THOMAS NYSTRÖM, MANUEL BALLESTEROS AND ÅSA FREDRIKSSON

BACTERIAL SENESCENCE AND THE OXIDATION PARADOX

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Abstract. Many Escherichia coli regulons, including the rpoS, fur, and oxyR ones, have a role in preventing premature oxidative deterioration of starving cells. Mutant cells lacking one or more of the master regulators of these networks exhibit increased levels of oxidatively damaged proteins and lose culturability prematurely during glucose limitation. However, the increased oxidation of target proteins also in wild-type cells subjected to glucose depletion suggests that the battery of stress regulons responding to carbon starvation is not sufficient to fully combat oxidative modification and the molecular reason for this increased oxidation is obscure. We have recently shown a correlation between synthesis of aberrant proteins and their oxidative modification. The level of aberrant proteins was elevated in Escherichia coli cultures by decreasing transcriptional or translational fidelity using specific mutations or drugs. Protein carbonylation, an oxidative modification, increased in parallel to the induction of the heat shock chaperone GroEL. As the protein turnover rates and level of intracellular oxidative stress remained unchanged, it appears that the carbonylation results from the increased susceptibility of the misfolded proteins. These studies show that the cellular protein oxidation is not only dictated by available reactive oxygen species, but by the levels of aberrant proteins.

1. INTRODUCTION

It has been proposed that aging result from random deleterious events and oxidative damage has been suggested to be one major contributor to such stochastic degeneration of organisms and their cells. Denham Harman was perhaps the first to suggest that free radicals produced during aerobic respiration cause cumulative oxidative damage, resulting in aging and death (Harman, 1956). The theory gained in credibility with the identification of superoxide dismutase, which provided compelling evidence of in vivo generation of superoxide anions (McCord and Fridovich, 1969). The hypothesis has later been supported by different experimental data demonstrating that steady-state levels of oxidatively damaged macromolecules, including DNA, protein, and lipids, increase with age in all species examined thus far, and that oxidatively modified proteins lose their catalytic activity and structural integrity. Also, there is a close association between oxidative damage of
macromolecules and life expectancy of houseflies. However, the strongest support of the theory comes from experiments in which the life-span of fruitflies was prolonged by overproducing antioxidants (Orr and Sohal, 1994), and recent identifications of gerontogenes (genes whose alteration causes life extension) in *Caenorhabditis elegans* further support the notion of a strong correlation between longevity and oxidative stress defence (Murakami and Johnson, 1998; Lin et al., 1998). In addition, the life-span of resting unicellular microbes, such as *Escherichia coli* (Dukan and Nyström, 1998; 1999) and *Saccharomyces cerevisiae* (Longo et al., 1996) appears to be limited by the cell’s ability to combat reactive oxygen species.

The causal factors behind the increased levels of oxidized macromolecules in resting and aging cells is a key question that has not been resolved. Some attempts have been made to correlate oxidation in aging cells with a reduced activity (or abundance) of the antioxidant defence and repair systems (or proteases involved in degradation of oxidized proteins). However, these attempts have generated conflicting results. For example, catalases have been demonstrated to either increase or decrease with age depending on the tissues analyzed (e.g. Sohal et al., 1995) and in other studies it has been demonstrated that some antioxidant defence proteins may increase while others decrease with age in the same tissues (e.g. Ji et al., 1991). Moreover, as pointed out by Beckman and Ames (1997), it is not clear whether an elevated abundance of an antioxidant defence system in any given cell indicates that there will be less oxidative damage in this cell or that it is experiencing an increasing oxidative load. In prokaryotic model systems, such as *E. coli*, the situation is paradoxical rather than conflicting since in a resting, starved, population of *E. coli* cells, it is known that the levels of both primary and secondary oxidative defence proteins increase markedly and that the population becomes increasingly resistant to external oxidative stresses (Jenkins et al., 1988). Yet, as will be reviewed here, the levels of oxidatively damaged proteins in such a resting *E. coli* population increase (Dukan and Nyström, 1998; 1999). The possible importance of and causation for this increased oxidation is the subject matter of this paper and recent findings indicating that the free radical hypothesis of aging may be applicable also to starving cells of *Escherichia coli* will be reviewed.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents.

Detection of carbonylated proteins was performed using the chemical and immunological reagents of the OxyBlot Oxidized Protein Detection Kit (Oncor). The chemiluminescence blotting substrate (POD) was obtained from Boehringer Mannheim and used according to instructions provided by the manufacturer. Immobilon-P polyvinylidene difluoride (PVDF) membrane was obtained from Millipore Corp.