GÖTEBORGS UNIVERSITET

On Aging and the Role of Ubp3 in Heterochromatic Gene Silencing and Protein Quality Control

David Öling

Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap med inriktning Biologi - Mikrobiologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 26:e September 2014 kl. 09:00 i Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg.

ISBN: 978-91-628-132-9

http://hdl.handle.net/2077/36643

GÖTEBORGS UNIVERSITET

Abstract

Aging is characterized by a build-up of damage in organisms ranging from protists to multi-cellular species. This damage adversely affects core components such as DNA and proteins which are necessary to sustain life. Remarkably, as an old yeast cell divides, its daughter cell is fully rejuvenated suggesting that age-related damage can be asymmetrically inherited and/or completely ameliorated. This thesis approaches the central question of how cells combat such damage to allow longevity.

Specific interest was directed towards the deubiquitinating enzyme Ubp3 which had already been shown to be tightly linked to regulation of transcription and proteome surveillance, both of which are essential in cells adaptation to stress. In this thesis, I show that cells lacking Ubp3 are short-lived despite displaying decreased unequal recombination at rDNA and increased silencing at all three heterochromatic regions in *S. cerevisiae* subjected to transcriptional silencing. These findings are at odds with existing aging-models in yeast, highlighting that increased silencing at rDNA is associated with long lifespan. Instead, our data suggest that premature aging in cells devoid of *UBP3* is caused by a pathway other than rDNA recombination/silencing. Indeed, I found that Ubp3 has an important dual role in protein quality control by saving or destroying aberrant protein species depending on the stage at which the damaged protein is committed for proteasomal destruction. Furthermore, in virgin and young cells lacking *UBP3*, aggregated proteins accumulated prematurely at a juxta-nuclear position whereas wild-type cells showed no indication of protein damage. In middle-aged and older cells in the same mutant, more aggregates accumulated at a peripheral location. This accumulation of peripheral aggregates correlated, in time, with a decline in mutant cell survival.

Similar to Ubp3, the well-characterized silencing-factor Sir2 is known to regulate other aging-processes unlinked to silencing. We show that Sir2-deficient cells display increased daughter cell inheritance of stress and age-induced misfolded proteins deposited in aggregates and inclusion bodies. This asymmetric inheritance has been argued to take place in a passive manner due to slow and random diffusion of aggregates. We present evidence that this is not a plausible scenario. The control of damage inheritance is more likely mediated by Sir2-dependent regulation of the chaperonin CCT which is required for folding actin and feeding the polarisome with properly folded substrates. We discuss data underlying these conflicting models and seek to understand which model best explains how damage asymmetry is achieved.

Keywords: Aging, protein damage, segregation, transcriptional silencing, rDNA, *UBP3*, *SIR2*, protein aggregates