Antioxidant micronutrients and gene expression

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In recent years there has been an explosion of interest in those components of the diet which have the potential to act as antioxidants. Much of the interest has arisen from epidemiological studies which have shown that the dietary intake of key antioxidant nutrients is inversely correlated with the incidence of common chronic disorders, such as cardiovascular disease or some cancers (Gey *et al.* 1987). In addition, biochemical studies have shown that key steps in the pathogenesis of these disorders appear to involve oxidation reactions mediated by free radical species, and to be inhibited by antioxidants.

Early studies of free radical interactions with cells concentrated on the manner in which these species could damage cells, leading to loss of cell viability or mutagenic changes (for example, see Slater *et al.* 1985), but more recently there has been an increasing awareness of the complex interactions which occur between free radicals and other processes in the pathophysiological mechanisms which lead to disease (for example, see McArdle & Jackson, 1997). In an analogous manner, our understanding of the way in which antioxidants interact with cells to preserve them against the potential deleterious effect of free radicals is becoming more sophisticated as our knowledge increases. Dietary antioxidants play a major role in the complex system which the body has to regulate free radical activity in tissues, but our knowledge of the overall effects of modification of any single part of this system is poor. Many studies have assumed that an increase in the dietary intake of the commonly-studied antioxidants (vitamin E, vitamin C, β-carotene etc.), and hence an increase in the tissue concentration of these compounds, is inevitably beneficial to the cell, but recent findings, such as those describing deleterious effects of β-carotene in carefully-controlled supplementation studies (Woodall *et al.* 1996), has raised doubts about this approach.

One possible explanation for these unexpected effects of antioxidant supplements is that the antioxidant micronutrients are having metabolic effects unrelated to their ability to inhibit free radical reactions, and there is widespread evidence that, for example, β-carotene has effects on cell–cell interaction (Zhang *et al.* 1991) and immune status (Bendich & Olsen, 1989). Additionally, it is becoming increasingly apparent that free radicals not only damage cells, but induce the cell to respond in a coordinated manner by changes in gene expression. Indeed, there is evidence that in some circumstances free radicals (or more generally oxidative reactions) may be involved in the physiological regulation of key changes in gene expression. Since antioxidants are able to regulate these free radical-mediated processes, they are likely to be important modulators of the expression of genes which are regulated in this manner.

The present short review will describe some of the work which underpins the idea that antioxidant micronutrients may regulate expression of key genes, and describe some potential situations where antioxidants may play an important role in regulation of gene expression *in vivo*.

Oxidative stress as a regulator of gene expression

Cells exposed to a non-lethal increase in free radical activity, induced by either environmental insult (e.g. H_2O_2 treatment) or a specific intracellular generation mechanism (e.g. tumour necrosis factor α-induced intracellular reactive oxygen species) have been shown to respond to these increases by the induction of a number of genes. These responses can be placed into two overlapping groups, the cellular adaptation to free radical insult, and the use of free radicals as intracellular messengers. The mechanism of induction of these genes and the absolute number of genes involved remains to be clarified. However, various studies have demonstrated some specific signalling pathways used and the genes which are activated.

The genes induced by increased free radical activity are activated by similar pathways, but the exact pathway differs between genes. Generally, an increase in free radical activity triggers a signal transduction pathway which terminates in the production of active transcription factors. These factors are then able to bind to the promoter region of their target genes and activate transcription. It would also appear that the final event of transcription factor binding to DNA can be modulated by the redox status of the cell.

The present review will concentrate on three transcription factors, nuclear factor kappa B (NFκB), activator protein-1 (AP-1), and heat shock factor (HSF), all of which have been shown to be modulated by oxidative stress (Fig. 1).

Abbreviations: AP-1, activator protein-1; HSF, heat shock factor; HSP, heat shock protein; NFκB, nuclear factor kappa B.

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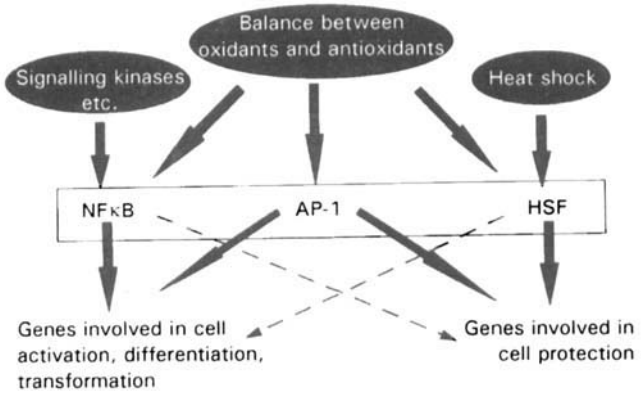

Fig. 1. Schematic representation of the effects of oxidants and antioxidants on transcription factors and resultant cellular adaptation. NFκB, nuclear factor kappa B; AP-1, activator protein-1; HSF, heat shock factor. (Redrawn from Storz & Polla, 1996.)

Nuclear factor kappa B

NFκB was first described as a B-cell specific factor; however, it has become apparent that it is functional in a wide range of cell types (Storz & Polla, 1996). NFκB can be activated by many stimuli, involving redox-sensitive and -insensitive pathways (Anderson *et al.* 1994; Jabber *et al.* 1994), and it has also been demonstrated that redox activation of NFκB can be blocked by various antioxidants (Storz & Polla, 1996). The genes activated by NFκB are diverse and include many used by the immune system; however, NFκB (perhaps in conjunction with AP-1, see p. 302) is responsible for the induction of Mn superoxide dismutase (*EC* 1.15.1.1), an important mitochondrial antioxidant enzyme (Das *et al.* 1995).

NFκB resides within the cytoplasm of cells in an inactive form and, on activation, involving phosphorylation of its suppresser subunit (known as IκB), moves to the nucleus and activates target genes (Latchman, 1995). Oxidative stress can result in NFκB-directed regulation of gene expression in at least two ways. First, it has been suggested by Whistler *et al.* (1994) that H_2O_2 can produce a direct conformational change in protein kinases at the start of the pathway which ultimately leads to phosphorylation of inhibitor IκB and activation of NFκB. This 'active' NFκB contains redox-sensitive cysteine residue(s) within its DNA-binding domain, and *in vitro* experiments have demonstrated that NFκB binding to DNA can be inhibited by reactive oxygen species. These two regulatory points in the NFκB activation pathway provide a rapid and sensitive method of NFκB activation and attenuation.

Activator protein-1

Like NFκB, AP-1 is a ubiquitous transcription factor capable of inducing a large number of genes. These genes are dependent on the cell type, age and stimulus; however, most are thought to be related to the control of proliferation and differentiation of cells (Latchman, 1995). AP-1 is a dimeric protein complex which comprises a number of related proteins of the Jun and Fos proto-oncogene families. AP-1 can consist of a Jun–Jun homodimer, or a Fos–Jun heterodimer and these have different DNA-binding affinities (Latchman, 1995).

The mechanisms of activation of AP-1 are similar to that of NFκB in that many stimuli involving redox-sensitive and -insensitive pathways are capable of producing active AP-1, and redox-sensitive activation can be blocked by low concentrations of hydrophilic antioxidants. However, in contrast to NFκB, if the levels of intracellular antioxidants are increased above a certain level, they act as a stimulus for AP-1 activation (Storz & Polla, 1996). Redox control of AP-1 also uses the activation of protein kinase (*EC* 2.7.1.37)–protein phosphatase (*EC* 3.1.3.16) pathways, but in this case activation is accompanied by the direct dephosphorylation of the Jun protein of AP-1 (Oehler *et al.* 1993). Once activated, the AP-1 complex is then capable of self induction by binding to promoter regions within the *c-jun* and *c-fos* genes. As with NFκB, there is a redox-sensitive cysteine residue within the DNA-binding domains of both Jun and Fos and this may also have a role in attenuating AP-1 activity (Oehler *et al.* 1993; Okuno *et al.* 1993).

It is interesting to note that the gene coding for Mn superoxide dismutase, an enzyme critical in maintaining the oxidative status of the cell, has response elements to both AP-1 and NFκB within its promoter region. Thus, a negative feedback loop may exist between AP-1 and NFκB and the genes they induce.

It should also be noted that NFκB and AP-1 are ubiquitous transcription factors involved in activating many more genes than those involved in cellular adaptation to oxidative stress. Thus, any attempt to modulate their activity by dietary antioxidants should be considered in the light of other processes in which they may be involved.

Heat shock factor

Cells respond to stress by the increased synthesis of a family of proteins known as stress proteins or heat shock proteins (HSP). These proteins were initially named due to their increased expression following a non-damaging hyperthermia of cells, but it is now known that their expression increases following a wide variety of cellular stresses (Voellmy, 1996). HSP are named according to their molecular weight and include the small HSP, HSP60, HSP70, HSP90 and HSP110. In a non-stressed cell, HSP associate with newly-synthesized proteins, preventing misfolding and aggregation and facilitating their transport to distant subcellular sites of action (Fig. 2). Increased intracellular content of HSP is thought to confer significant protection against subsequent cellular stress by associating with and stabilizing proteins until the conditions within the cell become more favourable. HSP are also able to resolubilize aggregated proteins, returning them to their active state (Li & Nussenzweig, 1996).

Increased transcription of heat shock genes following stress is mediated by HSF. HSF are activated by a wide range of stresses, such as hyperthermia, ischaemia–reperfusion, changes in intracellular pH, viral infection and inhibition of energy metabolism. The vast majority of chemical and physiological inducers of the stress response

result in the production of abherant, non-native proteins within the cell (Voellmy, 1996). Free radicals play a major role in this induction, either by direct interaction with cellular proteins, or indirectly by changing the redox state of the cell. Thus, a heat shock response has been described following changes in the cellular GSSG : GSH values, or changes in protein thiolation (Voellmy, 1996). Several different HSF have been isolated from various species and named HSF1–4. In the present review, we will concentrate on HSF1 and HSF2, known to be expressed in vertebrates (for review, see Morimoto *et al.* 1996). The different HSF appear to respond to different stimuli in different ways. HSF1 is the most potent transcriptional activator (Sistonen *et al.* 1992) and activity corresponds to the rapidly-activated stress response. HSF2 appears to be more associated with developmental changes in gene expression (Sistonen *et al.* 1992; Morimoto *et al.* 1996). HSF1 contains cysteine residues which may predispose this factor to detecting oxidative stress, whereas HSF2 contains threonine and tyrosine residues, suggesting a phosphorylation–dephosphorylation-mediated activation. HSF1 can also be phosphorylated at serine residues, although the role of this phosphorylation is not clearly understood (Sarge *et al.* 1993; Cotto *et al.* 1996; Morimoto *et al.* 1996).

In non-stressed cells, HSF1 is found as an inactive monomer in both the cytoplasm and the nucleus (Sarge *et al.* 1993). Within seconds of a stressful event, HSF1 forms a homotrimer which binds to the heat shock promoter element of the heat shock genes, although oligomerization and DNA binding alone may not result in transcriptionally competent HSF (Zuo *et al.* 1995; Voellmy, 1996).

A study of current literature has led to the proposal of the following scheme of events (Morimoto *et al.* 1996). In the unstressed cell, HSF1 is weakly associated with HSP70. Stress leads to the production of increased levels of misfolded proteins and HSP70 has a higher affinity for binding these destabilized proteins. This sequestration of HSP70 releases HSF1 from its inactive state allowing translocation of the monomer to the nucleus. On translocation, HSF1 forms a trimer and binds to the heat shock promoter element of the DNA. This complex remains transcriptionally inactive until HSF1 is phosphorylated and transcription of target genes ensues. As the stress is removed, HSP70 re-associates with HSF1 and facilitates release of HSF1 from the heat shock promoter element. HSF1 trimers are converted back to monomers and the monomers are once again associated with HSP70. Thus, cellular repair has a negative feedback effect on activated HSF1.

In summary, the redox state of the cell plays a major role in the induction of the stress response and changes in the redox state of the cell may interfere with this.

It may appear that NFκB and AP-1 act independently of HSF. However, adaptation of the cell is dependent on all three. For example, although NFκB and AP-1 are capable of inducing an increased expression of Mn superoxide dismutase, this is a mitochondrial enzyme encoded in the nuclear genome. In the hostile environment of an oxidatively-stressed cell, a newly-synthesized enzyme needs protection from oxidative damage and to be chaperoned to its site of action within the mitochondria. This role is fulfilled by the stress-induced HSP.

There are, therefore, clear indicators that substances which influence the redox state of the cell can modulate expression of genes activated by these transcription factors. Evidence that changes in the dietary intake of the antioxidant nutrients can mediate such changes at the cellular level in man, however, is lacking. We are currently studying two areas in which there is some evidence that free radicals play a role in the cell responses to stress, and in which there is increasing evidence that antioxidants may play a regulatory role.

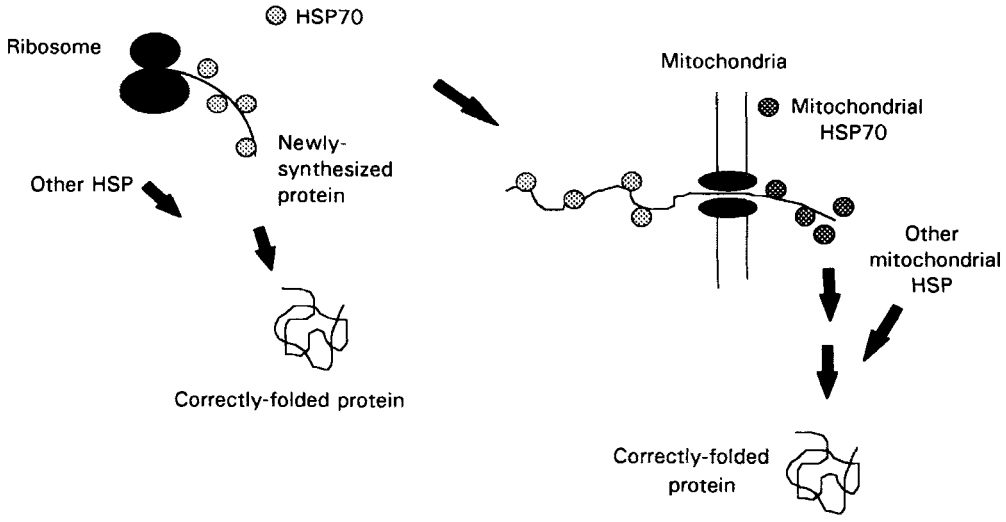

Fig. 2. Schematic representation of the role of heat shock proteins (HSP) as molecular chaperones facilitating both the correct folding of newly-synthesized proteins and their translocation to intracellular organelles.

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u.v.-Induced changes in skin cell proliferation

Acute exposure of human skin to u.v. light causes localized erythema and chronic exposure leads to skin ageing and cancer. The mechanisms underlying these u.v.-induced changes are unknown, but cell-culture studies indicate that u.v. exposure causes rapid activation of a number of genes involved in the control of cell proliferation. Thus, expression of the regulatory oncogene (tumour suppressor gene) P53 has been reported to be up-regulated in cells exposed to u.v.B (Fritsche *et al.* 1993), as is expression of early-response genes such as *c-fos* and the transcription factors AP-1 and NFκB (Cerutti, 1991; Devary *et al.* 1992). A number of studies indicate that many of these changes are mediated by u.v.-generated free radicals (Cerutti, 1991; Devary *et al.* 1992; Shah *et al.* 1993), although this has only been demonstrated in cell culture. The effect of common dietary antioxidants on these systems does not appear to have been extensively studied.

There is some evidence that similar mechanisms play a role in human skin *in vivo*. u.v. Exposure appears to induce a rapid elevation in indicators of free radical activity in human skin, associated with a depletion of the major lipid-soluble antioxidant, vitamin E (Wilton *et al.* 1995). Furthermore, the erythemal response to u.v. was associated with a loss of skin polyunsaturated fatty acids by lipid peroxidation, and was reduced by supplementation of subjects with *n*-3 polyunsaturated fatty acids (Rhodes *et al.* 1994). In this situation *n*-3 polyunsaturated fatty acids appear to be acting as an oxidizable buffer, preferentially reacting with u.v.-generated free radicals, and thus protecting other cellular constituents from damage. There is evidence, therefore, that free radicals appear to mediate some of the direct damaging effects of u.v.B light, although the potential effect of dietary antioxidants on the adaptive changes in gene expression was not studied. Alternative cell-culture studies appear to indicate that dietary antioxidants can modify the adaptive response of fibroblasts to u.v. light (Jones *et al.* 1998), but confirmation of similar effects *in vivo* is lacking.

Exercise and expression of muscle stress protein

There is now extensive literature indicating that certain forms of exercise lead to increased free radical production in skeletal muscle (for review, see Jackson, 1996). Most studies have examined the possibility that this increased activity leads to damage to muscle cells, but recent findings indicate that exhaustive exercise results in an increase in the synthesis of muscle stress protein, and this has been reported to be triggered by the increased oxidative stress associated with exercise (Salo *et al.* 1991; Locke & Noble, 1995). All cells respond to oxidative and other stresses by up-regulation of stress proteins or HSP. These proteins provide short-term cytoprotection to the cell and facilitate adaptations, including the up-regulation of antioxidant enzymes. In tissues such as the heart, exposure to a short period of sub-lethal oxidative stress (induced by ischaemia and reperfusion) results in protection against a subsequent bout of (normally lethal) oxidative stress (induced by a long period of ischaemia and reperfusion). This has been attributed to the stimulation of increased expression of HSP (Currie *et al.* 1988; Donnely *et al.* 1992; Yellon *et al.* 1992; Marber *et al.* 1995). Our recent findings show that a short period of exercise leads to an increase in production of stress protein and provides protection against subsequent excessive exercise (McArdle *et al.* 1995*a*, 1997), suggesting similar mechanisms may occur in skeletal muscle.

An important initiator of the stress response is oxidative stress. In particular, oxidation of protein thiol groups results in the induction of stress proteins (Freeman *et al.* 1995). Recent findings have also indicated that oxidation of protein thiol groups occurs rapidly in skeletal muscle following exercise, suggesting a possible role in stimulation of production of HSP (McArdle *et al.* 1995*b*). However, previous supplementation of animals with vitamin E suppressed these changes. Again, evidence for equivalent effects of dietary antioxidants in man is lacking.

Conclusions

The present short review has highlighted the basic role that redox regulation can play in the activation of key transcription factors, and pointed out example situations where such processes may be important *in vivo*. The possibility that changes in the dietary intake of antioxidant micronutrients can influence these processes in man is the subject of considerable speculation, but firm evidence for such a role is currently awaited.

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