Peroxisomes in yeast ageing

Kumar, Sanjeev

Summary

The evolution of eukaryotes from prokaryotes is marked by the development of distinct membrane bound compartments, called organelles. The compartmentalization of cytosolic functions allows eukaryotes to operate more effectively. Each organelle has a its own specific structural and functional characteristics. Although, originally thought to be independent evidence is now accumulating that suggest that these organelles function together in close association. Among these, peroxisomes are remarkable cellular organelles that display a wide variety of functions depending on cellular requirements. Important known functions of peroxisomes include fatty acid β-oxidation, the glyoxylate cycle, plasmalogen synthesis, photorespiration and purine biosynthesis and this list is not complete yet. Along with mitochondria peroxisomes are important contributors to the generation of intracellular reactive oxygen species. In recent years studies have implicated the importance of these organelles in cellular ageing.

In the recent times the average human life expectancy has increased and so did the age-related complications. In order to minimize these complications it is imperative to have a thorough understanding of the molecular mechanisms of ageing processes. Research performed in various simple short-lived organisms has contributed significantly to our current knowledge of this process. Yeast have been widely used as model organism to study ageing and most known longevity pathways were first discovered in this simple organism. In yeast, two types of ageing paradigms have been established, namely replicative and chronological ageing. The replicative lifespan is defined as the number of daughter cells produced by yeast mother cell before they die, and this is considered a model system for dividing cells in higher eukaryotes. The chronological lifespan represents the time that yeast cells can survive in their non-dividing state and this is regarded as model for non-dividing cells of higher eukaryotes. Conventional methods used to study yeast ageing have been informative, however with the recent introduction of new systems level approaches yeast is becoming an increasingly attractive model to elucidate the molecular mechanism of ageing. The research described in this thesis focuses on the two paradigms of yeast ageing with special emphasis on significance of peroxisomes in this process.

Chapter 1 presents a summary of our current knowledge of peroxisomes. Special emphasis has been given to the processes that are required for peroxisomal quality control. In addition, we present an outline of the current our knowledge of the role of peroxisomes in the yeast ageing.

In Chapter 2 we studied the differences among peroxisomes in cells of the yeast *Saccharomyces cerevisiae*. For this purpose we used the concept of differences in maturation times of fluorescent proteins to define the age of proteins. A fusion construct of DsRed1, a red fluorescent protein with a maturation time of approximately

11 hrs, and sfGFP, with a very short maturation time (approximately 10 min), was targeted to peroxisomes. Analyses of fluorescence intensity ratios of DsRed1/sfGFP of peroxisomes revealed that organelles with proteins of different ages are present in a single cell. Peroxisomes with a relatively old matrix protein content showed a reduced capacity for further matrix protein import. This most likely was related to a reduction of Pex14, a component of the matrix protein importomer, on these organelles. Interestingly, peroxisomes with a relatively young matrix protein content imported matrix proteins more effectively concomitant with the preferential sorting of Pex14 to these organelles. However, this selective targeting of peroxisomal membrane proteins (PMPs) was not a general phenomenon because Pex3 was sorted to all peroxisomes irrespective of the age of their matrix content. The organelles with the relatively old content (the mature ones) are selectively retained by the mother cell, whereas the nascent ones with relatively young content are preferentially transported to the developing buds. The asymmetric inheritance of peroxisomes was facilitated by the localized distribution of the peroxisome inheritance proteins, Inp1 and Inp2. Inp1, implicated in anchoring the organelles in the mother cell, was concentrated on mature peroxisomes present in the mother cells. Inp2 that facilitates migration of the organelle to developing buds was enriched on the nascent peroxisomes that were destined for the daughter cells. Interestingly, the tight asymmetry in peroxisomes inheritance was maintained throughout the replicative lifespan of yeast cell. Finally, distinct from what is generally assumed, our studies established that yeast peroxisomes are important in replicative ageing, also when cells are grown on glucose.

Nicotinamidase, Pnc1, has been implicated in replicative ageing of *S. cerevisiae*. In Chapter 3 we analyzed the mechanism of Pnc1 targeting to peroxisomes and its relation to the replicative lifespan of yeast. We show that Pnc1 physically interacts with the peroxisomal targeting signal-2 (PTS2) containing protein Gpd1, which allows its piggyback import to peroxisomes. Western blotting revealed that both proteins are not present at constant ratios in the cell, suggesting that they do not form a stable complex with fixed stoichiometry. Although these proteins form a complex we found that the stability of one protein is unaffected in the absence of the other, suggesting that both proteins interact primarily for the targeting purpose.

Previous studies indicated that Gpd1 relocalizes to the cytosol upon exposure of cells to various stress conditions. Using quantitative fluorescence microscopy, we analyzed the levels of peroxisomal and cytosolic Gpd1 and Pnc1 prior and after exposure of cells to different stress conditions. These studies revealed that both proteins were confined to peroxisomes in unstressed cells. Upon exposure of cells to stress the levels of both proteins strongly increased, together with the appearance of cytosolic Gpd1 and Pnc1. However, at the same time also the level of peroxisomal

protein increased. A similar distribution pattern was observed for a non-stress related peroxisomal protein (thiolase), when produced under control of the *GPD1* promoter. These analyses suggest that the appearance of cytosolic Gpd1 and Pnc1 may be related to the inefficiency of the PTS2 import machinery.

Previously, it was suggested that Pnc1 functions in the nucleus by lowering nicotinamide levels and hence activates Sir2 activity that has a positive effect on replicative ageing of yeast. We analyzed single yeast cells during multiple cell divisions using a microfluidics device, which showed that during replicative ageing Pnc1 never concentrated in the nucleus. When we forced the localization of Pnc1 exclusively to peroxisomes by adding a PTS1 (-SKL.COOH) to the C-terminus, we still did not observe shortening of yeast replicative lifespan relative to wild type control cells. Our findings do not support a role of nucleus-bound Pnc1 in determining yeast replicative lifespan.

In Chapter 4 we analyzed the significance of peroxisomal metabolism in yeast aging. We used the methylotrophic *Hansenula polymorpha* in this study because it can utilize a wide variety of carbon and nitrogen sources that are metabolized by peroxisomal enzymes. We found that these cells had an enhanced chronological lifespan when methanol or ethanol, which both require peroxisomal enzymes for growth, were used as carbon source relative to growth on glucose, which does not require peroxisomal metabolism. We also found that when using D-alanine or methylamine as sole nitrogen sources, which also need peroxisome functions, had positive effects and led to an increased lifespan relative to the commonly used nitrogen source ammonium sulphate. Further analysis revealed that the lifespan extension on methylamine as nitrogen source was linked to its oxidation by the peroxisomal enzyme amine oxidase into formaldehyde. Further oxidation of formaldehyde into CO_2 generates two molecules of NADH that can provide cells with surplus energy during the stationary phase. These data show that additional energy provided during stationary phase of the culture has positive effects on the chronological lifespan. Therefore, while analyzing non-dividing cultures, the energy status of these cells must be taken into account.

In Chapter 5 we investigated the role of the Fis1/Dnm1 organelle fission machinery that is involved in both mitochondrial and peroxisomal fission, especially in relation to the role of peroxisome fission in the chronological lifespan of *S. cerevsiae*. As reported earlier, first we confirmed that *Δfis1Δvps1* cells are fully blocked in peroxisome and mitochondrial fission and have an increased chronological lifespan. When we selectively blocked mitochondrial fission in this strain by a introducing a Fis1-Pex15 fusion protein that selectively sorts all Fis1 protein to peroxisomes, we did not observe an extension in the chronological lifespan. Based on this we concluded that inhibition in mitochondrial fission does not affect yeast chronological lifespan.

Deletion of *FIS1* in cells devoid of peroxisomes (Δ*pex3*) did also not have a positive effect on yeast chronological lifespan. This further confirms our conclusion that the positive effects on yeast lifespan in Fis1/Dnm1 deficient cells is predominantly related to the block in peroxisome fission rather than in mitochondria.