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Respiratory chain cysteine and methionine usage indicate a causal role for thiyl radicals in aging

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ABSTRACT

The identification of longevity-related structural adaptations in biological macromolecules may yield relevant insights into the molecular mechanisms of aging. In screening fully sequenced animal proteomes for signals associated with longevity, it was found that cysteine depletion in respiratory chain complexes was the by far strongest predictor on the amino acid usage level to co-vary with lifespan. This association was though restricted to aerobic animals, whereas anaerobic animals showed variable cysteine accumulation. By contrast, methionine accumulation, a prominent feature of mitochondrially encoded proteins affording competitive antioxidant protection, was not predictive of longevity, but rather paralleled aerobic metabolic capacity. Hence, the easily oxidized sulfur-containing amino acids cysteine (a thiol) and methionine (a thioether) show doubly diametrical behaviour in two central paradigms of respiratory oxidative stress. From this comparison, it is concluded that only the one–electron oxidation of thiols to thiyl radicals contributes to aging, whereas other forms of sulfur oxidation, especially even-electron oxidation of both thiols and thioethers, are less critically involved, presumably as their consequences may be much more easily repaired. Thiyl radicals may yet act as chain-transfer agents to entail an irreversible intramembrane cross-linking ("plastination") of some of the a priori most hydrophobic and insoluble proteins known, the respiratory chain complexes.

1. Introduction

Exceptional longevities in the animal kingdom of 100 years or more have long attracted the interest of the scientific biomedical community. Various aspects about these extreme longevities appear to be astonishing: first, they have been realized several times independently during evolution, in numerous phylogenetically unrelated species, e.g. among mammals, fish, or reptiles. Second, they are often accompanied by proportionately extended developmental periods without reproduction, making the survival for such extended timeframes an evolutionary necessity rather than an individual luxury. Third, even the exceptionally long-lived animals do one day show signs of aging that very closely resemble those signs to be found in short-lived animals just much earlier. These and other observations pose the question whether it is actually "simple" for a species to acquire exceptional longevity, which one might feel to infer, or whether longevity and thus long developmental and reproductive periods rather constitute the outcome of a perennial, fierce interplay of mutation and selection for this very trait.

There are essentially two alternatives on how increased longevity might be accomplished. It might either result from an investment into improved replacement and repair capacity, which also includes the restricted use of terminal differentiation (as in sponges, cnidaria, or plants, for instance), or it might result from a stepwise structural optimization of biochemical building blocks that for some reason cannot be replaced or repaired with the required fidelity or velocity or at an affordable energetic cost.

In recent years, fascinating experimental results have demonstrated once again the importance of genetically encoded mechanisms of replacement and repair on various biological levels. First, on a cellular level, immortality is basically possible. Obviously, there must be mechanisms that enable germ cells to fully escape the forces of temporal attrition. Moreover, differentiated cells that have reached their replicative limit and show numerous signs of senescence can still be rejuvenated by transformation with oncogenes. In addition, a multitude of middle-aged, differentiated cells types have been experimentally rejuvenated by transgenic protein expression of various reprogramming factors, resulting in their metamorphosis into induced pluripotent stem cells (Yamanaka, 2008). Above all, highly differentiated cells of the immune system, human B cells, have been shown to become conditionally immortal in culture by simple stimulation with an extracellular ligand (CD40L), without the need of oncogenic transformation or other types of genetic manipulation (Wiesner et al., 2008). Lacking any signs of senescence, these cells maintained their functional capacity in terms of antigen presentation and T cell activation for more than 4 years in continuous culture and in continuous need of the

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extracellular ligand. Hence, the achieving of immortality on a cellular level appears to be a rather straightforward task. But also on the organismal level, numerous strategies to significantly extend longevity in different laboratory animal species have been described. For instance, the genetic manipulation of signalling pathways such as the IGF-1 cascade has led to surprisingly durable and long-lived animals (Bartke, 2008; Kuningas et al., 2008). Interestingly, many of these manipulations have just brought about a simple impairment of the targeted signalling pathway and may thus be considered to constitute rather artless alterations in a functional sense. Combinedly, these results seem to indicate that longevity was a modifiable biological property like many others and thus not fundamentally different from simple anatomical characteristics such as body size or adipose tissue mass, the default targets of the IGF-1 cascade.

Given these observations, what is the role of primary building block stability in attaining longevity? In contrast to modifications in individual signal transduction pathways, which might already become optimized by a single amino acid substitution, structural stability optimizations have to work on a much more collective level. For a single cell to become resistant to heat, radiation, or starvation, numerous proteins, lipids, and other cellular constituents have to be optimized in a stepwise fashion, by variation and selection. Hence, if collective structural adaptations were detectable in higher animals in definite relation to increased longevity, such a finding would strongly argue for the indispensable importance of the corresponding structural stability aspect, because in such a case, one would have to conclude that all other conceivable adaptations on more easily accessible levels have failed to achieve the same result. In other words, why should tedious and time-consuming bit-by-bit evolutionary optimizations on a structural stability level have taken place if simpler, more straightforward mechanisms of signal transduction modulation (e.g., impairment of the IGF-1 cascade), enhanced cellular replacement (e.g., from stem cells) or accelerated molecular repair (e.g., by faster re-synthesis) were already sufficient to extend longevity in real life in the wild?

In the following, one such step-by-step evolutionary optimization on the structural stability level is described, i.e. the loss of the amino acid cysteine in respiratory chain complexes with increasing species longevity. Regarding humans as a case-in-point, this adaptation comprises approximately 80 different amino acid substitutions, demonstrating the immense evolutionary pressure against cysteine in the inner mitochondrial membrane of long-lived animals.

2. Mitochondrial cysteine depletion and longevity

In the animal kingdom, global cysteine usage is characterized by little variation. Irrespective of phylogeny or particular life history traits, nuclear-encoded cysteine usage amounts to approximately 2.0–2.5% of all encoded amino acids (Moosmann and Behl, 2008). Mitochondrially encoded cysteine usage, however, varies substantially, and accounts for only ~0.5% of all amino acids in certain chordates, while contributing ~4.5% of all amino acids in some platyhelminthes. It was recently demonstrated that this surprising variation is attributable to two particular life history traits: first, in a qualitative fashion, aerobicity, and second, in a quantitative fashion, longevity. Focusing on free-living, aerobic taxa, i.e. chordates and several classes of arthropods, it was found that mitochondrially encoded proteins of these animals were characterized by severe cysteine depletion compared to nuclear-encoded proteins, the degree of which was correlated with the maximum lifespan of each species in a highly significant manner. This result is visualized in Fig. 1A, which shows that mitochondrial cysteine usage seems to constitute an interphyletic, universal correlative of longevity in animals. The specificity of this correlation was established by controlling for potentially confounding variables including body mass and phylogenetic interdependence (Moosmann and Behl, 2008). Interestingly,

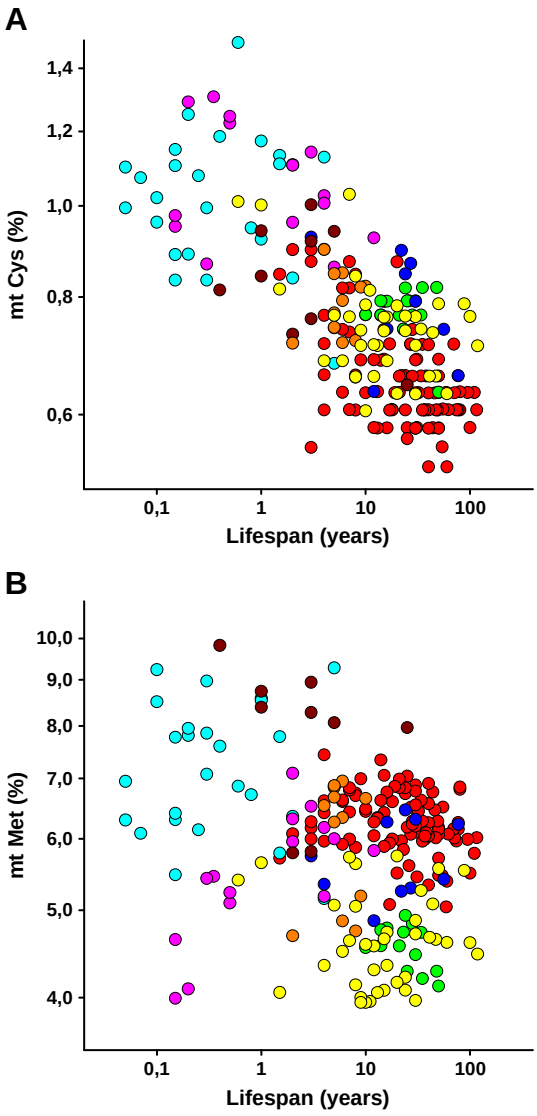

Fig. 1. Mitochondrially encoded, proteomic cysteine (A) and methionine (B) contents of 218 animals in relation to their maximum longevity. The selection of the depicted species (chordates and arthropods) was done as described (Moosmann and Behl, 2008). The color code denotes: mammals (red), birds (green), reptiles (blue), amphibians (orange), fish (yellow), insects (cyan), crustaceans (pink), and arachnids (brown). There is a highly significant relationship between mitochondrial cysteine usage and longevity in animals, whereas mitochondrial methionine usage and longevity are not correlated. (A) is modified from Moosmann and Behl, 2008.

even a very short-lived species like *Caenorhabditis elegans* still displays approximately 40% cysteine underrepresentation in its mitochondrially encoded proteome as compared to its nuclear-encoded counterpart. These observations led to the assumption that cysteine as a very oxidant-labile amino acid might be avoided in the inner mitochondrial membrane of long-lived species to avoid any possible harmful effects of spontaneous amino acid oxidation. This hypothesis was tested by investigating a diverse set of parasitic species which after maturation seem to largely or completely avoid respiration and thus avoid the main pathway of reactive oxygen species formation. It was found that these anaerobic–parasitic worms of the nematode, platyhelminth, and acanthocephalan phyla were characterized by much higher mitochondrially encoded cysteine levels than almost any chordate or arthropod (Fig. 2A). Moreover, a correlation of cysteine usage with lifespan was undetectable. Aerobic worms of the nematode, annelid, and chaetognath classes, however, followed the cysteine-lifespan correlation similarly as the higher