Tracking the big ones : novel dynamics of organelles and macromolecular complexes during cell division and aging

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Summary

When proteins would be static entities, instead of the highly dynamic entities they are, life would never have evolved. It is the complex interplay of protein synthesis, folding, degradation, modification, movement and interactions that makes a cell something alive instead of a lifeless bag full of proteins. Understanding these protein dynamics is therefore pivotal to understand life and is essential to understand the causes and treatment of many human diseases. In this Thesis we address two important aspects of protein dynamics; protein synthesis and distribution upon cell division and dynamics of the protein degradation machinery.

Adequate synthesis and distribution of proteins upon cell division is important to make sure that both new cells get enough starting material to support life. This is particularly true for the proteins inside organelles and macromolecular complexes, since these large cell structures are essential for the cell and often synthesized in a template-based manner. Therefore, components of the existing organelles and macromolecular complexes need to be adequately shared between both new cells (inheritance) and complemented with synthesis of new components. Synthesis of organelles and macromolecular complexes can occur either *de novo* or template-based. The mechanisms underlying the inheritance and synthesis of organelles and macromolecular complexes have been extensively studied in budding yeast, yet important questions remained unanswered. Like whether both new cells get an equal share of existing and new components and to what extend some of these structures are synthesized in a *de novo* or template-based manner.
To address these questions one needs to distinguish and simultaneously track old and new proteins. We therefore developed the Recombination-Induced Tag Exchange (RITE) technique in **Chapter 2**. RITE is a genetic method that induces a permanent epitope-tag switch in the coding sequence after a transient hormone-induced activation of Cre recombinase. Old and new proteins can thus be simultaneously tracked by their different tags and the variety of available tags allows detection by a wide range of methods.
In **Chapter 3** we applied RITE to make a comprehensive analysis of the synthesis and inheritance of organelles and macromolecular complexes upon cell division. Asymmetric inheritance of these large cell structures are of interest since they can induce lineage differences and play a role in cell differentiation. Our analysis shows that, in general, old and new components of organelles and macromolecular complexes are symmetrically inherited. Apparently the age of these components does not play a role in asymmetric inheritance and does not induce lineage differences. An interesting exception to this common rule is the Spindle Pole Body (SPB), the yeast centrosome, which shows predominant inheritance of old components in the young daughter cell. The biological consequence of this asymmetrical inheritance remains to be determined. Our RITE-based analysis also shows that all membrane-containing organelles are synthesized in a template-based manner in budding yeast. This was known or expected for most organelles, but still debated for peroxisomes. Also most macromolecular complexes showed a template based synthesis, with the interesting exception of the nuclear pore complex (NPC). Our data not only shows *de novo* synthesis of NPC's, but also suggests that there is no exchange of old for new subunits in this very stable complex over many generations.

The dynamics of the protein degradation machinery is of interest since it affects the

degradation of damaged proteins in aging cells. The accumulation of damaged proteins in aging cells suggests that insufficient protein degradation is an important factor in cellular aging and is implicated in several age-related diseases. An important mechanism for the degradation of damaged proteins is the ubiquitin-proteasome system (UPS). The activity of the UPS is found to decrease during the aging of several model organisms and this decrease is suggested to play a causative role in cellular aging. Also, enhanced UPS activity seems to correlate with enhanced longevity. These observations fueled a growing interest in the role of UPS activity in the aging process and raise the possibility of curing age-related diseases by enhancing this activity.

In **Chapter 4** we present data that suggest that not only the activity of the UPS, but also the localization of this activity may play a role in cellular aging. We found a correlation between the localization of the proteasome and the replicative age of starving budding yeast cells, a frequently used model for cell aging. Also, a genome-wide screening identified a role for N-terminal acetylation in both proteasome localization and fitness during cellular aging. All in all our data justifies further research on the role of proteasome localization in aging and age-related diseases.

One reason for the decrease in UPS activity during aging may be a decreasing 'fitness' of the pool of proteasomes. Being a protein complex itself, the proteasome is vulnerable to protein damage which may compromise its activity. Therefore, analogous to damaged proteins, one would expect damaged proteasomes to be cleared from the cell. The scope and mechanisms of such proteasome quality control however, remained to be determined.

In **Chapter 5** we present the first sketches of a potential proteasome quality control mechanism. Our data is consistent with a model in which damaged proteasomes are degraded by some form of autophagy. The specifics and specificity of this degradation remain to be established and is of high interest since it is likely to play a role in both cellular aging and age-related diseases.

In summary, this Thesis provides a comprehensive analysis of the synthesis and inheritance of organelles and macromolecular complexes upon cell division and uncovers novel aspects of proteasome dynamics that may play a role in cellular aging.