Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Theoretical Biology 225 (2003) 531–540
Journal of Theoretical Biology
www.elsevier.com/locate/jtbi

A unifying view of ageing and disease: the double-agent theory

Nick Lane*

Department of Surgery, Royal Free and University College Medical School, Pond Street, LondonNW3 2QG, UK

Received 17 June 2003; received in revised form 24 July 2003; accepted 29 July 2003

Abstract

The quest for therapies based on molecular genetics (pharmacogenomics, DNA microarrays, etc.) drives pharmaceutical research into individual diseases of old age, but has failed to deliver an unequivocal clinical breakthrough. Attempts to treat most age-related diseases using antioxidant supplements have been equally disappointing, despite the clear benefits of a healthy diet. The double-agent theory is a new, unifying synthesis that draws on flaws in three leading theories of ageing. It argues that there is a tradeoff between oxidative stress as a critical redox signal that marshals genetic defences against physiological stress (such as infection) and oxidative stress as a cause of ageing and age-related disease.

The stress response and ageing are linked by redox-sensitive transcription factors, such as NFκB. Ageing is a function of rising intracellular oxidative stress, rather than chronological time, but this relationship is obscured because free-radical leakage from mitochondria also tends to rise with age. Mitochondrial leakage produces a genetic response which mirrors that following infection, but because mitochondrial leakage is continuous the shift in gene expression is persistent, leading to the chronic inflammation characteristic of old age. Age-related diseases are thus the price we pay for redox control of stress-gene expression. Because the selective pressure favouring the stress response in youth is stronger than that penalising degenerative diseases after reproductive decline, we may be homeostatically refractory to antioxidant supplements that 'swamp' the redox switch. Furthermore, because genetic selection takes place predominantly in the reductive homeostatic environment of youth, alleles associated with age-related diseases are not inherently damaging (they do not inevitably express a negative effect over time), but are simply less effective in the oxidising conditions of old age. Gene therapies for age-related diseases are unlikely to succeed unless oxidative stress can be controlled physiologically, thereby altering the activity and function of potentially hundreds of genes.

Keywords: Double-agent theory; Oxidative stress; Mitochondria; Ageing; Redox switch

1. Introduction

Age-related diseases are often considered to be distinct pathologies, rather than an inevitable part of 'normal' ageing. We seek genes or other factors that raise our susceptibility to a disease and celebrate completion of the human genome project because it provides us with more detailed information about which genes can be held 'responsible'. In this paper I put forward a contrary view. I argue that ageing and age-related diseases are linked by a simple but refractory evolutionary tradeoff. The diseases of old age are the price we pay for the way that we are set up to handle physiological stresses, such as infections, in our youth. Infections and age-related diseases are linked by oxidative stress (an imbalance in the production and elimination of oxygen and nitrogen free radicals, and other related species) but the outcomes are opposed: resistance to stress in youth and vulnerability to disease in old age. The duplicitous role of oxidative stress is central to each so I term the hypothesis the double-agent theory (Lane, 2002).

Although the idea that oxidative stress is important in ageing is an old one, the double-agent theory is novel in that it reconciles two apparently contradictory positions, both of which are supported by a wealth of circumstantial evidence: (i) that rising oxidative stress is a primary cause of ageing (Halliwell and Gutteridge, 1999); and (ii) that antioxidants do not prevent ageing (Gutteridge and Halliwell, 2000; Parthasarathy et al., 2001). When taken together, these two unambiguous statements are usually interpreted to mean that (i) oxidative stress is only one factor among many that

*Tel.: +44-20-7794-0500x4190.
E-mail address: n.lane@rfc.ucl.ac.uk (N. Lane).

0022-5193/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0022-5193(03)00304-7

contribute to ageing, and (ii) that antioxidants are only one factor among many that help to slow down the process, hence the minimal perceived effect. The double-agent theory contradicts this position, stating instead that (i) rising oxidative stress is a primary cause of both ageing and age-related diseases; and (ii) that antioxidant supplements do not and cannot slow ageing in people with a balanced diet. From this new perspective, it is also clear that the aim of gene therapy and pharmacogenomics as a treatment for age-related diseases is misguided, and will not deliver substantial health benefits in old age: the negative effects of most 'susceptibility' genes depend on the redox state of their intracellular environment and not on inherent differences in sequence per se.

2. Strengths and weaknesses of the main theories of ageing

Of the numerous theories of ageing, only three are widely credited by evolutionary biologists and gerontologists. Evolutionary biologists often refer to the theory of antagonistic pleiotropy and the disposable soma theory. Each provides an evolutionary rationale for ageing, but neither specifies a proximate mechanism. In contrast, gerontologists often cite the free-radical theory of ageing, which offers a proximate mechanism—accumulating wear and tear—but not an evolutionary rationale; the theory was explicitly criticised as 'non-evolutionary' by Rose (1991). All three theories are backed by strong evidence, but marred by flaws. The double-agent theory not only explains these flaws, but also connects the three theories in a simple and satisfying manner.

The theory of antagonistic pleiotropy was introduced by Williams (1957). The basic idea is that selection pressure falls over time, as the cumulative risk of 'accidental' death rises. In other words, it pays to reproduce quickly, before death (by predation, disease or accident) makes reproduction statistically unlikely. An organism that focuses its resources on reproducing in youth will therefore be more likely to pass on its genes than one that does not focus its energies in this way. Genes that offer an advantage in youth but are detrimental later on (i.e., antagonistic pleiotropy) will be selected if the early advantage outweighs the later disadvantage.

This theory explains why some genetic polymorphisms are linked with age-related diseases, but presents ageing as little more than the cumulative effect of many late-acting detrimental alleles. There are two difficulties with this view. First, it implies the *rate* of ageing should be fairly inflexible: to extend life requires the coordinated mutation of numerous genes, each of which might have a detrimental effect on vigour in youth—and this should take many generations even in a large population. In fact, not only is lifespan quite flexible in animals (for example, the lifespan of *Drosophila* can be doubled by selection over a few generations (Rose, 1999) but single genes can have a big effect on the rate of ageing. Thus, mutations in *age-1*, *daf-2* and *daf-16* double or even treble the lifespan of nematode worms (Kimura et al., 1997; Morris et al., 1996; Ogg et al., 1997). Homologous genes exert similar, albeit less pronounced, effects in *Drosophila* (Clancy et al., 2001).

The second difficulty with the theory of antagonistic pleiotropy is that it implies that the diseases of old age are fundamentally genetic in nature, and that they should ultimately be found to be caused by particular polymorphisms or combinations of polymorphisms. In reality, most susceptibility genes have a limited penetrance, and the entire genetic contribution is often less than 50% (Finch and Tanzi, 1997). Most age-related diseases are at least influenced by poorly defined 'environmental' factors (Finch and Tanzi, 1997). The theory of antagonistic pleiotropy fails to address the relationship between genes and environment—what is the common factor linking environmental and genetic causes of disease? Antagonistic pleiotropy therefore offers an unsatisfactory explanation of age-related diseases and a poor explanation of the plasticity of the underlying ageing process.

The disposable soma theory was proposed by Kirkwood (1977). This is sometimes claimed to be a special case of antagonistic pleiotropy, but in fact suffers the opposite problems. The theory argues that there is a tradeoff between resources dedicated to reproduction, and those earmarked for survival. Given finite resources, the more an animal expends on bodily maintenance, the less it can expend on reproduction, and vice versa (Holliday, 1995). In general there is a good negative correlation between the fecundity and lifespan of different species. If lifespan rises over generations, fecundity falls. For example, Austad has shown that island opossums shielded from predation for approximately 5000 years age more slowly than opossums exposed to predation on the mainland—but they also have fewer offspring per litter (Austad, 1993). The disposable soma theory implies that there should be a direct correlation between longevity and the resources dedicated to cellular and bodily maintenance, and this is broadly true. Thus, cells cultured from long-lived species have better stress resistance than those from short-lived species (Kapahi et al., 1999; Kirkwood et al., 2000). Similarly, in nematode worms, *age-1* and *daf-2* induce the expression of genes that confer stress resistance (Honda and Honda, 1999; Barsyte et al., 2001), doubling lifespan at the cost of fecundity, which falls by 75% in the case of some *age-1* mutants (Friedman and Johnson, 1988). The 'Indy' (I'm not dead yet) mutation in drosophila apparently does prolong lifespan

without affecting fecundity or organismal performance, though only under favourable conditions (Marden et al., 2003).

The disposable soma theory therefore presents ageing as the outcome of a resource-use dilemma and shows why the rate of ageing should be flexible given a shift in resource use, as happens in calorie restricted mammals (Speakman et al., 2002; Barja, 2002a) and Indy mutants (Marden et al., 2003). The trouble with this is that the disposable soma theory does not explain the contribution of individual 'susceptibility' genes to age-related diseases. Unlike the theory of antagonistic pleiotropy, it does not explain why certain alleles should exert negative effects later in life in some people but not in others. Why should one person suffer from heart disease, and another from cancer, and a third from nothing at all, while all three age at much the same rate? What is it about the underlying process of ageing that unmasks the late-acting negative effects of some, but not other, polymorphic alleles? A general shift in resource use might be expected to slow the rate of ageing, and delay the onset of age-related diseases in general, but the quirky pattern of susceptibility to diseases that we actually experience does seem to be related to our particular genetic dispositions, even if the 'environment' plays an important role. While the contribution of 'susceptibility' genes can be explained by antagonistic pleiotropy, the disposable soma theory has little practical to say about the real clinical problem of age-related diseases.

This brings us to the free-radical theory of ageing, which is better known to gerontologists. This might be seen as a proximate mechanism of the disposable soma theory. If the trick to longevity is maintaining bodily integrity, then the question arises, against what? Of all the contenders, the most successful has been oxygen free radicals, generated during metabolism. The rate of ageing generally varies with metabolic rate (Speakman et al., 2002). Birds are often cited as an exception, as they live up to ten times longer than mammals with equivalent basal metabolic rates (Holliday, 1995), but this objection is false: Barja and colleagues have shown an impressively tight correlation between the rate of free-radical leakage and lifespan in numerous birds and mammals (Barja, 1998). In other words, it is not the metabolic rate that is important but the rate of leakage of free radicals, and this, in general but not always, tends to be faster in metabolically active species (Barja, 2002b). In birds, where a fast metabolic rate is coupled with an anomalously long lifespan, the discrepancy is explained by 'well-sealed' mitochondria, which leak up to ten-fold fewer free radicals in vitro (Barja, 1998, 2002b). The selective pressure to improve the 'sealing' of mitochondria in birds probably had nothing to do with ageing, but rather the requirement for respiratory efficiency to achieve the high power to weight ratio necessary for flight; gram per gram, the metabolic rate of a hovering hummingbird is 10 times greater than that which can be achieved by human athletes exercising at their maximum aerobic metabolic rate, $\dot{V}O_{2max}$ (Maina, 2000; Suarez, 1998).

An attractive aspect of the free-radical theory is that it can potentially explain both ageing and age-related diseases. Free radicals are incriminated in the pathogenesis of many degenerative diseases, including diabetes cardiovascular disease, cancer and Parkinson's disease (Aviram, 2000; Kovacic and Jacintho, 2001; Maassen et al., 2002; Preston et al., 2001; Poulton, 1998; Schapira, 2002; van der Walt et al., 2003). The importance of mitochondria in both ageing and age-related disease is testified by research from Tanaka et al. (1998) who showed that nearly two-thirds of Japanese centenarians have a mitochondrial gene variant known as Mt5178A. This variant codes for a subunit of NADH dehydrogenase, at complex I of the respiratory chain. Not only were people with the Mt5178A variant more likely to survive to a hundred, but they were half as likely to be hospitalised for *any* age-related disease as people without the variant: a strong link between ageing and age-related disease (Tanaka et al., 1998). Given that complex I is a major source of superoxide radicals in vitro (Barja, 2002b), it may be that the Mt5178A variant is associated with a low leakage of free radicals from mitochondria. This hypothesis is supported by further work from Tanaka's group, showing that women with the Mt5178A variant have fewer mitochondrial DNA mutations in their oocytes, which suggests that the variant is indeed associated with less free-radical leakage (Tanaka et al., 2000). Interestingly, the substitution of an A for a C at Mt5178 results in a change in the amino acid sequence from leucine to methionine. The sulphur moiety of the methionine residue is relatively easily oxidised to the sulphoxide, which can be reduced back to methionine by the abundant enzyme methionine sulphoxide reductase, pin-pointing antioxidant protection to a critical point of free radical escape from the respiratory chain (Levine et al., 1996b, 1999; Tanaka et al., 2000). Although Mt5178A is not common outside Japan, it is not the only mitochondrial variant to be associated with longevity: several other mitochondrial DNA variants have since been detected at relatively high levels in centenarians (de Benedictis et al., 1999; Zhang et al., 2003); whether they, too, restrict oxidative damage is a moot point .

The idea that free radicals cause ageing and age-related disease frames the well-known but ultimately disappointing hypothesis that antioxidants should prolong lifespan. Half a century of frustration has convinced most impartial researchers that, while antioxidant supplements may correct for dietary deficiencies, they have little impact on the underlying rate of

ageing in people with a well-balanced diet (Gutteridge and Halliwell, 2000; Lane, 2002; Parthasarathy et al., 2001). Today, most researchers would concur with Gutteridge and Halliwell (2000): "By the 1990s it was clear that antioxidants are not a panacea for ageing and disease, and only fringe medicine still peddles this notion." So the problem with the free-radical theory is that, in its usual formulation, it has failed to stand up to experimental verification—a damning indictment of any theory.

In summary, then, the theory of antagonistic pleiotropy partially explains age-related diseases but not ageing; the disposable soma theory explains ageing but not age-related diseases; and the free-radical theory may explain both ageing and age-related diseases, but the poor efficacy of antioxidant supplements undermines its explanatory power. The double-agent theory draws on the flaws in each of these theories to explain the link between ageing and age-related diseases (it is not a novel theory of ageing).

3. The double-agent theory of ageing and disease

The double-agent theory posits that a change in redox state—a reversible rise in oxidative stress—is required to trigger the stress and inflammatory response in youth, and so is evolutionarily conserved. Numerous transcription factors, including SoxRS, OxyR, NFκB, AP-1, Nrf-2 and P53, are known to be redox sensitive, that is, their activity is governed by redox-sensitive groups like thiols, which can be oxidised or nitrosylated by oxygen and nitrogen free radicals (Arrigo, 1999; Barouki and Morel, 2001; Gonzalez-Flecha and Demple, 2000; Ishii et al., 2000; Karin et al., 2001; Martindale and Holbrook, 2002). In effect, these free radicals behave like smoke, activating a smoke detector (the redox-sensitive transcription factor) in the event of a fire (infection or other physiological stress). The smoke detector activates the fire service (the genetic stress and inflammatory response) to quell the flames. Just as it is critical to the safety of a building to detect smoke as a surrogate for fire, so too it is critical for cells, and the body as a whole, to detect free radicals as a surrogate for infection or other physiological stress. To smother free radicals with antioxidants is to deactivate the fire alarm.

During ageing, oxidative stress rises insidiously, as free radicals leak continuously in the course of mitochondrial respiration, and slowly undermine cellular integrity (Harman, 1972; Kirkwood and Kowald, 1996; Lane, 2002). Free-radical damage to mitochondrial proteins and DNA probably exacerbates leakage and oxidative damage to the cell in general (Sohal, 2002). In effect, there is smoke but no fire (free radicals but no infection). The fire service is activated nonetheless, to dispatch the phantom target. The same transcription factors that rally our genetic response to stress and infection bring about a shift in gene expression in response to ageing. Because mitochondrial leakage cannot be resolved (at least not easily) the inflammation is chronic (Fig. 1) and this in itself exacerbates oxidative stress. However, as these negative effects take place in old age, they exert less selective pressure than the positive benefits of redox signalling in youth.

It is important to distinguish between the intracellular and extracellular compartments. Stress-gene transcription is influenced by the redox state inside the cell, which may respond to external oxidative stress, inflammatory mediators or infection, but also reflects internal factors such as mitochondrial leakage. The difficulty is that the body acts to maintain a flexible and responsive *intracellular* redox poise, enabling a swift genetic response to stress. In other words, the body as a whole prevents the intracellular redox state from being 'swamped' by antioxidant supplements. As we age, the intracellular redox state tends towards the oxidised, regardless of extracellular conditions (such as infection or chronic inflammation), as free-radical leakage from mitochondria increases from within, while the cell is 'buffered' from without via a homeostatic regulation of intracellular antioxidant levels. This means that the primary cause of inflammation with advancing age is the endogenous stress response, induced by the loss of redox poise within cells.

By placing oxidative stress at the centre of ageing and disease, the double-agent theory resolves the difficulties with the theories discussed above, making predictions of its own. First, the inflexibility implied by antagonistic pleiotropy depends on *time*—time to accidental death, time to reproduction, time to low selection pressure, etc.—which are set by selection and so written in the genetic sequence (Williams, 1999, 2000). This means that the rate of ageing can only change slowly over generations of selection. But if the negative effects of pleiotropic genes depend on oxidative stress, not time, then to prevent age-related diseases we must lower oxidative stress. The double-agent theory argues that beneficial polymorphic alleles are selected in the *reductive* homeostatic environment of youth. Persistent oxidative stress in old age changes the behaviour of gene products that would be beneficial under reductive conditions, leading to patterns of vulnerability to disease that we ascribe to late-acting 'susceptibility' genes. This means that genetic susceptibility to age-related disease is unmasked by advancing biological, not chronological, age. This unifying explanation also explains how 'environmental' factors such as smoking and inadequate diet may contribute to 'genetic' risk: any factors that persistently raise oxidative stress can potentially unmask the negative effects of susceptibility genes. Presumably the best way to redress these negative effects is not via

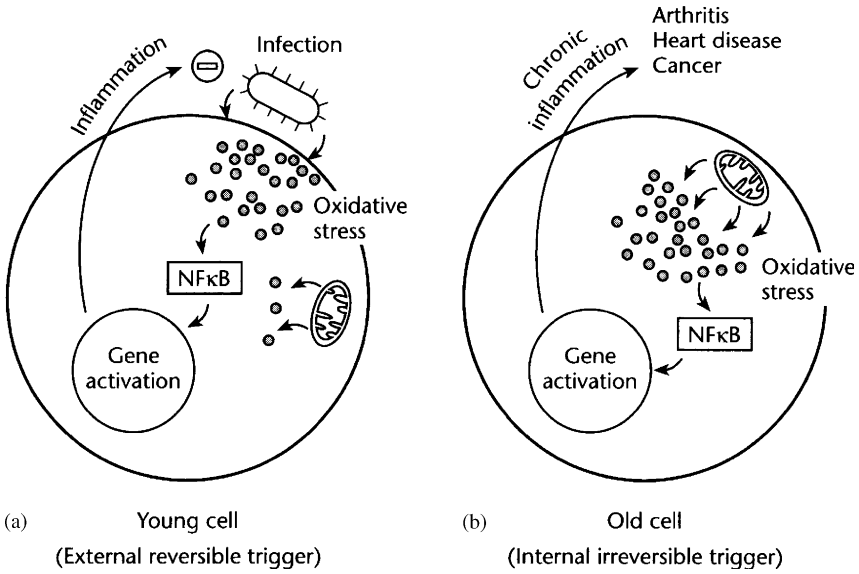

Fig. 1. Schematic representation of the double-agent theory. In the young cell (a), infections or stress outside the cell (depicted by the bacterium) elicit a rise in *intracellular* oxidative stress, which activates NFκB (and other redox-sensitive transcription factors, not shown here), leading to transcription of stress and inflammatory genes, such as nitric oxide synthase and haem oxygenase-1. Importantly, the young cell (a) represents any cell-type, including epithelial cells and immune cells: the actual genetic response varies according to the cell-type, but the underlying activation of stress genes via a rise in oxidative stress occurs in epithelial cells, endothelial cells, phagocytes and lymphocytes. Active inflammation outside the sensor cell resolves the infection, and intracellular oxidative stress returns to normal. In the old cell (b), an equivalent rise in oxidative stress is brought about by free-radical leakage from mitochondria within the cell, which also activates NFκB (and other transcription factors) and the inflammatory response. In this case, it is not possible to resolve the trigger, so the inflammatory response is chronic. Continuous oxidative stress shifts gene expression, and this brings about the negative effects of 'susceptibility' genes, rather than the polymorphisms themselves. Because oxidative stress is critical to our recovery from infections in youth it is positively selected by natural selection, to our own detriment in old age. Figure reproduced from Oxygen: The Molecule that made the World, by Nick Lane, with permission from Oxford University Press.

pharmacogenomics or gene therapy, but by lowering oxidative stress in a physiological manner.

Second, the disposable soma theory does not indicate a proximate mechanism to connect the underlying ageing process with age-related diseases. Oxidative stress offers such a mechanism. If genetic susceptibility to age-related disease depends on oxidative stress, but not time, then similar 'genetic' diseases will occur at different times in different species, when the level of oxidative stress is similar. This is true. Old mice suffer similar ailments to old people: their joints stiffen, their skin wrinkles, they lose their ability to remember and learn, their immune system degenerates, and they have a rising incidence of heart disease and cancer; but mice pass through the sequence of age-related changes in 4 years, whereas we take 70 (Holliday, 1995). Smaller variations take place within species. Radiation poisoning and smoking speed up the rate of ageing, as well as the likelihood of age-related diseases like cancer and heart disease (Ross et al., 2001). Accelerated ageing syndromes, such as Werner's syndrome, are associated with an early onset of age-related diseases, including cataracts, muscle atrophy, bone loss, diabetes, atherosclerosis and cancer (Gray et al., 1997). Conversely, a balanced diet delays the onset of many age-related diseases, including heart disease and cancer (Key et al., 1996). So too does calorie restriction—which in rodents at least slows the decline in physical activity, behaviour, learning, immune response, enzyme activity, gene expression, hormone action, protein synthesis and glucose tolerance (Merry, 2000). The double-agent theory postulates that bodily maintenance equates to the maintenance of redox poise; and when this is lost, rising oxidative stress unmasks the negative effects of late-acting alleles.

Third, the double-agent theory explains the failure of antioxidant therapies to delay ageing and disease in people with a balanced diet (Lane, 2002; Gutteridge and Halliwell, 2000; Parthasarathy et al., 2001). If crossing a threshold of oxidative stress is *necessary* to elicit a stress response, then antioxidant loading should in principle suppress the stress response and delay resolution of acute infections or other physiological stress—a disadvantage likely to have been selected against, for example by favouring the evolution of homeostatic mechanisms that regulate the levels of antioxidants in the body. Thus, the double-agent theory predicts that we should be actively *refractory* to antioxidant loading, hence the minimal perceived effect of antioxidant supplements.

It is critical to realise that stress proteins like nitric oxide synthase (NOS) and haem oxygenase-1 (HO-1)

exert far more potent synergistic effects than dietary antioxidants, and help to resolve infections as well as protect bystander cells from destruction (Morse and Choi, 2002; Foresti and Motterlini, 1999). For example, heightened expression of NOS and HO-1 in malaria seems capable of protecting against cerebral malaria and inducing malarial tolerance (Taramelli et al., 2000; Taylor-Robinson et al., 1996; Schluesener et al., 2001). A similar response has been invoked clinically in the use of preconditioning to protect against surgical stress, reperfusion injury, and xenograft rejection (Koti et al., 2002; Amersi et al., 1999; Lin et al., 1999). All these effects are far more powerful than any that have been achieved using even massive doses of antioxidants. In other words, to protect against sudden episodes of physiological stress, such as infection or surgery, the genetic stress response is far more effective than any combination of dietary antioxidants. Given the way the stress response is actually elicited (by a rise in oxidative stress) loading up with ineffective antioxidants therefore runs the risk of undermining the effectiveness of the genetic stress response.

Importantly, even if antioxidant supplements are potentially detrimental, it remains possible to lower oxidative stress in a physiological manner, either by modulating the stress response itself, or by restricting primary mitochondrial leakage (as in birds, and possibly in the case of the Mt5178A variant). Thus, while the double-agent theory predicts that we should be refractory to antioxidants it does not rule out the possibility of lowering oxidative stress in a more physiological manner, by modulating the stress response itself, or by lowering leakage at source.

4. Specific predictions of the double-agent theory

Two testable predictions emerge from this hypothesis:

1. Oxidative stress is *necessary* to coordinate the stress and immune response, and so we should have homeostatic mechanisms that are actively refractory to antioxidant loading.
2. Mitochondrial leakage produces a rise in intracellular oxidative stress and a shift in gene expression, which is responsible for the negative effects of 'susceptibility' genes in old age.

If oxidative stress is *necessary* to trigger our response to physiological stress, then the production of free radicals cannot be a secondary phenomenon that depends on the recruitment and activation of immune cells—the respiratory burst of phagocytes, for example—but must be a *primary* response of all cells. In other words, the stress response takes place regardless of immune-system involvement, and is in fact the main stimulus for immune activation via the production of inflammatory mediators (Murtaugh and Foss, 2002). There is now good evidence that this is the case.

Influenza is a good example of the primary importance of oxidative stress in the response to infection. The influenza protein haemagglutinin produces a rise in oxidative stress in cultured cells, probably by overloading the endoplasmic reticulum with viral proteins (Pahl and Bauerle, 1997), leading to the transcription of stress and inflammatory genes via the redox-sensitive transcription factor NFκB (Pahl and Bauerle, 1995; Janssen-Heininger et al., 2000). A similar dependency on oxidative stress has been shown in other viral and bacterial infections (Pahl and Bauerle, 1995, 1997).

In many infections, epithelial cells are the first bastion of the innate immune response, as they have the first contact with pathogenic microorganisms (Naumann, 2000). Epithelial cells actively participate in mucosal immunity and inflammation, through the transcription of genes for inflammatory cytokines and intracellular adhesion molecules (such as ICAM-1). In vivo, the rapid local cytokine response to urinary tract infection (within 30 min) suggests that bacteria induce mucosal cytokine production directly (Hedges et al., 1995). This inference is confirmed by studies of cell lines from the human urinary tract (Naumann, 2000). These studies showed that activation of NFκB takes place within 10 min of infection by *Neisseria gonorrhoea*, the causal agent of gonorrhoea, and controls the up-regulation of pro-inflammatory cytokines, including TNF-α, GM-CSD, IL-6, IL-8 and MCP-1 (Naumann, 2000). NFκB-dependent cytokine production has been demonstrated in a number of other epithelial cell lines, including renal cells and HeLa cells (Wesselborg et al., 1997), alveolar epithelial cells (Rahman, 2000) and gastric epithelial cells (Keates et al., 1997). In cell cultures (as opposed to in vivo), the entire inflammatory response can be abolished by antioxidants such as dithiothreitol, which suppress the activation of NFκB (Pahl and Bauerle, 1995; Janssen-Heininger et al., 2000; Morgensen et al., 2003).

The fact that it is possible to suppress NFκB and cytokine production in vitro just by adding antioxidants implies that cultured cells cannot exercise full control over internal redox state, and are not refractory to antioxidant supplementation. This is not surprising, as redox-sensitive gene transcription depends on changes in the *internal* redox state of the cell. If this were to be refractory to change, then it would counter the cell's ability to respond to threats. Thus, to ensure that the intracellular redox state is sensitive to stress, and can induce flexible and rapid changes in gene transcription, the major homeostatic mechanisms that govern our antioxidant levels should take place at the level of tissues, organs and the body as a whole, rather than the cellular level. There are some indications that this is

true. For example, plasma ascorbate levels are tightly controlled by intestinal absorption and renal excretion, rendering megadose oral ascorbate futile (Levine et al., 1996a). The levels of other antioxidants, such as α-tocopherol (which is excreted in the bile), are also tightly regulated (Herrera and Barbas, 2001). Iron status, which can profoundly affect oxidative stress via the Fenton reaction, is very tightly regulated by transferrin and ferritin (Cairo et al., 2002). Some poorly understood homeostatic mechanisms seem to maintain an appropriate antioxidant balance in the body as a whole, even to the detriment of particular cells and tissues. For example, supplementation with α-tocopherol can lead to a reduction in plasma levels of γ-tocopherol (Dieber-Rotheneder et al., 1991; Mahabir et al., 2002) whereas supplementation with β-carotene may reduce serum lutein and zeaxanthin levels (to 40% of baseline levels in chicks), which in the long-term may even lower carotene levels in the macula of the eye (Snodderly, 1995).

Although there may be some variation in blood levels—long-term supplementation with antioxidants generally does raise blood levels—I am not aware of any research showing how long-term supplementation affects intracellular levels of antioxidants. In this context, the double-agent theory makes a clear prediction: even if long-term supplementation raises blood levels of antioxidants, this will have a limited affect on intracellular levels or redox status, as homeostatic mechanisms will correct for the rise at the physiological level, possibly by down-regulating cell membrane receptors, such as the dehydroascorbate receptor. The degree to which intracellular antioxidant levels are under genetic control, and the degree of variability between individuals, richly deserves further exploration.

The second prediction of the double-agent theory is that intracellular oxidative stress shifts gene expression, and it is this that brings about the negative effects of 'susceptibility' genes, rather than the polymorphisms themselves. In other words, selection acts on genes expressed in the reductive cellular environment of youth more those expressed in the oxidised environment of old age. Two sets of evidence are needed to corroborate this hypothesis: (i) proof that there is a gradual transition to oxidising conditions with age; and (ii) proof that it is the oxidising conditions, rather than any intrinsic property of the genetic polymorphisms themselves, that is responsible for the negative effects of 'susceptibility' genes.

A transition to oxidising conditions undoubtedly occurs with ageing, and this is coupled with a change in gene expression. To give a single example, a study of ageing in rhesus monkeys showed a two-fold or more shift in the expression of 450 genes with age (Kayo et al., 2001). This shift in transcription was associated with oxidative stress, and was more pronounced in metabolically active tissues (Kayo et al., 2001). Similar shifts have been reported in people (Stadtman, 2001).

The effect of oxidative stress on the behaviour of 'susceptibility' genes is exemplified by Alzheimer's disease. An important therapeutic target is the *ε4* allele of the *ApoE* gene, which is associated with an 8–16-fold greater risk of dementia (Cummings and Cole, 2002). If the negative effects of *ApoE4* depend on oxidative stress, not on time, then they may be averted by lowering oxidative stress in a physiological manner. In contrast, antioxidant supplements like selegiline and vitamin E have a limited effect on the progression of disease (Sano et al., 1997), as predicted by the double-agent theory (i.e., the body is refractory to high doses of dietary antioxidants). The double-agent theory specifically predicts that people with low oxidative stress should have a low risk of dementia, even if they carry two *ApoE4* alleles. There is some intriguing evidence that this might be the case. Studies of dementia in Ibadan, Nigeria, show that the risk of dementia is *half* that of age-matched Nigerians living in Indianapolis (Hendrie et al., 2001). (These data were corrected against the higher mortality rate in Africa, and reflect a genuinely lower incidence of Alzheimer's disease.) Importantly, *ApoE4* is not an independent risk factor in the African populations, for unknown reasons (Farrer, 2001; Hendrie et al., 2001). The reason may relate to the endemic distribution of malaria in Nigeria. Malarial tolerance is widespread—that is, many people have asymptomatic hyperparasitaemia. Tolerance is produced by suppression of the immune response, which in turn lowers *intracellular* oxidative stress, apparently via the long-term expression of HO-1 (Taramelli et al., 2000). The low incidence of dementia in tropical Africa may therefore be linked with malarial tolerance, in which low oxidative stress mitigates the negative effects of *ApoE4*. The double-agent theory predicts that this kind of relationship between 'susceptibility' genes and the level of oxidative stress will be common to many other age-related diseases (Lane, 2002).

5. Implications of the double-agent theory for medical research

The double-agent theory constrains our view of age-related diseases: we cannot alter the progression of disease by fiddling with 'susceptibility' genes if the underlying cause of disease is oxidative stress. Conversely, antioxidant supplementation could potentially suppress the stress response, and to guard against this the body may be refractory to antioxidant loading. Such a response could explain why a balanced diet is healthier than antioxidant supplementation: the combination of mild toxins and antioxidants in fruit and vegetables may modulate a flexible and ongoing stress response.

In this context, there is some evidence that the stress response is far from a simple 'on-off' system (Motterlini

et al., 2002). Deficiency of stress proteins like HO-1 (which are expressed *only* in response to stress) has serious long-term consequences in knock-out mice and one known clinical case, implying that the stress response rises and falls in tune with fluctuating circumstances (Poss and Tonegawa, 1997; Yachie et al., 1999). People who are vulnerable to illness in their youth (who presumably have an ongoing stress response) tend to be relatively robust in old age (Yashin et al., 2000). If they survive their youth at all they are more likely to live to a hundred, which suggests that an up-regulated stress-response earlier in life may protect against age-related diseases later on, by modulating immune and inflammatory activity (Yashin et al., 2000).

Modulating the stress response itself, however, inevitably involves a tradeoff between our vulnerability to infections or to age-related disease. If we wish to escape this catch-22, to prolong healthy lifespan, we must find ways of lowering primary mitochondrial leakage, like the birds. As suggested by the studies of Japanese centenarians by Tanaka et al. (1998) it might indeed prove possible to suppress the leakage of free radicals from mitochondria at the genetic level, by substituting one amino acid for another. Whether we might be able to alter the sequence of mitochondrial gene products by adapting the RNA editing system present in some mitochondria (such as trypanosomes; Madison-Antenucci et al., 2002; Simpson, 2000) will be an interesting question for the future. In the meantime, it may be possible to reduce mitochondrial free-radical leakage in a more physiological manner. For example, calorie restriction reduces mitochondrial leakage in rodents (Gredilla et al., 2001). More realistically, in our pill-popping age, hormones such as oestrogen have recently been shown to suppress mitochondrial leakage in female rats to half the levels seen in male rats (Borras et al., 2003), a finding that might explain the difference in lifespan between males and females in humans as well as rats (and possibly the lower risk of cancer and heart disease in pre-menopausal women compared with men). While the benefits of hormone-replacement therapy have been questioned by a number of important trials (Rymer et al., 2003), many of the unwanted side-effects relate to the powerful hormonal properties of oestrogen. If but a fraction of the research effort dedicated to studying genetic predisposition to disease were diverted to mitochondrial medicine, such drawbacks would surely be solved.

Acknowledgements

Thanks to Dr. Ana Hidalgo, Dr. Roberto Motterlini and Dr. Roberta Foresti, at the Northwick Park Institute for Medical Research, and to Professor Barry Fuller at the Royal Free and University College Medical School, for stimulating discussions; thanks to the anonymous referees for many helpful comments.

References

Amersi, F., Buelow, R., Kato, H., et al., 1999. Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. J. Clin. Invest. 104, 1631–1639.

Arrigo, A.P., 1999. Gene expression and the thiol redox state. Free Radic. Biol. Med. 27, 936–944.

Austad, S.N., 1993. Retarded senescence in an insular population of Virginia opossums (*Didelphis virginiana*). J. Zool. 229, 695–708.

Aviram, M., 2000. Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. Free Radic. Res. 33 (Suppl.), S85–S97.

Barja, G., 1998. Mitochondrial free radical production and ageing in mammals and birds. Ann. NY Acad. Sci. 854, 224–238.

Barja, G., 2002a. Endogenous oxidative stress: relationship to ageing, longevity and caloric restriction. Ageing Res. Rev. 1, 397–411.

Barja, G., 2002b. The quantitative measurement of H_2O_2 generation in isolated mitochondria. J. Bioenerg. Biomembr. 34, 227–233.

Barouki, R., Morel, Y., 2001. Oxidative stress and gene expression. J. Soc. Biol. 195, 377–382.

Barsyte, D., Lovejoy, D.A., Lithgow, G.J., 2001. Longevity and heavy-metal resistance in *daf-2* and *age-1* long-lived mutants of *Caenorhabditis elegans*. FASEB J. 15, 627–634.

Borras, C., Sastre, J., Garcia-Sala, D., Lloret, A., Pallardo, F.V., Vina, J., 2003. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic. Biol. Med. 34 (5), 546–552.

Cairo, G., Recalcati, S., Pietrangelo, A., Minotti, G., 2002. The iron regulatory proteins: targets and modulators of free radical reactions and oxidative damage. Free Radic. Biol. Med. 32 (12), 1237–1243.

Clancy, D., Gems, D., Harshman, L.G., Oldham, S., Stocker, H., Hafen, E., Leevers, S.J., Partridge, L., 2001. Extension of lifespan by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science 292, 104–106.

Cummings, J.L., Cole, G., 2002. Alzheimer disease. J. Am. Med. Assoc. 287, 2335–2338.

de Benedictis, G., Rose, G., Carrieri, G., et al., 1999. Mitochondrial DNA inherited variants are associated with successful ageing and longevity in humans. FASEB J. 13, 1532–1536.

Dieber-Rotheneder, M., Puhl, H., Waeg, G., Striegl, G., Esterbauer, H., 1991. Effect of oral supplementation with D-alpha-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. J. Lipid Res. 32 (8), 1325–1332.

Farrer, L.A., 2001. Intercontinental epidemiology of Alzheimer disease. A global approach to bad gene hunting. J Am. Med. Assoc. 285, 796–798.

Finch, C.E., Tanzi, R.E., 1997. Genetics of aging. Science 278 (5337), 407–411.

Foresti, R., Motterlini, R., 1999. The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. Free Radic. Res. 31, 459–475.

Friedman, D.B., Johnson, T.E., 1988. A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. Genetics 118, 75–86.

Gonzalez-Flecha, B., Demple, B., 2000. Genetic responses to free radicals. Homeostasis and gene control. Ann. NY Acad. Sci. 899, 69–87.

Gray, M.D., Shen, J.C., Kamath-Loeb, A.S., Blank, A., Sopher, B.L., Martin, G.M., Oshima, J., Loeb, L.A., 1997. The Werner syndrome protein is a DNA helicase. Nat. Gen. 17, 100–103.

Gredilla, R., Barja, G., Lopez-Torres, M., 2001. Effect of short-term calorie restriction on H_2O_2 production and oxidative DNA damage in rat liver mitochondria and location of the free radical source. J. Bioenerg. Biomembr. 33 (4), 279–287.

Gutteridge, J.M.C., Halliwell, B., 2000. Free radicals and antioxidants in the year 2000: a historical look to the future. Ann. NY Acad. Sci. 899, 136–147.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine, 3rd Edition.. Oxford University Press, Oxford, England.

Harman, D., 1972. The biologic clock: the mitochondria? J. Am. Geriatr. Soc. 20 (4), 145–147.

Hedges, S.R., Agace, W.W., Svanborg, C., 1995. Epithelial cytokine responses and mucosal cytokine networks. Trends Microbiol. 3, 266–271.

Hendrie, H.C., Ogunniyi, A., Hall, K.S., et al., 2001. Incidence of dementia and Alzheimer disease in two communities: Yoruba residing in Ibadan, Nigeria, and African Americans residing in Indianapolis, Indiana. J. Am. Med. Assoc. 285, 739–747.

Herrera, E., Barbas, C., 2001. Vitamin E: action, metabolism and perspectives. J. Physiol. Biochem. 57, 43–56.

Holliday, R., 1995. Understanding Ageing. Cambridge University Press, Cambridge, England.

Honda, Y., Honda, S., 1999. The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. FASEB J. 13, 1385–1393.

Ishii, T., Itoh, K., Takahashi, S., et al., 2000. Transcription factor Nrf2 co-ordinately regulates a group of oxidative stress inducible-genes in macrophages. J. Biol. Chem. 275, 16023–16029.

Janssen-Heininger, Y.M., Poynter, M.E., Baeuerle, P.A., 2000. Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. Free Radic. Biol. Med. 28 (9), 1317–1327.

Kapahi, P., Boulton, M.E., Kirkwood, T.B., 1999. Positive correlation between mammalian lifespan and cellular resistance to stress. Free Radic. Biol. Med. 26, 495–500.

Karin, M., Takahashi, T., Kapahi, P., et al., 2001. Oxidative stress and gene expression: the AP-1 and NF-kappaB connections. Biofactors 15, 87–89.

Kayo, T., Allison, D., Weindruch, R., Prolla, T.A., 2001. Influences of ageing and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. Proc. Natl. Acad. Sci. USA 98, 5093–5098.

Keates, S., Hitti, Y.S., Upton, M., Kelly, C.P., 1997. Helicobacter pylori infection activates NF-kappa B in gastric epithelial cells. Gastroenterology 113 (4), 1099–1109.

Key, T.J., Thorogood, M., Appleby, P.N., Burr, M.L., 1996. Dietary habits and mortality in 11,000 vegetarians and health-conscious people: results of a 17-year follow up. Br. Med. J. 313, 775–779.

Kimura, K.D., Tissenbaum, H.A., Ruvken, G., 1997. daf-2, an insulin-recptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science 277, 942–946.

Kirkwood, T.B.L., 1977. Evolution of ageing. Nature 270, 301–304.

Kirkwood, T.B., Kowald, A., 1996. A network theory of ageing: the interactions of defective mitochondria, aberrant proteins, free radicals and scavengers in the ageing process. Mutat. Res. 316, 209–236.

Kirkwood, T.L., Kapahi, P., Shanley, D.P., 2000. Evolution, stress, and longevity. J. Anat. 197, 587–590.

Koti, R.S., Seifalian, A.M., McBride, A.G., Yang, W., Davidson, B.R., 2002. The relationship of hepatic tissue oxygenation with nitric oxide metabolism in ischemic preconditioning of the liver. FASEB J. 16, 1654–1656.

Kovacic, P., Jacintho, J.D., 2001. Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. Curr. Med. Chem. 8, 773–796.

Lane, N., 2002. Oxygen: The Molecule that made the World. Oxford University Press, Oxford, UK.

Levine, M., Conry-Cantilena, C., Wang, Y., et al., 1996a. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc. Natl. Acad. Sci. USA 93, 3704–3709.

Levine, R.L., Mosoni, L., Berlett, B.S., Stadtman, E.R., 1996b. Methionine residues as endogenous antioxidants in proteins. Proc. Natl. Acad. Sci. USA 93, 15036–15040.

Levine, R.L., Berlett, B.S., Moskovitz, J., Mosoni, L., Stadtman, E.R., 1999. Methionine residues may protect proteins from critical oxidative damage. Mech. Ageing Dev. 107, 323–332.

Lin, Y., Soares, M.P., Sato, K., Takigami, K., Csizmadia, E., Smith, N., Bach, F.H., 1999. Accomodated xenografts survive in the presence of anti-donor antibodies and complement that precipitate rejection of naive xenografts. J. Immunol. 163, 2850–2857.

Maassen, J.A., Janssen, G.M., Lemkes, H.H., 2002. Mitochondrial diabetes mellitus. J. Endocrinol. Invest. 25 (5), 477–484.

Madison-Antenucci, S., Grams, J., Hajduk, S.L., 2002. Editing machines: the complexities of trypanosome RNA editing. Cell. 108 (4), 435–438.

Mahabir, S., Coit, D., Liebes, L., Brady, M.S., Lewis, J.J., Roush, G., Nestle, M., Fry, D., Berwick, M., 2002. Randomized, placebo-controlled trial of dietary supplementation of alpha-tocopherol on mutagen sensitivity levels in Melanoma patients: a pilot trial. Melanoma Res. 12 (1), 83–90.

Maina, J.N., 2000. What it takes to fly: the structural and functional respiratory refinements in birds and bats. J. Exp. Biol. 203, 3045–3064.

Marden, J.H., Rogina, B., Montooth, K.L., Helfand, S.L., 2003. Conditional tradeoffs between aging and organismal performance of Indy long-lived mutant flies. Proc. Natl. Acad. Sci. USA 100 (6), 3369–3373.

Martindale, J.L., Holbrook, N.J., 2002. Cellular response to oxidative stress: signaling for suicide and survival. J. Cell Physiol. 192, 1–15.

Merry, B.J., 2000. Calorie restriction and age-related oxidative stress. Ann. NY Acad. Sci. 908, 180–198.

Mogensen, T.H., Melchjorsen, J., Hollsberg, P., Paludan, S.R., 2003. Activation of NF-kappaB in virus-infected macrophages is dependent on mitochondrial oxidative stress and intracellular calcium: downstream involvement of the kinases TGF-beta-activated kinase 1, mitogen-activated kinase/extracellular signal-regulated kinase, kinase 1, and Ikappab kinase. J. Immunol. 170 (12), 6224–6233.

Morris, J.Z., Tissenbaum, H.A., Ruvkun, G., 1996. A phosphotidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature 382, 536–539.

Morse, D., Choi, A.M., 2002. Heme oxygenase-1: the 'emerging molecule' has arrived. Am. J. Respir. Cell. Mol. Biol. 27, 8–16.

Motterlini, R., Green, C.J., Foresti, R., 2002. Regulation of heme oxygenase-1 by redox signals involving nitric oxide. Antioxid. Redox Signal. 4, 615–624.

Murtaugh, M.P., Foss, D.L., 2002. Inflammatory cytokines and antigen presenting cell activation. Vet. Immunol. Immunopathol. 87, 109–121.

Naumann, M., 2000. Nuclear factor-κB activation and innate immune response in microbial pathogen infection. Biochem. Pharmacol. 60, 1109–1114.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A., Ruvkun, G., 1997. The fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature 389, 994–999.

Pahl, H., Baeuerle, P., 1995. Expression of influenza virus hemagglutinin activates transcription factor NF-kappa B. J. Virol. 569, 1480–1484.

Pahl, H.L., Baeuerle, P.A., 1997. The ER-overload response: activation of NF-kappa B. Trends Biochem. Sci. 22, 63–67.

Parthasarathy, S., Khan-Merchant, N., Penumetcha, M., Khan, B.V., Santanam, N., 2001. Did the antioxidant trials fail to validate the oxidation hypothesis? Curr. Atheroscler. Rep. 3, 392–398.

Poss, K.D., Tonegawa, S., 1997. Heme oxygenase-1 is required for mammalian iron re-utilization. Proc. Natl. Acad. Sci. USA 94, 10919–10924.

Poulton, J., 1998. Does a common mitochondrial DNA polymorphism underlie susceptibility to diabetes and the thrifty genotype? TIG 14 (10), 387–389.

Preston, T.J., Abadi, A., Wilson, L., Singh, G., 2001. Mitochondrial contributions to cancer cell physiology: potential for drug development. Adv. Drug Delivery Rev. 49, 45–61.

Rahman, I., 2000. Regulation of nuclear factor-kappa B, activator protein-1, and glutathione levels by tumor necrosis factor-alpha and dexamethasone in alveolar epithelial cells. Biochem. Pharmacol. 60 (8), 1041–1049.

Rose, M.R., 1991. Evolutionary Biology of Aging. Oxford University Press, Oxford, UK.

Rose, M.R., 1999. Genetics of ageing in Drosophila. Exp. Gerontol. 34, 577–585.

Ross, J.S., Stagliano, N.E., Donovan, M.J., Breitbart, R.E., Ginsburg, G.S., 2001. Atherosclerosis and cancer: common molecular pathways of disease development and progression. Ann. NY Acad. Sci. 947, 271–292.

Rymer, J., Wilson, R., Ballard, K., 2003. Making decisions about hormone replacement therapy. Br. Med. J. 326, 322–326.

Sano, M., Ernesto, C., Thomas, R.G., et al., 1997. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's disease cooperative study. New Engl. J. Med. 336, 1216–1222.

Schapira, A.H., 2002. Primary and secondary defects of the mitochondrial respiratory chain. J. Inherit. Metab. Dis. 25 (3), 207–214.

Schluesener, H.J., Kremsner, P.G., Meyermann, R., 2001. Heme oxygenase-1 in lesions of human cerebral malaria. Acta Neuropathol. 101, 65–68.

Simpson, L., Thiemann, O.H., Savill, N.J., Alfonzo, J.D., Maslov, D.A., 2000. Evolution of RNA editing in trypanosome mitochondria. Proc. Natl. Acad. Sci. USA 97, 6986–6993.

Snodderly, D.M., 1995. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am. J. Clin. Nutr. 62 (6 Suppl.), 1448S–1461S.

Sohal, R.S., 2002. Role of oxidative stress and protein oxidation in the ageing process. Free Radic. Biol. Med. 33, 37–44.

Speakman, J.R., Selman, C., McLaren, J.S., Harper, E.J., 2002. Living fast, dying when? The link between ageing and energetics. J. Nutr. 132 (Suppl. 2), S1583–S1597.

Stadtman, E.R., 2001. Protein oxidation in ageing and age-related diseases. Ann. NY Acad. Sci. 928, 22–38.

Suarez, R.K., 1998. Oxygen and the upper limits to animal design and performance. J. Exp. Biol. 201, 1065–1072.

Tanaka, M., Gong, J.S., Zhang, J., Yoneda, M., Yagi, K., 1998. Mitochondrial genotype associated with longevity. Lancet 351, 185–186.

Tanaka, M., Gong, J., Zhang, J., Yamada, Y., Borgeld, H.J., Yagi, K., 2000. Mitochondrial genotype associated with longevity and its inhibitory effect on mutagenesis. Mech. Ageing Dev. 116, 65–76.

Taramelli, D., Recalcati, S., Basilico, N., Olliaro, P., Cairo, G., 2000. Macrophage preconditioning with synthetic malaria pigment reduces cytokine production via heme iron-dependent oxidative stress. Lab. Invest. 80, 1781–1788.

Taylor-Robinson, A.W., Severn, A., Phillips, R.S., 1996. Kinetics of nitric oxide production during infection and reinfection of mice with Plasmodium chabaudi. Parasite Immunol. 18, 425–430.

van der Walt, J.M., Nicodemus, K.K., Martin, E.R., et al., 2003. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am. J. Hum. Genet. 72 (4), 804–811.

Wesselborg, S., Bauer, M.K., Vogt, M., Schmitz, M.L., Schulze-Osthoff, K., 1997. Activation of transcription factor NF-KappaB and p38 mitogen-activated protein kinase is mediated by distinct and separate stress effector pathways. J. Biol. Chem. 272 (19), 12422–12429.

Williams, G.C., 1957. Pleiotropy, natural selection and the evolution of senescence. Evolution 11, 398–411.

Williams, G.C., 1999. The 1999 Crafoord Prize lectures. The Tithonus error in modern gerontology. Q. Rev. Biol. 74, 405–415.

Williams, G.C., 2000. The Raymond Pearl memorial lecture, 1997: the quest for medical normalcy—who needs it? Am. J. Human Biol. 12, 10–16.

Yachie, A., Niida, Y., Wada, T., et al., 1999. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. J. Clin. Invest. 103, 129–135.

Yashin, A.I., de Benedictis, G., Vaupel, J.W., et al., 2000. Genes and longevity: lessons from studies of centenarians. J. Gerontol. 55A, B319–B328.

Zhang, J., Asin-Cayuela, J., Fish, J., et al., 2003. Strikingly higher frequency in centenarians and twins og mtDNA mutation causing remodeling of replication origin in leukocytes. Proc. Natl. Acad. Sci. USA 100, 1116–1121.