624–3632.

[79] D.R. Edwards and L.C. Mahadevan (1992) Protein synthesis inhibitors differentially superinduce c-fos and c-jun by three distinct mechanisms: lack of evidence for labile repressors. *EMBO Journal*, **11**, 2415–2424.

[80] C. Abate, L. Patel, F.J. Rauscher III and T. Curran (1990) Redox regulation of fos and jun DNA-binding activity *in vitro*. *Science*, **249**, 1157–1161.

[81] Y.J. Suzuki, H.J. Forman and A. Sevanian (1997) Oxidants as stimulators of signal transduction. *Free Radicals in Biology and Medicine*, **22**, 269–285.

[82] C.R. Hoyal, A.P. Thomas and H.J. Forman (1996) Hydroperoxide-induced increases in intracellular calcium due to annexin VI translocation and inactivation of plasma membrane Ca^{2-}-ATPase. *Journal of Biological Chemistry*, **271**, 29 205–29 210.

[83] H. Okuno, A. Akahori, H. Sato, S. Xanthoudakis, T. Curran and H. Iba (1993) Escape from redox regulation enhances the transforming activity of Fos. *Oncogene*, **8**, 695–701.

[84] P. Hainaut, N. Rolley, M. Davies and J. Milner (1995) Modulation by copper of p53 conformation and sequence-specific DNA binding: role for Cu(II)/Cu(I) redox mechanism. *Oncogene*, **10**, 27–32.

[85] P. Hainaut and J. Milner (1993) Redox modulation of p53 conformation and sequence-specific DNA binding *in vitro*. *Cancer Research*, **53**, 4469–4473.

[86] T.R. Hupp, D.W. Meek, C.A. Midgley and D.P. Lane (1993) Activation of the cryptic DNA binding function of mutant forms of p53. *Nucleic Acids Research*, **21**, 3167–3174.