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

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Chapter Six

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Conclusions and Discussion

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When proteins would have been static entities, instead of the highly dynamic entities they are, life would never have evolved. It is the complex interplay of protein synthesis, repair, degradation, modification, movement and interactions that makes a cell a living entity instead of a bag full of proteins. The ability to monitor these protein dynamics is therefore crucial for the discovery of the cellular processes underlying life.

The RITE tool to monitor protein dynamics

To study cellular processes, monitoring the dynamics of the total pool of a protein is informative. However, more detailed information can often be obtained by monitoring specific sub-populations within the total pool. A decrease in the overall level of a protein, for example, may be caused by enhanced degradation or decreased synthesis. These possibilities can be distinguished when one can track the dynamics of a sub-population of this protein, e.g. by labeling a defined population with radioactive labeled amino acids as one does in a pulse-chase assay. Sub-populations may also be defined by functional differences. The overall levels of a kinase, for example, may not change upon a certain stimulus, whereas the sub-population of phosphorylated (and thus activated) kinases may. Antibodies specific for the (un) phosphorylated form are therefore of great additive value to study the dynamics of kinases.

To distinguish sub-populations of proteins based on their age (synthesized long ago versus recently), we developed a technique that we named Recombination-Induced Tag Exchange (RITE) [1,2]. RITE allows the distinction and simultaneous tracking of old and new proteins and has been successfully used to address the exchange of histones in chromatin [1], inheritance of histones and plasma-membrane proteins [3,4], synthesis and inheritance of organelles and macromolecular complexes [5], and the origin of nuclear proteasomes [6]. Here we discuss the strong and weak points of this technique as encountered in this Thesis as well as some future perspectives.

A strong point of the RITE technique is that it allows tracking of old and new proteins simultaneously. Amongst others, this capability enabled us to distinguish *de novo* and template-based synthesis of peroxisomes and nuclear enrichment of proteasomes due to localized synthesis or retention of existing proteasomes [5,6]. Whereas these studies relied on fluorescent tags, a series of different RITE cassettes and the modular design of these cassettes allows easy adjustment of the tags to the experimental needs [2]. In Chapter 2, for example, epitope tags are used for immunodetection and purification of old and new histones [1]. Old and new proteins may also be distinguished by using tags with a different size. This allows simultaneous Western-blot detection with a single antibody (e.g. against LoxP or the tagged protein), which enables quantitative comparison of the levels of old and new proteins [2]. Adequate detection of old and new proteins in this Thesis is supported by a high recombination efficiency of the RITE cassette upon induction and low levels of non-induced background recombination. Application of RITE in these studies is further strengthened by the endogenous expression of the tagged proteins and the lack of a non-tagged (WT) protein pool. The genomic integration of the RITE cassette and the efficient and irreversible recombination make RITE suitable for many downstream applications including high-throughput screening. This was successfully exploited in a microscopy-based screening for factors involved in proteasome dynamics and a CHIP-Seq based screening for factors modulating histone turnover in the chromatin [6,7].

A limitation of RITE is the time needed to complete recombination in the total population of cells, which is ~2 h under both nutrient-rich and starvation conditions [1,2]. This makes RITE less suitable to track fast protein dynamics, since synthesis of RITE tagged proteins during this time period will yield proteins with both new and old tags. In this Thesis we minimized this problem by inducing the recombination in nutrient-starved cells, which show only limited transcription and translation [8]. In this system the old protein pool is mainly produced before nutrient starvation and a pool of new proteins is emerging slowly after recombination when starvation is prolonged. On the other hand, a strong increase in new proteins can be observed when releasing cells in nutrient rich medium, which quickly induces new transcription and translation. Recombination under starved conditions and release in fresh medium not only expands the 'age-gap' between old and new proteins, but also results in a more or less synchronized first cell division. This principle was used in Chapter 3 to study the inheritance of organelles and macromolecular complexes.

Whereas RITE was successfully used in Chapter 3 and 4 to address the localization and synthesis of old and new proteins, it was unable to detect degradation of old proteasomes in Chapter 5. This may be due to a very high stability of the proteasome and/or insufficient sensitivity of the RITE technique. The sensitivity of RITE is probably higher for synthesis then for degradation, as synthesis adds new signal to a small population and degradation removes signal from a big population. Still, there is no reason to suspect that RITE cannot be used to address the degradation of other (less stable) proteins. In fact, RITE has been successfully used to detect the disappearance of old histones from the chromatin in growth arrested yeast cells [1,7].

All in all, we can conclude that RITE is a valuable tool to distinguish and simultaneously track old and new proteins with a variety of different read-outs under a variety of different conditions. Especially its compatibility with screening methods makes it an interesting tool to further study factors involved in the dynamics of specific proteins, protein complexes and organelles. In this Thesis, we present applications of RITE in budding yeast only. However, the universal nature of the RITE cassettes and the available Cre-recombinases for other organisms make RITE in principle applicable to a broad range of model systems. The emergence of techniques to tag proteins endogenously in higher eukaryotes will even further increase the potential of RITE in these model systems [9].

Organelle dynamics upon cell division

In Chapter 3 we applied RITE to make a comprehensive analysis of the synthesis and inheritance of organelles and macromolecular complexes upon cell division. The inheritance of old and new components of these structures can be addressed by RITE and may be important to define lineage differences. Furthermore, RITE can address the biogenesis of these structures, as it is able to distinguish *de novo* and template-based synthesis. Here we discuss the general conclusions from this analysis and some organelle specific issues.

Our comprehensive analysis shows that, in general, old and new components of organelles and macromolecular complexes are symmetrically inherited upon cell division [5]. Apparently the age of these components does not play a role in asymmetric inheritance or lineage differences. This suggestion is supported by the observed mixing of old and new components in all tested organelles and the nucleolus [5]. The mixing of old and new components of mitochondria, in particular, illustrates this notion as yeast cells do show asymmetrical inheritance and lineage differences based on the functionality of this

organelle [10,11]. An interesting exception to the generally observed mixing of old and new components is the nuclear pore complex (NPC). Since the NPC is formed *de novo* and is very stable, the age of its components reflects the age of the entire complex and may thus influence its functionality and inheritance [12]. Still we find symmetrical inheritance of old and new NPCs, suggesting that the age of the complex is unconnected to its inheritance. Symmetrical NPC inheritance also resolves ongoing debates about whether or not daughter cells obtain their nuclear pores by synthesis and inheritance or by *de novo* synthesis only [13,14]. Our data based on two RITE tagged subunits of the nuclear pore strongly support the first model and was later strengthened by similar observations for three other subunits [15]. All in all we can conclude that symmetrical inheritance of old and new components of organelles and macromolecular complexes is a common rule in budding yeast. Whether this is also the case in mammalian cells remains an interesting question.

An interesting exception to this common rule is the spindle pole body (SPB), the yeast centrosome, which shows an unexpected mode of asymmetrical inheritance [5,16]. Based on our data, we propose the following model. Upon cell division, a small portion of this macromolecular complex splits to initiate the duplication process. New components are added to this template to build a new SBP and a similar amount of new components is added to the existing SPB, which is therefore bigger. Interestingly, the bigger SPB (containing the majority of the old components) is inherited by the daughter cell (the younger lineage), whereas the smaller SPB (containing mostly new components) stays in the mother [5]. Apparently the old components of the SPB contain functionalities that are favorable for the daughter cell or the daughter needs a bigger SPB. As a consequence of this inheritance pattern, the SPB in the mother cell will shrink after each cell division. Whether a shrinking SPB is limiting the replicative potential of a mother cell or whether mother cells synthesize new components to compensate for this loss at a later time point is an interesting question for further research.

Another aspect that we addressed in our RITE-based analysis of organelles and macromolecular complexes is whether they are synthesized *de novo* or template-based. Template-based synthesis is known or expected for most membrane containing organelles [17], though is still debated for peroxisomes [18,19]. In our RITE assays, template-based synthesis should result in mixing of old and new components within the same compartment. This was indeed observed in the nucleolus and all membrane containing organelles, including the peroxisome. Our data not only supports a model of template-based synthesis of peroxisomes, but also excludes *de novo* synthesis since no peroxisomes were observed with only new components. In summary, our work suggests that all membrane containing organelles are synthesized in a template-based manner in budding yeast.

The macromolecular complexes in our analysis displayed more variation, since the nucleolus and the SPB showed template-based synthesis and the NPC showed *de novo* synthesis. *De novo* synthesis of NPCs is apparent since old and new NPC components do not co-localize in our assays, although they are present in the same nucleus. Even several divisions after the recombination of the RITE cassette, old components appear to exclude new components indicating that the tagged subunits do not exchange visibly in these very stable complexes. Interestingly, old and new nuclear pores appear to cluster in their own areas on the nuclear envelope. The reason for this phenomenon is unknown, but may be related to the assembly of this complex. The clustering of NPCs and their stability across generations is, unlike their *de novo* synthesis, most likely not conserved in mammalian

cells, since these cells completely disassemble their nuclear envelope and NPCs during cell division [12].

Overall we can conclude that template-based growth and symmetric distribution of old and new components upon cell division are common aspects of organelle and macromolecular complex dynamics. Two important exceptions are the nuclear pore complex, that is formed *de novo*, and the spindle pole body, that showed asymmetric inheritance.

PSGs; storage granules or protein quality control compartments?

In Chapter 4 and 5 we used budding yeast to address novel aspects of proteasome dynamics [6]. A characteristic aspect of this dynamics in yeast is the sequestration of proteasomes in cytoplasmic foci upon glucose depletion (starvation). These foci are stable when starvation is prolonged, though when nutrients are re-added they rapidly dissolve to restore the normal (non-starved) proteasome localization. These observations made the initial discoverers suggest that the main function of these foci is storage of the proteasome, hence they named them Proteasome Storage Granules (PSGs) [20]. However, when the term PSG was coined, another research group reported stress-induced cytoplasmic proteasome foci that they named JUxta-Nuclear Quality control (JUNQ) compartments. JUNQ compartments are induced by protein stress and are suggested to enhance Protein Quality Control (PQC) by sequestering proteasomes and their substrates in the same compartment [21]. The discovery of JUNQ compartments raised the question whether PSGs are indeed storage compartments (as initially suggested) or compartments with high PQC activity (like JUNQ compartments) [22]. Although a definite answer to this question is missing, the data presented in this Thesis strongly favors the storage function of PSGs.

First of all, it is unlikely that JUNQ compartments and PSGs represent the same structure induced under different conditions (protein stress and glucose depletion respectively). One reason is that JUNQ compartments are in close proximity to the nuclear or ER membrane [21], whereas PSGs do not show any membrane association [20] (and our unpublished results). Another reason is that JUNQ compartments show a strictly mother-biased segregation upon cell division [21,23], which is not compatible with the higher prevalence of PSGs in virgin cells that we observed [6]. However, so far there are no reports on the presence of marker proteins (BLM10 for PSG [24] and HSP104 and soluble misfolded proteins for JUNQ compartments [21]) in both structures.

Although it is unlikely that PSGs are JUNQ compartments, they may still represent compartments with high PQC activity. If so, one would expect to find active proteasomes in PSGs. The presence of both 20S and 19S (base and lid) subunits in the same PSGs suggests the presence of (active) 26S proteasomes [20,25]. However, native gel analysis of starved yeast cells yields conflicting data. Whereas Laporte *et al* shows predominantly 26S proteasomes in these cells, Weberuss *et al* convincingly shows PSG-containing cells with predominantly 20S proteasomes [20,24]. Starvation-induced 26S proteasome dissociation was also observed by Bajorek *et al* and suggested to be caused by a drop in ATP levels [26]. On the other hand, a subunit of the 19S lid complex was found to be required for PSG formation, which suggests sequestering of intact 26S proteasomes [27]. Also, proteasome dissociation does not appear to be required for PSG formation [27,28]. These apparently conflicting data may suggest a model in which the dissociation of the 26S and the formation of PSGs are independent processes.

Other than discussing proteasome conformation in PSGs, the issue of whether or not proteasomes are active in PSGs may also be addressed by using model substrates. Simply assessing the decreasing levels of these substrates is not informative; since it cannot be excluded that the proteasomes outside the PSGs are responsible for the observed degradation. A rapid exchange of (fluorescent) model substrates between PSGs and its surroundings or ubiquitin dependent recruitment of substrates to the PSGs, may be a better indication of active proteasomes [21].

Two additional observations argue against PSGs as compartments with high PQC activity. First, if the proteasome would be sequestered with high amounts of substrates one would expect their degradation before proteasomes are released from the PSGs. This seems incompatible with the rapid release of proteasomes (within 10 minutes) upon addition of fresh nutrients [20,24] (our unpublished results). A second argument relates to the drop in cellular ATP levels during the starvation process. This could lead to 26S disassembly [26], but may also necessitate the cell to shut down the energy consuming activity of the UPS in favor of less energy consuming processes, like autophagy or the sequestration of damaged proteins by small Heat Shock Proteins [8,29]. It may therefore very well be that PSGs convey PQC activity when cells have sufficient energy, but that this activity ceases rapidly during the starvation process.

Whereas it is not trivial to identify PQC functionalities of PSGs, PSGs show many similarities with known protein storage compartments in budding yeast. Several metabolic enzymes, a histone deacetylase and F-actin are known to form cytoplasmic foci upon the depletion of specific nutrients during yeast starvation [30–33]. Like PSGs, these cytoplasmic foci do not show any association with membranes and rapidly dissolve upon re-addition of nutrients. The formation of these foci, as for PSGs, is suggested to be induced by a drop in cytosolic pH that accompanies starvation due to glucose limitation [25,32]. The storage function of these foci is deduced from their very high stability in starvation and their rapid dissociation when the cell gets re-activated with new nutrients.

As reported for other storage compartments, we found a very high stability of PSGs since their prevalence hardly decreases during a two week starvation period (our unpublished results). Also, RITE experiments presented in Chapter 5 are consistent with a very high stability of the proteasome during starvation. This may suggest that storage in PSGs protects proteasomes from degradation. This hypothesis is also consistent with the positive correlation between PSG prevalence and proteasome abundance in starvation, which was found when studying a yeast knock-out library (our unpublished results).

The term 'storage' entails that the stored proteins are functional once released from their storage compartment. In many studies, including those describing PSGs [20,31,33], the functionality of stored proteins after release is deduced from their re-localization to their original (non-starved) location. Application of the RITE technology provided us with more direct evidence of the functionality of proteasomes after release from PSGs. When recombination of the RITE cassette was induced after two days of starvation, little or no synthesis was detected in the following three days and the old proteasome was sequestered in PSGs. Upon addition of fresh medium, PSGs rapidly dissolve and proteasomes regain their nuclear localization while cells prepare to start a new cell division. New proteasomes were only detected during this first cell division, indicating that the activity of the old (stored) proteasomes was at least sufficient to support this first cell cycle, for which they should be active (our unpublished results).

The data presented in this Thesis shed new light on the question whether PSGs are storage

compartments or PQC compartments. First, the higher prevalence of PSGs in virgin cells, as presented in Chapter 4, argues against being a PQC compartment. Second, application of RITE technology suggests that PSGs are bona fide storage compartments.

A role for proteasome localization and quality control in aging cells?

Accumulation of damaged proteins is a hallmark of cellular aging and implicated in several age-related diseases [34,35]. This suggests that the degradation of damaged proteins gets limiting during cellular aging. Limiting degradation of damaged proteins may (in part) be caused by the age-dependent decline in proteasome activity, as observed in several aging model systems [36–38]. Increasing proteasome activity may solve the limiting degradation during aging and is therefore expected to attenuate cellular aging. The validity of this idea was proven in budding yeast [39,40] and fuelled a growing interest in ways to enhance proteasome activity as potential treatment for neuro-degenerative and other age-related diseases [41]. In this Thesis we addressed novel aspects of proteasome dynamics that may also play a role during cellular aging; proteasome localization and proteasome quality control. Here we discuss their potential role in cellular aging.

In Chapter 4 we report a correlation between the localization of the proteasome and replicative age in starving budding yeast. This observation may indicate a role for proteasome localization in cellular aging. During the starvation process, budding yeast transports its nuclear proteasomes to the cytoplasm and stores them in cytoplasmic PSGs. Whereas this scheme applies to most replicative young cells during starvation, old cells are more likely to maintain their nuclear proteasome pool and not to form PSGs [6]. This may be caused by an age-dependent defect in the proteasome re-localization machinery, but may also reflect a response to the reported accumulation of protein damage in old cells [42,43]. Perhaps damage accumulation requires the maintenance of a larger pool of active proteasomes in old cells, which would be consistent with the low prevalence of PSGs. The nuclear enrichment of the proteasome may help old cells to clear protein damage in the nucleus [44,45], but may also support protein quality control in the cytoplasm [46,47]. The latter is especially important for quality control of (partially) unfolded cytoplasmic proteins, since one of the dominant E3s of this pathway (San1) is localized in the nucleus [46,47]. In summary, the data presented in Chapter 4 are consistent with a model in which the proteasome localization is adapted to the load of protein damage during aging. The exact nature of this damage and possible conservation of the age-related localization of the proteasome in mammalian cells requires further study.

Mammalian cells harbor proteasomes in both the nucleus and the cytosol [48,49], while not showing the nuclear enrichment as observed in budding yeast [50]. Similar to the situation in yeast, nuclear proteasomes in mammalian cells are implicated in nuclear protein quality control [45,51], as well as the degradation of misfolded cytosolic proteins [52]. The conservation of these processes may underlie conservation of age-related proteasome localization. However, proteasome localization in mammalian cells is more difficult to address than in budding yeast. Not only does a substantial population of free 20S proteasomes exist in several mammalian cell lines, there is also a compartment-dependent attachment of different regulatory particles [53,54]. Assessing proteasome localization therefore requires tracking of both the 20S and its regulatory particles. This principle has been illustrated by a study of age-dependent proteasome localization in human liver samples. Whereas the localization of several 20S subunits does not correlate with age, the cytoplasmic levels of the 11S complex (a regulatory particle like the 19S) specifically drop in the cytoplasm and

not the nucleus [55]. Another study showed a correlation between the nuclear localization of both 20S and 19S subunits and the neuronal vulnerability for Alzheimer's Disease and Creutzfeld-Jacobs Disease in human brains [56]. These correlations and our data in budding yeast justify a more extensive study on proteasome localization during aging in mammalian cells.

In Chapter 5 we present the first sketches of a potential quality control system for the 20S proteasome. Such a system is likely to play a role in the aging of long-lived non-dividing cells, like neurons, since its limiting activity could lead to a less functional pool of proteasomes. This, in turn, may contribute to the accumulation of damaged proteins that is implicated in cellular aging and several age-related neurodegenerative diseases. The data presented in Chapter 5 is consistent with a model in which damaged proteasomes are degraded by some form of autophagy. The specifics and specificity of this autophagic degradation remain to be established and may yield new insights in the mechanisms underlying cellular aging

Knowledge of the proteasome quality control mechanisms may also yield new treatment modalities for age-related diseases. Currently the idea of treating these diseases by enhancing proteasome activity gets increasing attention [57]. Potential compounds for these treatments are IU1, a small molecule inhibitor of the proteasome associated de-ubiquitination enzyme USP14 [58], and sulforaphane, which induces the expression of proteasome subunits by inducing the transcription factor Nrf2 [59]. An alternative for these methods to enhance proteasome activity may be enhancing the quality control mechanisms of the proteasome, which therefore deserve further attention.

Degradation of 20S proteasomes is also interesting from a more philosophical point of view. Not only because this complex is very stable, but also considering the protease activity inside the 20S. During 20S assembly, protease activity is only activated inside the 20S barrel when the complex assembly has been completed, thus assuring that the cell is protected from unwanted and uncontrolled protein degradation [60]. The mechanisms degrading the 20S can be expected to involve similar precautions. In the proteasome degradation mechanism that we propose in Chapter 5, this requirement is met since the proteasome is degraded inside the lysosome as the result of an autophagy-like process. The acidic conditions in a lysosome may then support unfolding of the 20S proteins as a prelude to complete destruction. The requirement for a protection mechanism makes proteasome degradation by another proteasome unlikely, since this would entail disassembly of the complex before degradation of its subunits. Also, so far no chaperones have been identified that would facilitate 20S disassembly. For the same reasons, it is unlikely that (damaged) subunits within the 20S are turned over individually instead of turnover of the complex as a whole. Whole complex turnover is also consistent with the very similar half-lifes found for the individual subunits of the 20S in mouse brains [61].

All in all, we uncovered novel aspects of proteasome dynamics that may play a role in cellular aging. Our data justifies further research on the role of proteasome localization and proteasome quality control in aging and age-related diseases.

N-acetylation in proteasome dynamics and aging

In Chapter 4 we show the involvement of three N-terminal acetylation complexes (NatA, NatB and NatC) in proteasome localization and fitness during aging of budding yeast [6]. Deletion of different Nat complexes has different effects on proteasome localization and longevity, implying that the N-terminal acetylation of specific (subsets of) proteins is

important for these phenotypes. This may suggest that acetylation of protein N-termini, like acetylation of lysine residues [62,63], is important in age-related processes. The relevant substrate(s) of these Nat complexes and the conservation of a role for N-terminal acetylation in aging in mammalian cells remain to be established.

Like in budding yeast, the major N-acetylation complexes in mammalian cells are NatA, B and C. Their subunit composition and their specificities for certain N-terminal sequences is largely conserved from yeast to human [64,65]. Also, the functionality of N-terminal acetylation shows many conserved aspects. One conserved aspect of N-terminal acetylation is being a modulator of the N-end rule. The N-end rule describes the influence of the N-terminal amino acid and its post translational modifications on the stability of the protein. Proteins can be stabilized by N-terminal acetylation of a (destabilizing) N-terminal amino acid in both yeast and mammalian cells [65,66]. On the other hand, for some proteins in yeast, N-terminal acetylation may also represent a destabilizing signal [67]. A mammalian version of this destabilizing signal remains to be characterized. Other conserved functions of N-terminal acetylation include preventing other N-terminal modifications and mediating protein-protein and protein-membrane interactions [65,68]. Although the Nat complexes and the functionality of N-terminal acetylation seem to be conserved from yeast to human, the very N-termini of substrate proteins are generally not conserved. Therefore it may be necessary to evaluate the N-terminal acetylation status of specific proteins rather than the Nat complexes themselves when considering conserved roles of N-acetylation in proteasome localization and aging.

To further address the (conservation of the) role of N-terminal acetylation in proteasome localization and aging, identification of the relevant Nat substrates would be very helpful. Several substrates tested negative in Chapter 4, but that does not exclude involvement of other (groups of) substrates or may indicate functional redundancy. In principle, loss of a Nat complex can affect proteasome localization by affecting its translocation machinery or by inducing protein damage. Induction of protein damage may also directly affect fitness during aging. A 'damage-induced model' would lead to the following hypotheses on how the different Nat complexes affect proteasome localization and aging.

A population of starved NatC-deficient cells showed WT-like prevalence of PSGs and higher prevalence of nuclear enrichment of the proteasome. Whereas PSGs were most prevalent in replicative young cells, old cells were more likely to retain their proteasomes in the nucleus. Enhanced nuclear retention in NatC-deficient cells may be caused by induction of nuclear protein damage or cytoplasmic protein misfolding. Mother-biased inheritance of this protein damage may allow replicative young cells to store their proteasomes in PSGs. Old cells on the other hand, may need their proteasome in the nucleus to cope with the enhanced load of damage. Enhanced protein damage in old, but not in young, cells may also explain the observed decrease in fitness during the aging of replicative old cells.

Loss of NatB activity strongly reduced the prevalence of PSGs in starved cells, whereas nuclear retention of the proteasome was more prevalent then in WT cells. The higher prevalence of nuclear proteasomes in replicative old cells suggests that the mother-biased inheritance of protein damage is intact when NatB activity is lost. Still the low prevalence of PSGs in replicative young cells suggests that the load of protein damage does not allow them to store their proteasomes in PSGs. Increased levels of protein damage in both young and old cells is also consistent with the observed drop in the fitness of both old and young cells.

Unlike NatC and NatB, loss of the NatA complex did not affect the localization of the

proteasome in the total population. This suggests that NatA deficiency does not affect the levels of nuclear protein damage or cytosolic protein misfolding. However, the mother-biased inheritance of this damage appeared to be affected, since no correlation between proteasome localization and replicative age was observed in NatA-deficient cells. The lower fitness of both young and old NatA-deficient cells may suggest a higher load of cell damage in both groups, but apparently not of the kind that affects the localization of the proteasome.

In summary, the model of 'damage-induced proteasome localization' is consistent with the data presented in Chapter 4, though further research will be necessary to validate this model. Our data suggest a role for N-terminal acetylation in proteasome localization and fitness during aging. The exact underlying mechanisms remain to be established and may yield novel insights in proteasome dynamics and aging.

Overall summary

In this Thesis we addressed two important aspects of protein dynamics; protein synthesis and distribution upon cell division and the dynamics of the protein degradation machinery. To analyze protein synthesis and distribution upon cell division, one needs to distinguish and simultaneously track old and new proteins. Therefore we developed Recombination-Induced Tag Exchange (RITE) in Chapter 2. In Chapter 3 we used RITE to make a comprehensive analysis of the synthesis and inheritance of all organelles and the major macromolecular complexes in budding yeast. We conclude that most of these large cell structures are synthesized by template-based growth and show symmetric distribution of old and new components upon cell division. Two important exceptions are the nuclear pore complex, that is formed *de novo*, and the spindle pole body, that showed asymmetric inheritance of its components.

The dynamics of the protein degradation machinery is of interest since it is implicated in aging and age-related diseases. In Chapter 4 and 5 we addressed two novel aspects of proteasome dynamics that may be of interest for aging research; proteasome localization and quality control of the proteasome. In Chapter 4 we showed that the localization of the proteasome, like its activity, correlates with fitness during aging. Also, genetic factors involved in proteasome localization and longevity in budding yeast were identified in a genome-wide screening. In Chapter 5 we presented data that is consistent with lysosomal degradation of damaged proteasomes, which may represent the first sketches of a quality control system for the proteasome.

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