On the role of protein oxidation and heat shock proteins in senescence and fitness

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Abstract

Similar to the ageing process of eukaryotes, oxidative damage to cellular macromolecules may be involved in deterioration of growth arrested (stationary phase) *Escherichia coli* cells; a process referred to as 'conditional cell senescence'.

In this work we demonstrate that the heat shock proteins (Hsps) are key players in the cellular defence against deleterious protein oxidation (carbonylation) during conditional senescence in *E. coli* cells, that such oxidation is linked to increased production of aberrant proteins caused by increased mistranslation, and that carbonylation of aberrant proteins, which are intrinsically sensitive to oxidation, can occur in the absence of increased oxidative stress. Hsp70 (DnaK), together with the Lon and ClpQY Hsp proteases, are shown to be major participants in protecting stationary phase cells against accumulation of carbonylated proteins. A further link between protein oxidation and Hsps were established by results showing that induction of the heat shock regulon in response to increased mistranslation requires oxidative modification of the malformed proteins. This is shown to be true both for cells entering stationary phase and for cells in which the ribosomes display reduced translational fidelity due to mutations in the ribosomal accuracy centre. In addition to affecting Hsp regulation, mistranslated and oxidized proteins, also affect stationary phase elevation of the transcription factor, SigmaS (σ^S) and induction of the σ^S regulon. Mechanistically, this effect of mistranslation on σ^S acts via titration of the ClpP-protease (ClpXP is responsible for σ^S degradation). σ^S is a key player in switching gene expression from growth/reproduction related activities towards those of maintenance and is essential, similar to the Hsps, to counteract protein oxidation upon entry of cells into stationary phase.

Furthermore, using *Salmonella enterica* serovar Typhimurium LT2 we demonstrate that random mutations achieved during evolution interact such that their combined effect on fitness is mitigated (antagonistic epistasis). The levels of DnaK and GroEL were elevated in lineages with many point mutations. Also, ectopic overproduction of GroEL was demonstrated to increase fitness in such strains. These data suggest that chaperones may buffer the cell against the fitness cost caused by the accumulated mutations and provides a mechanistic, physiological, explanation for antagonistic epistasis.

Keywords: *Escherichia coli*, senescence, fitness, protein oxidation, protein carbonylation, heat shock proteins, Hsp70, DnaK, GroEL, Lon, ClpXP, proteolysis, Sigma32, SigmaS, antagonistic epistasis, *Salmonella*

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