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

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If you are told that a bag full of marbles could be alive, you would never believe it. Still, this is exactly what a cell is; a bag full of lifeless material that is very much alive!

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Cover: Microscopic images of budding yeast cells from a screening for genes involved in proteasome dynamics. The localization of old and new proteasomes is visualized by the green en red color respectively and the blue color indicates the location of the nucleus. Each group of cells represents a knock-out mutant in a specific growth condition.

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Scope of the Thesis

Although Heraclitus did most likely not think about proteins when he made his famous statement "Panta Rhei", this saying applies very well to proteins inside cells. Cellular proteins are highly dynamic due to continuous synthesis, degradation, modification, translocation and interactions with other cellular components [1–3]. Protein dynamics is an absolute requirement for life and aberrations of it are implicated in several diseases. Understanding the processes underlying protein dynamics is pivotal to understanding these diseases and developing effective treatments [2–4]. 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.

Upon cell division, a cell needs to distribute its components in such a way that both of the new cells get enough starting material to support a successful life. This is accomplished by sharing the existing components of the mother cell (inheritance) and by the synthesis of new components. Synthesis of macromolecular complexes and organelles can occur *de novo*, or by growth and division of existing structures (template-based). The template-based synthesis of some of these essential cell structures makes the inheritance of the existing components particularly important. The mechanisms underlying the inheritance of organelles and macromolecular complexes have been successfully studied in budding yeast and are often found conserved in mammalian cells [5]. Budding yeast has the advantage of easy genetics and a distinguishable 'mother' and 'daughter cell upon cell division and is therefore used as a model organism in this Thesis.

In this Thesis we applied a new tool, Recombination-Induced Tag Exchange (RITE) [6,7], to visualize novel aspects of inheritance and synthesis of organelles and macromolecular complexes in budding yeast. RITE is able to distinguish and simultaneously track old and new proteins and can thus be used to study the inheritance of existing (old) cell components and the synthesis of new components [6,7]. Using RITE, we made a comprehensive analysis of the inheritance and synthesis of all organelles and the major macromolecular protein complexes in cells. Our data resolves ongoing debates on the synthesis of certain organelles (*de novo* or template-based) and visualizes patterns of symmetric and asymmetric inheritance [8]. Asymmetric inheritance of organelles is of interest since it may define lineage differences and play a role in the differentiation of mammalian cells [9,10].

The dynamics of the protein degradation machinery is of interest since it affects the degradation of damaged proteins in aging cells. Damaged proteins tend to accumulate in aging cells and are implicated in several age-related diseases [3]. This suggests that insufficient degradation of damaged proteins is a dominant factor in cellular aging. An important mechanism for the degradation of damaged proteins is the ubiquitin-proteasome system (UPS) [11]. 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 [12–14]. Also, enhanced UPS activity seems to correlate with enhanced longevity [15,16]. 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 this Thesis 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 tracked the localization of the degrading entity of the UPS (the proteasome) in starving budding yeast cells, a frequently used aging model. We observed a correlation between the localization of

the proteasome and the age of the cell and identified genetic factors controlling both proteasome localization and the fitness of aging cells [17].

One reason for insufficient UPS activity in aging cells may be a less 'fit' pool of proteasomes. Being a protein complex itself, the proteasome is also vulnerable to protein damage. 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, remain to be determined. Earlier studies suggested that the proteasome is an extremely stable complex with a reported half-life ranging from 5 to 12 days [18–20]. Studies in rat liver suggest that proteasomes are degraded in the lysosomal compartment [20]. In this Thesis we present data supporting lysosomal degradation of proteasomes in budding yeast and HeLa cells, which are better suited model systems for further study then rat livers. Also, our findings in HeLa cells are consistent with a model in which damaged proteasomes are delivered to the lysosome by autophagy.

In summary, this Thesis addresses synthesis and distribution of proteins upon cell division and the dynamics of the protein degradation machinery. Application of RITE technology in budding yeast gave us the unique opportunity to track both the age of proteins and of cells, which yielded novel insights relevant for cell differentiation and cellular aging. In the study of a possible degradation mechanism for the proteasome, we extended our findings in yeast to a mammalian cell line.