Peroxisomes in yeast ageing

Kumar, Sanjeev

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Peroxisomes in yeast ageing

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by

Sanjeev Kumar

born on 18 December 1983
in Bihar, India

Supervisor
Prof. I.J. van der Klei

Assessment Committee
Prof. O.P. Kuipers
Prof. B.M. Bakker
Prof. H.A.B. Wosten

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Contents

Aim and outline

Eukaryotic cells are subdivided into specialized membrane bound organelles that are characterized by their unique structure and functions. Among these, peroxisomes are single membrane bound, ubiquitous organelles which display a wide range of physiological functions depending on organism, environmental conditions and developmental stage. An important feature of peroxisomes is the presence of oxidases, which generate hydrogen peroxide that has been shown to be important in cellular ageing. Ageing is defined as a multifactorial process that is characterized by a progressive decline in cellular and organismal functions. Various model organisms, among which yeast, have been instrumental in the advancement of our understanding of the molecular principles of the ageing process.

Using yeast as model organism, the aim of the research described in this thesis was to understand the significance of peroxisomes in cellular ageing processes.

In Chapter 1 an overview of our current understanding of peroxisome biology has been presented. Special emphasis has been given to our knowledge of peroxisomal quality control mechanisms and the roles of peroxisomes in ageing.

Chapter 2 describes the heterogeneity of peroxisomes in wild type cells of the yeast *Saccharomyces cerevisiae* that exists with respect to their matrix protein composition. By using specific fluorescent proteins that display different maturation times, we show that peroxisomes containing relatively old or new matrix protein content are present in one and the same cell. We showed that peroxisomes harboring a relatively old matrix protein content have a reduced capacity to import newly synthesized matrix proteins. This was related to reduced levels of Pex14, a peroxisomal membrane protein involved in matrix protein import, on mature peroxisomes. Further analysis revealed that mature peroxisomes are retained by the mother cells whereas peroxisomes with a younger peroxisomal matrix protein content are preferentially transported to the daughter cells.

Chapter 3 describes studies on the sorting of two stress related proteins, Pnc1 (nicotinamidase) and Gpd1 (glycerol-3-phosphate dehydrogenase) to peroxisomes in yeast. We show that Pnc1 is transported to peroxisomes by piggy-backing on the peroxisomal targeting signal-2 (PTS2) containing enzyme Gpd1. Our data indicate that Gpd1 and Pnc1 physically interact and most likely form a transient complex during import into peroxisomes. Previous reports indicated that Gpd1 relocalises to the cytosol upon exposure of cells to osmotic stress. Quantitative analysis of the distribution of Gpd1 and Pnc1 over the cytosol and peroxisomes revealed that both proteins are predominantly localized to peroxisomes in unstressed cells. Upon exposure of cells to stress the levels of both peroxisomal and cytosolic Gpd1 and Pnc1 increased. A similar behaviour was observed for the non-stress related

peroxisomal control protein thiolase produced under control of the *GPD1* promoter. This observation suggests that presence of peroxisomal matrix proteins in the cytosol of cells exposed to stress is a result of reduced matrix protein import efficiency.

Chapter 4 describes the effects of different carbon and nitrogen sources on the chronological lifespan of the methylotrophic yeast *Hansenula polymorpha*. We found that this lifespan is enhanced when cells are grown on methanol or ethanol as sole carbon sources, compounds that require peroxisomal enzymes for their metabolism, relative to cultivation on glucose, which is metabolized independent of peroxisomal enzymes. We also observed that the use of methylamine as sole nitrogen source has a positive effect on yeast chronological lifespan relative to ammonium sulphate. Methylamine is initially metabolized inside peroxisomes by the enzyme amine oxidase. The lifespan extension by the utilization of methylamine was shown to be due to the surplus energy generated by the oxidation of methylamine during the stationary phase.

Mitochondrial fragmentation mediated by the Dnm1/Fis1 fission machinery has been suggested to accelerate cellular ageing. However, this Dnm1/Fis1 fission machinery is also involved in fission of peroxisomes. In Chapter 5 we describe studies on the importance of peroxisomal fission in yeast ageing. We show that lifespan extension in *FIS1* deletion cells is mainly caused by the defects in peroxisome fission and not due to blockage of mitochondrial fragmentation. The deletion of *FIS1* in peroxisome deficient *pex3* cells did not result into lifespan extension, which further underlines the importance of peroxisome fission in yeast ageing.