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Origin and evolution of the protein-repairing enzymes methionine sulphoxide reductases

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ABSTRACT

The majority of extant life forms thrive in an O_2-rich environment, which unavoidably induces the production of reactive oxygen species (ROS) during cellular activities. ROS readily oxidize methionine (Met) residues in proteins/peptides to form methionine sulphoxide [Met(O)] that can lead to impaired protein function. Two methionine sulphoxide reductases, MsrA and MsrB, catalyse the reduction of the S and R epimers, respectively, of Met(O) in proteins to Met. The Msr system has two known functions in protecting cells against oxidative damage. The first is to repair proteins that have lost activity due to Met oxidation and the second is to function as part of a scavenger system to remove ROS through the reversible oxidation/reduction of Met residues in proteins. Bacterial, plant and animal cells lacking MsrA are known to be more sensitive to oxidative stress. The Msr system is considered an important cellular defence mechanism to protect against oxidative stress and may be involved in ageing/senescence. MsrA is present in all known eukaryotes and eubacteria and a majority of archaea, reflecting its essential role in cellular life. MsrB is found in all eukaryotes and the majority of eubacteria and archaea but is absent in some eubacteria and archaea, which may imply a less important role of MsrB compared to MsrA. MsrA and MsrB share no sequence or structure homology, and therefore probably emerged as a result of independent evolutionary events. The fact that some archaea lack *msr* genes raises the question of how these archaea cope with oxidative damage to proteins and consequently of the significance of *msr* evolution in oxic eukaryotes dealing with oxidative stress. Our best hypothesis is that the presence of ROS-destroying enzymes such as peroxiredoxins and a lower dissolved O_2 concentration in those *msr*-lacking organisms grown at high temperatures might account for the successful survival of these organisms under oxidative stress.

Key words: methionine sulphoxide reductase, MsrA, MsrB, Archaea, S and R epimers, reactive oxygen species (ROS), oxidative stress, protein repair, evolution.

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I. INTRODUCTION

For the first half of the earth's 4.5-billion-year history, the atmosphere contained almost no oxygen (O_2), mainly consisting of carbon dioxide, water vapour, and methane (Kasting, Liu & Donahue, 1979). Methane-producing archaea may have inhabited the earth as early as 3.8 billion years ago. During this period, oxidation of biological molecules (interaction with molecular oxygen) would not have been a prominent process for the primitive life forms present on the "O_2-free" earth. The emergence of cyanobacteria marked the beginning of an oxygenic world. Cyanobacteria became the first photosynthetic organisms to use solar energy to convert carbon dioxide and water into sugars with evolution of O_2 as a by-product, perhaps as early as 3.5 billion years ago and certainly by 2.7 billion years ago (Brocks *et al.*, 1999). The "Great Oxidation Event" occurred some 2.3 billion years ago when the O_2 level in earth's atmosphere increased dramatically as a result of photosynthesis (Holland, 2006). The O_2-rich atmosphere has since fostered a vast diversity of life on earth. However, the abundant presence of O_2 (currently at about 21% of the earth's atmosphere) also creates an unavoidable oxidative stress to life processes. A group of reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion (O^-_2) and hydroxyl radical ($HO^\bullet$), are generated by cells as by-products of normal respiration and metabolism. These ROS interact with reactive molecules in the cells and cause damage to macromolecules such as DNA, proteins and lipids, thereby impairing cellular functions. Although cells have mechanisms to destroy ROS and repair damage caused by ROS, oxidative damage is considered to be one of the major factors responsible for age-related diseases and the ageing process.

Oxidation of proteins is no doubt a frequently occurring process in all aerobic microbes, plants and animals. One common result of oxidation is fragmentation and/or carbamylation of the peptide backbone (Vogt, 1995). ROS reactions can also cause specific modifications of amino acid side chains, resulting in structural changes in proteins/enzymes. Two sulphur-containing amino acids, methionine (Met) and cysteine (Cys), are particularly susceptible to oxidation. Met as a universal initiating amino acid for protein synthesis has additional importance to cellular functions. Both free Met and protein-based Met are readily oxidized by ROS to form methionine sulphoxide [Met(O)] that could alter protein structure and/or function as has been shown for a large number of peptides and proteins (Brot & Weissbach, 1991). Oxidative damage to proteins has been implicated in many studies on oxidative stress in a wide variety of organisms (Vogt, 1995; Moskovitz *et al.*, 1995, 2001; Berlett & Stadtman, 1997; Davies, 2005). In response to oxidative stress, living organisms have evolved ROS-scavenging and repair mechanisms. The ROS-scavenging systems include enzymes such as peroxiredoxins, superoxide dismutases, catalases and glutathione peroxidases. The DNA repair system is an example of how cells repair damage to macromolecules. In the case of proteins it is known that degradation of damaged proteins by proteases (proteosomes) can occur (Davies, 1993). This is energetically inefficient since the cell then would have to resynthesise the protein. However, in the case of Met oxidation, a more energy-efficient mechanism involves the action of the methionine sulphoxide reductase system (Msr) which catalyses the reduction of Met(O) to Met in proteins (Brot *et al.*, 1981), repairing the oxidized proteins without the need for new protein synthesis (Fig. 1). In so doing, it has been noted that one equivalent of ROS is destroyed for every Met residue repaired. Thus, because of the Msr system Met residues in proteins can also act as catalytic antioxidants by removing ROS (Levine, Berlett & Stadtman, 1996).

Met oxidation by ROS should lead to the formation of equal amounts of the two epimers of Met(O) called Met-S-(O) and Met-R-(O) (see review by Weissbach *et al.*, 2002; Fig. 1). In order to restore the maximal function of a damaged protein, the Msr enzymes must be able to repair both epimers. In all eukaryotic and most prokaryotic organisms, two different enzymes, MsrA and MsrB, reduce Met-S-(O) and Met-R-(O) in proteins, respectively (Moskovitz *et al.*, 2000; Grimaud *et al.*, 2001; Lowther *et al.*, 2000*b*; Weissbach *et al.*, 2002; Kryukov *et al.*, 2002; Rouhier *et al.*, 2006). In some bacteria, the two activities are present in one protein as MsrA/B (Lowther *et al.*, 2002; Olry *et al.*, 2002).

MsrA and MsrB are not structurally related but catalyse a similar reaction using as substrate the appropriate epimer of Met(O). Overall, MsrA and MsrB are separately conserved across life domains/kingdoms (Kumar *et al.*, 2002; Rouhier *et al.*, 2006; Ding *et al.*, 2007; Fukushima *et al.*, 2007). The signature Cys-containing motifs (GCFWG in MsrA and RXCXN near the C-terminus of MsrB) are essential for the catalytic activity and therefore invariably conserved (Kumar *et al.*, 2002; Rouhier *et al.*, 2006; Ding *et al.*, 2007). Some Msr pre-proteins in eukaryotes have N-terminal signal peptides that target the enzymes to different cellular compartments such as mitochondria, endoplasmic reticulum and chloroplasts (Sadanandom *et al.*, 2000; Hansel *et al.*, 2002; Kim & Gladyshev, 2004*a*,*b*, 2005*b*). The sequence similarity between archaea, eubacteria and eukaryotes indicates that *msr* genes are very ancient,

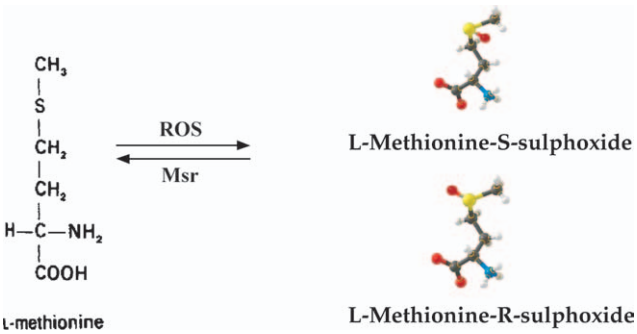

Fig. 1. Oxidation of methionine to methionine sulphoxide and its reduction. A mixture of R and S isoforms of methionine sulphoxide is produced after chemical oxidation of methionine by reactive oxygen species (ROS). The methionine sulphoxide reductase (Msr) system can reduce the methionine sulphoxide in proteins back to methionine.

perhaps first appearing along with the earliest O_2-requiring life form. This would also support the view that the Msr system has an important cellular role in protecting organisms against oxidative damage.

A novel group of naturally occurring selenoprotein Msr enzymes have been identified whose redox-active Cys is replaced with a selenocysteine (Kryukov *et al.*, 2002; Kim *et al.*, 2006). These enzymes exhibited as much as 100-fold higher activity than their Cys mutant forms. In contrast to the ubiquitous presence of *msrA*, the selenoprotein *msrA* sequences were found only in a few eukaryotes and one bacterium but not in vertebrates and archaea (Kim *et al.*, 2006), whereas selenoprotein *msrB* genes were identified only in vertebrates (Kim & Gladyshev, 2004*a*, 2005*a*). Due to their limited distribution, the selenoprotein *msr* coding genes are likely to have evolved from the "normal" *msr* and have a more recent history. The evolutionary mechanisms and significance of the selenoprotein Msr enzymes remain to be investigated further.

Since O_2 is a necessary precursor of all ROS and the reduction of Met(O) epimers in proteins requires both MsrA and MsrB, several questions arise as to the evolution and importance of the *msr* genes. We here address the possible role of the *msr* genes in organisms that emerged as the atmospheric free oxygen became abundant and the independent origins and convergent evolution of these two Msr enzymes.

II. PHYLOGENETIC DISTRIBUTION OF *MSR* GENES

The phylogenetic distribution and evolution of *msr* genes among representative life domains indicates a complex pattern of paralogy and lateral gene transfer events (Delaye *et al.*, 2007). Without exception, all extant eukaryotes and cyanobacteria examined contain *msrA* and *msrB* genes but no *msrA/B*. All eubacteria contain either a distinct *msrA* gene alone, or *msrA* and *msrB* genes, or the bifunctional gene *msrA/B* (Table 1). The universal presence of the *msr* genes in organisms where Met oxidation in proteins is a constant product of cellular metabolism might be expected since reduction of Met(O) should be an important repair reaction. However, exceptions do exist. First, some endoparasites (e.g. *Rickettsia spp.* and *Chlamydia spp.*) and endosymbionts (e.g. *Buchnera spp.* and *Tropheryma spp.*) do not possess *msr* genes in their diminutive genomes. This *msr* gene loss may be due to (1) secondary adaptation to the anaerobic intracellular environment of their hosts as suggested by Delaye *et al.* (2007) and/or (2) available protection against oxidative damage shared from the activities of the host Msr enzymes. Secondly, and more intriguingly, a survey of the complete sequences of representative archaea genomes indicates that while five species possess *msrA* only, eight have both *msrA* and *msrB*, one archaeon has both *msrA* and *msrA/B*, and notably 12 archaea do not have *msrA* or *msrB* genes (Table 1). This conclusion was reached by close examination of the predicted protein sequences, which confirmed the absence of the invariable MsrA signature motif "GCFWG/C" (Lowther *et al.*, 2000a; Kumar *et al.*, 2002; Rouhier *et al.*, 2006) and the lack of the MsrB conserved catalytic site (RXCXN) (Lowther *et al.*, 2002; Kryukov *et al.*, 2002; Ding *et al.*, 2007) in all the predicted open reading frames throughout the genomes of these *msr*-lacking archaea.

Table 1. Distribution of *msr* genes in representative extant organisms

	msrA	*msrB*	*msrA/B*
Archaea			
Aeropyrum pernix K1	N	N	N
Archaeoglobus fulgidus DSM 4304	N	N	N
Methanocaldococcus jannaschii DSM 2661	N	N	N
Methanopyrus kandleri AV19	N	N	N
Nanoarchaeum equitans Kin4-M	N	N	N
Pyrobaculum aerophilum str. IM2	N	N	N
Pyrococcus abyssi GE5	N	N	N
Pyrococcus furiosus DSM 3638	N	N	N
Pyrococcus horikoshii OT3	N	N	N
Sulfolobus tokodaii str. 7	N	N	N
Thermoplasma acidophilum DSM 1728	N	N	N
Thermoplasma volcanium GSS1	N	N	N
Ferroplasma acidarmanus	Y	N	N
Methanococcus maripaludis S2	Y	N	N
Picrophilus torridus DSM 9790	Y	N	N
Sulfolobus acidocaldarius DSM 639	Y	N	N
Sulfolobus solfataricus P2	Y	N	N
Haloarcula marismortui ATCC 43049	Y	Y	N
Halobacterium salinarum NRC-1	Y	Y	N
Methanococcoides burtonii DSM 6242	Y	Y	N
Methanosarcina acetivorans C2A	Y	Y	N
Methanosarcina barkeri str. Fusaro	Y	Y	N
Methanosarcina mazei Go1	Y	Y	N
Methanothermobacter thermautotrophicus str. Delta H	Y	Y	N
Natronomonas pharaonis DSM 2160	Y	Y	N
Thermococcus kodakarensis KOD1	Y	N	Y
Cyanobacteria	Y	Y	N
Eubacteria	Y	Y/N	Y/N
Eucarya	Y	Y	N

Y: presence; N: absence.

Archaea are unique, mostly hyperthermophilic organisms that often live in extreme environments (Table 2). While many archaea contain genes coding for MsrA, it is surprising that others do not contain any *msr* genes (Table 1). One possibility is that *msr*-lacking archaea may have lost the ancestral *msr* gene but have evolved a functionally equivalent system similar to the ferritin-like antioxidant protein characterized in *Sulfolobus solfataricus* (Wiedenheft *et al.*, 2005). It should be pointed out that *S. solfataricus* P2 does contain a *msrA* gene (Table 1), even though this gene is speculated as resulting from horizontal gene transfer (Delaye *et al.*, 2007). This possibility is most likely to apply to those strict/facultative anaerobic archaea living in low-O_2 environments. For aerobic archaea, high levels of O_2 are both unavoidable and necessary for growth.

Table 2. Brief summary of characteristics of some *msr*-lacking archaea

	Characteristics
Aeropyrum pernix	aerobic hyperthermophilic growth at up to 100°C
Archaeoglobus fulgidus	sulphur-metabolizing and strictly anaerobic, growing at 60 ~ 95°C
Methanopyrus kandleri	methane-producing, growing at near or above 100°C
Nanoarchaeum equitans	symbiotic or parasitic growth on *Ignicoccus* cells at 70 ~ 98°C, pH ~ 6 and ~ 2% NaCl
Pyrobaculum aerophilum	facultatively aerobic nitrate-reducing hyperthermophilic (up to 104°C)
Sulfolobus tokodaii	oxidizing hydrogen sulphide to sulphate, optimum growth at pH 2 ~ 3 and 75 ~ 80°C in terrestrial volcanic hot springs
Thermoplasma acidophilum, *Thermoplasma volcanium*	heterotrophic growth at 55 ~ 60°C and pH 0.5 ~ 4, obtaining energy anaerobically *via* sulphur respiration and scavenging other organisms. Cells lyse at a neutral pH

For example, the growth of *Thermoplasma acidophilum* whose genome does not encode *msr* requires constant aeration (vigorous shaking) at 55°C and pH 2, which would conceivably generate ROS and induce oxidative stress. Yet, no Msr enzyme activity was detected in a protein extract (X.-H. Zhang & H. Weissbach, unpublished data). Fukushima *et al.* (2007) pointed out that most hyperthermophilic bacteria and archaea whose optimal growth temperatures are 80°C or above do not have *msr* genes. They proposed that low O_2 solubility at high temperatures [according to our calculation, the saturated dissolved O_2 concentration in water (atmospheric pressure at sea level) is 234 μmol l^{-1} at 30°C, 91 μmol l^{-1} at 80°C and 53 μmol l^{-1} at 90°C] would depress ROS production and hence Met oxidation in those hyperthermophiles and thus make the presence of *msr* unnecessary. Yet, even at 90°C, it is possible there is enough dissolved O_2 to support cellular metabolism (e.g. respiration) and conceivably therefore induce ROS formation. Also, the suggestion of Fukushima *et al.* (2007) cannot explain fully how those *msr*-lacking organisms deal with Met oxidation when the environmental conditions change, such as during growth at lower temperatures. In fact, many of these hyperthermophilic archaea can survive a wide range of temperatures, pH or salt concentrations, within which the levels of cellular ROS and oxidative stress conceivably will also change. Therefore, the best test for the hypothesis of Fukushima *et al.* (2007) is to examine whether those *msr*-lacking archaea contain protein-based Met(O). Fukushima *et al.* (2007) observed high rates of nonenzymatic Met(O) reduction in the presence of 20 mmol l^{-1} dithiothreitol (DTT), as temperature increased to 100°C. Without DTT, the nonenzymatic Met(O) reduction did not occur. They suggested that this nonenzymatic Met(O) reduction with the help of reduced thioredoxin at high temperatures may explain the absence of *msr* genes in most hyperthermophiles. This is a theoretical possibility only and there is no evidence that such non-physiological levels of dithiols exist in any organisms.

An alternative explanation for the absence of *msr* genes is that those archaea contain unique protein structures that protect their Met residues from exposure to ROS, or have mechanisms that protect their proteins from oxidation. It should be noted that many *msr*-containing and *msr*-lacking archaea, both aerobic and anaerobic, share similar growth environments. It seems difficult to argue that only those *msr*-free species have evolved special mechanisms. Nevertheless, it would be interesting to investigate whether there are structural and functional distinctions regarding Met oxidation among these archaea. Another possibility is that these archaea contain a *msr*-containing plasmid that may complement the lack of *msr* in the genomes. However, there is no evidence of natural plasmids in these organisms. For those archaea known to contain plasmids, *msr* is almost always located in the genome, not on the plasmid. Alternatively, these *msr*-lacking archaea may have developed efficient systems to degrade oxidized proteins and replace them with newly synthesised proteins. This strategy would be an energy-expensive way of dealing with damaged proteins and there is no evidence to support it. Recently, Lin *et al.* (2007) reported a novel free Met-R-(O) reductase (fRMsr) from a $MsrA^-B^-$ knockout of *Escherichia coli*, which is structurally different from MsrB. Interestingly, fRMsr homologues can be found in archaea including species lacking *msr*, raising the possibility that these *msr*-lacking archaea can reduce the oxidized free Met-R-(O). Questions remain, however, as to (1) whether the fRMsr is functional in hyperthermophilic archaea, (2) whether a yet-to-be-identified Met-S-(O) specific reductase (fSMsr) also exists in archaea, and (3) what reduces the protein-based Met(O) since fRMsr acts only on free residues (Lin *et al.*, 2007).

Finally, it is important to note that based on our analysis, all life forms (Eucarya, Bacteria and Archaea including *msr*-lacking species) possess coding genes for peroxiredoxins/peroxidases and in most cases also (alkyl) hydroperoxide reductases, catalases and/or superoxide dismutases. Since reactive oxygen species (ROS) are the main cause of oxidative stress resulting in Met(O) formation, these ROS-destroying enzymes would form the first line of defence. The presence and function of these enzymes in *msr*-lacking organisms may generate a low-ROS environment that diminishes the frequency of Met oxidation. As noted above, ROS production would be further hindered in hyperthermophilic archaea, many of them *msr*-free, that grow in high-temperature environments with a low soluble O_2 level (Fukushima *et al.*, 2007).

The fact that Archaea can be categorised into two broad groups based on the presence/absence of Msr

(Table 1) suggests a divergent evolutionary scenario for this heterologous group of organisms that are distinct from eukaryotes and eubacteria. Since the majority of living organisms contain Msr, *msr*-lacking species appear to be an evolutionary "aberration" resulting from the loss of *msr*-encoding DNA. It should be stressed that additional *msr*-lacking archaea or other microbes may be identified as more genome sequences become available.

III. CONVERGENT EVOLUTION OF MSRA AND MSRB ENZYMES

Genome analysis has shown that, with the exception of the *msr*-lacking archaea discussed above, all extant organisms contain *msrA*; all eukaryotes and most eubacteria also possess *msrB* genes although some eubacteria apparently do not have *msrB*-coding sequences in their genomes (Tables 1 and 3). However, without exception, no *msrB*-containing organism exists without *msrA* (Table 1). This suggests that (1) MsrA and MsrB evolved independently and emerged early (such as in the ancestors of Archaea and cyanobacteria), and (2) MsrA alone is sufficient to perform this specific protein repair process (or at least was during early geological periods with lower O_2 levels) while MsrB may have evolved to facilitate cellular responses to increasingly greater levels of oxidative damage seen in more complex later life forms such as animals and plants.

As noted above, Met oxidation leads to a racemic mixture of two epimers, Met-S-(O) and Met-R-(O). That *msrB* is not as ubiquitous as *msrA* may suggest a biological difference between the R and S forms of Met(O) in proteins. Perhaps there was an enzymatic stereospecific oxidation of Met in proteins resulting in a greater abundance of the S epimer early in evolution. This difference could have resulted in a selection pressure to preferentially reduce the S epimer and might explain why *msrA* genes are found in almost all living organisms and MsrA enzymes are physiologically more important than MsrB as discussed below. It is difficult to determine whether *msrA* and *msrB* evolved simultaneously or whether *msrA* preceded *msrB*. The absence of *msrB* in some prokaryotes may indicate a gene loss event. Yet why would such gene loss include either *msrB* only (as in some prokaryotes, Table 1) or both *msrA* and *msrB* (as in some archaea, Table 1), but not *msrA* alone? Is it possible that *msrB* emerged later in the ancestors of many microbes and all eukaryotes, but not in those that gave rise to the extant *msrA*-containing, *msrB*-lacking organisms? Another possibility is that lateral transfer of *msrB* occurred and then was fixed and evolved in most organisms, but lost in others due to the environmental oxidative conditions they experienced.

Not only are the DNA and amino acid sequences of *msrA* and *msrB* different but in most organisms, with the exception of a few prokaryotes, the organisation of the *msrA* and *msrB* genes also differs. That the emergence of *msrA* and *msrB* occurred convergently may be inferred from the massive *msrB* gene duplication seen in the plant *Arabidopsis thaliana*. Among nine putative *msrB* genes, one is located on chromosome 1, and the other eight on chromosome 4, with two 12-17 kbp clusters (consisting of four *msrB* genes each) separated by approximately 9,133 kbp (X.-H. Zhang, unpublished analysis). In stark contrast, *msrA* genes in this plant do not show the similar pattern of gene duplications. While *msr* gene duplication in other higher plants is not as dramatic as in *A. thaliana*, the available information indicates that all plants and algae investigated contain multigene *msr* families. For example, rice, poplar (*Populus trichocarpa*) and the green alga *Chlamydomonas reinhardtii* contain four, five and four *msrA* genes, and three, four and three *msrB* genes, respectively (Rouhier *et al.*, 2006). Even the lower plant moss (*Physcomitrella patens*) may possess at least seven *msrA* homologues and at least one *msrB* gene, based on a preliminary analysis of the unfinished genome sequence data (X.-H. Zhang, personal observations). By comparison, animals and bacteria contain fewer *msr* genes: humans and mouse have one *msrA* and three *msrB* genes (Cabreiro *et al.*, 2006), and the fruit fly *Drosophila melanogaster* and *E. coli* have one gene each for *msrA* and *msrB* (Kim & Gladyshev, 2006; Ezraty, Aussel & Barras, 2005). The variation in *msr* gene family size among different life forms clearly suggests divergent and complex pathways of *msr* gene evolution. The fact that two obviously unrelated (both in sequence and structure) genes evolved to produce two different enzymes that act stereospecifically on the same Met(O) reflects a clear evolutionary necessity to adapt to protein-based Met oxidation.

IV. AEROBIC GROWTH AND PRESENCE OF *MSR* GENES

It is probably reasonable to assume that it was the rapid increase in atmospheric O_2 level, brought about by the initiation of oxygenic photosynthesis some 3 billion years ago, that triggered Msr evolution (Fig. 2). All eukaryotes apparently require both O_2 and Msr activity. However, not all the extant *msr*-containing organisms are aerobic and not all the *msr*-lacking archaea are anaerobic (Table 2). In fact, several strictly anaerobic bacteria also possess at least one type of *msr* (Table 3), while the vigorous aerobic, high-temperature and low-pH-requiring *T. acidophilum* does not have either *msr* genes or Msr activity (X.-H. Zhang & H. Weissbach, personal observations). Therefore, the requirement for O_2 and the functioning of *msr* genes are not necessarily linked, raising the possibility that *msr* coding sequences could have originated before the formation of a substantial level of atmospheric O_2. A key question will be whether ROS production and Met oxidation occur in extant strictly anaerobic microbes inhabiting extremely low free O_2 environments, and if so how those *msr*-lacking organisms deal with this oxidative stress through ROS-destroying enzymes such as peroxiredoxins or other mechanisms, as compared to their *msr*-containing counterparts. Perhaps the Msr enzymes in these anaerobic *msr*-containing organisms have another, as yet, unknown function.

Table 3. Presence/absence of *msr* genes in some strict anaerobic organisms

	msrA	*msrB*	*msrA/B*
Archaea			
Archaeoglobus fulgidus DSM 4304	N	N	N
Methanothermobacter thermautotrophicus str. Delta H	Y	Y	N
Eubacteria			
Bifidobacterium longum NCC2705	N	N	Y
Clostridium tetani E88	Y	N	N

V. EVOLUTIONARY ROLE OF MSR ENZYMES

Studies with microbial, animal and plant systems all indicate that Msr can protect cells against oxidative damage and may be involved in ageing (reviews by Weissbach *et al.*, 2002; Weissbach, Resnick & Brot, 2005). For example, overexpression of *msrA* nearly doubled the lifespan of fruit flies *Drosophila melanogaster* (Ruan *et al.*, 2002) and protected PC12 cells against hypoxia/reoxygnenation-induced injury (Yermolaieva *et al.*, 2004), whereas *msrA*-knockout mice exhibited a shorter lifespan, hyperbaric sensitivity and a neurological defect (Moskovitz *et al.*, 2001). Even though *E. coli* mutants lacking *msrA* grew normally, they were more sensitive to ROS (Moskovitz *et al.*, 1995; St. John *et al.*, 2001). In yeast under aerobic conditions, a *msrA* mutant had reduced viability while overexpressing *msrA* increased viability (Koc *et al.*, 2004). Similarly, plant MsrA and MsrB are implicated in oxidative responses, particularly in preventing chloroplast proteins from Met sulphoxidation (Gustavsson *et al.*, 2002; Bechtold, Murphy & Mullineaux, 2004; Vieira Dos Santos *et al.*, 2005). In *Arabidopsis thaliana*, down-regulation of a plastid *msrA* had no phenotypic effects under normal growth conditions. However, under oxidative stresses, *msrA*-overexpressing *A. thaliana* contained a lower level of sulphoxide in chloroplasts and was more resistant to oxidative stress as compared to the down-expressers (Romero *et al.*, 2004).

Indeed, *msr* genes have been placed on the list of a minimal gene set necessary and sufficient to sustain cellular life of *Mycoplasma genitalium* (Fraser *et al.*, 1995) and *Haemophilus influenzae* (Mushegian & Koonin, 1996). By contrast, Kobayashi *et al.* (2003) did not find *msr* among

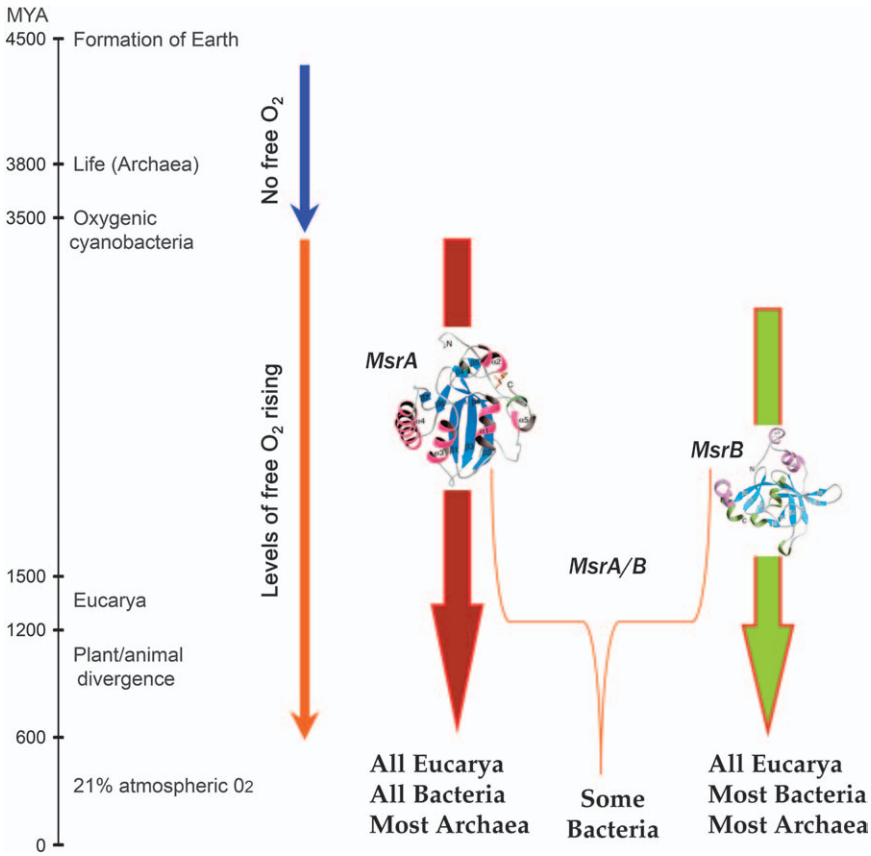

Fig. 2. Schematic model of the evolution of methionine sulphoxide reductases (Msr). The accumulation of free atmospheric O_2 that began some 3 billion years ago may have resulted in the evolution of Msr enzymes to deal with the unavoidable oxidation of Met in proteins. The distinct presence of MsrA and MsrB suggests convergent evolution, while the formation of MsrA/B in certain bacteria is likely the result of gene recombination/fusion. MYA: million years ago. The images of MsrA and MsrB proteins are adapted from Lowther *et al.* (2000*b*, 2002).

a minimum set of genes essential for *Bacillus subtilis* to sustain life in nutritious conditions, and as discussed above, some parasites and archaea lack Msr-coding genes. No matter whether the *msr* system is "essential" for life, there is little doubt that the convergent evolution of two sets of *msr* genes leading to MsrA and MsrB has given organisms living in an O_2-rich environment an effective mechanism for combating one aspect of oxidative stress, Met oxidation (Fig. 2). The ancestral appearance of the *msr* system may have conferred an evolutionary advantage to a majority of the extant life forms that face constant environmental stress due to oxidative damage unavoidable in this O_2-rich world.

VI. CONCLUSIONS

(1) Methionine sulphoxide reductases, particularly MsrA, are important for the life processes of a vast majority of extant organisms. Rapid accumulation of genome sequence information, especially of Archaea, has provided new insights into the origin and evolution of the ubiquitous Msr.

(2) With exception of a few parasites and archeae, MsrA is ubiquitous in all extant eukaryotes and prokaryotes. MsrA likely evolved in aerobic life forms to deal with the oxidative stress that accompanied the increase in atmospheric O_2 level that began in the Precambrian. MsrA may be one of the earliest and essential proteins for oxic life forms (Fig. 2).

(3) All eukaryotes contain both *msrA* and *msrB* genes. By contrast, some prokaryotes have *msrA* but no *msrB*, yet no MsrB-containing organism exists without MsrA. Probably, MsrA and MsrB emerged independently as a result of convergent evolution early in life such as in the ancestors of Archaea and Cyanobacteria, although the timelines and order of appearance of this pair of enzymes are unknown. MsrA alone is functionally sufficient while MsrB, as a product of perhaps more recent evolutionary events, may facilitate cellular responses to increasingly greater oxidative challenge faced by more complex life forms.

(4) That MsrB is not as ubiquitous as MsrA may suggest a biological bias for the two epimers of Met(O) in proteins. Perhaps there was an enzymatic stereospecific preference for S epimers in cellular Met oxidation during evolution, or perhaps Met-S-(O) in proteins is functionally more damaging than Met-R-(O). These possibilities could have resulted in early selection for MsrA. Alternatively, MsrA and/or MsrB may have other unknown biochemical functions besides protein repair, which would account for their unequal distribution in extant genomes. Clearly, many unanswered questions remain.

(5) The apparent absence of Msr-coding genes in some archaea presents interesting questions regarding the biological significance of Met oxidation and the role of Msr in the evolution of life. Whether these organisms originally had *msr* genes that were subsequently lost is not clear. If these archaea experience Met oxidation, it is not known how they repair the affected proteins without Msr enzymes. The universal presence of genes for peroxiredoxins and other ROS-degrading enzymes and low free O_2 levels in the local environments due to high temperatures may result in negligible Met oxidation in these *msr*-lacking, heat-loving archaea. Further biochemical investigations of these organisms, involving searches for the presence of ROS-degrading enzymes and Msr-like activities, and measuring cellular levels of ROS and protein-based Met(O), will help answer some of these questions. These studies may reveal novel aspects of cellular processes involved in ROS responses, protein repair and ageing/senescence and the origin and evolution of O_2-dependent forms of life.

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