Protein Damage Control during Embryonic Stem Cell Differentiation:
Role of the Proteasome

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AKADEMISK AVHANDLING

För filosofie doktorsexamen i Naturvetenskap, inriktning biologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras onsdagen den 1 juni 2011 kl. 10.00 i Carl Kylberg, Institutionen för Cell- och Molekylärbiologi, Medicinaregatan 7B, Göteborg.

Fakultetsopponent: Professor Bertrand Friguet, Université Pierre et Marie Curie - Paris 6 (UPMC), France

The thesis is based on the following papers:

I. *Elimination of damaged proteins during differentiation of embryonic stem cells.*
Hernebring M*, Brolén G*, Aguilaniu H, Semb H & Nyström T. (2006) Proc Natl Acad Sci U S A. 103(20): 7700–7705.
* contributed equally to this work

II. *Identification of Hsc70 as target for AGE modification in senescent human fibroblasts.*
Unterluggauer H, Micutkova L, Lindner H, Sarg B, Hernebring M, Nyström T & Jansen-Dürr P. (2009) Biogerontology. 10(3):299-309.

III. *The proteasome activator PA28 is required for the removal of damaged proteins during differentiation of mouse embryonic stem cells*
Hernebring M, Fredriksson Å, Norrman K, Rivett J, Wiseman J, Semb H & Nyström T. Manuscript.

IV. *Effects of aging and reproduction on proteostasis in soma and gametes of* Drosophila melanogaster.
Fredriksson Å, Krogh-Johansson E, Hernebring M, Pettersson E & Thomas Nyström. Manuscript.

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ABSTRACT

During the lifespan of organisms ranging from yeast to humans, there is an accumulation of macromolecular damage. However, these organisms produce youthful progeny with low damage levels. This thesis focuses on how this is accomplished.

I have analyzed whether the levels of oxidatively damaged proteins change in mouse embryonic stem (ES) cells during the initial steps of cell specification (differentiation) from the pluripotent state. The results show that ES cells contain high levels of proteins modified by carbonyls and advanced glycation end products and that the identity of these damaged proteins, including chaperones and proteins of the cytoskeleton, are the same as those of aged tissues. However, early differentiation is accompanied by a dramatic drop in the damage of such proteins, both in cultured ES cells and in the blastocyst *in vivo*. In addition, differentiation of ES cells triggers production and assembly of the proteasomal complex PA28-20S/20Si. Experiments using proteasome inhibitors and RNAi technology suggest that both the 20S proteasome and its regulator PA28 are required for the clearance of damaged proteins during differentiation of ES cells.

The data point to previously unknown roles of PA28 both in protein homeostasis and during early embryogenesis. Moreover, the results support a model in which the restoration of low levels of protein damage at the start of each generation is achieved, in part, by a maintained capacity of the germ line to rid itself from such damage.

Similar to mouse ES cells, studies using *Drosophila melanogaster* indicate that the main reduction in protein damage at the start of a new generation may depend on the proteasome. However, in contrast to the situation in mice, this reduction of protein damage appears to take place prior to fertilization. The data also indicate that mating has a negative effect on protein damage control and highlight egg production as a potential culprit in the trade-off between somatic maintenance and reproductive success.

Key words: Embryonic Stem Cells, Proteasome, Proteasomal Activator PA28, Protein Carbonylation, Advanced Glycation End products, Cell Differentiation, Oxidative stress, Aging, RNAi

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