Development and Breakdown of Peroxisomes in the Yeast Hansenula polymorpha in Response to a Changing Environment

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CHANGES IN THE OSMOTIC PRESSURE OF *E. COLI* W7 DURING THE CELL CYCLE

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During the cell cycle of exponentially growing *Escherichia coli* cell mass and length are doubled. Because the surface-to-volume ratio changes during the cell cycle it has often been supposed that the cell density or the cellular osmotic pressure change likewise. However, experimentally very little attention has been paid to this matter. In the present study the ability of cells to plasmolyze in 0.5 M sucrose has been used as an indication for the osmotic pressure inside the cell. Cells have been classified according to length in order to follow plasmolysis during the cell cycle.

For the applied sucrose concentration the percentage of plasmolyzed cells of the whole population showed a maximum at 5 min after the addition of sucrose. Thereafter, deplasmolysis, though delayed by the addition of sodium azide, starts to predominate. It was inferred that for a given sucrose concentration (i.e., osmolarity of the medium) cells have a certain probability of showing plasmolysis. The probability of *non-dividing* cells to plasmolyze increases with cell length. Comparison of the distributions of plasmolyzed and non-plasmolyzed *dividing* cells shows that they have more or less a constant probability to plasmolyze for cell length classes.

We conclude, provided that plasmolysis is a good measure, that cellular osmotic pressure decreases during the growth in length of non-dividing cells. Furthermore, it might be that if some critical osmotic pressure has been attained cell constriction initiates. This is accompanied by a sudden increase in osmotic pressure which remains more or less constant during the constriction process. After cell separation osmotic pressure starts to decrease again.

CHANGE IN CELL WIDTH DURING THE LIFE CYCLE OF ROD-SHAPED BACTERIA

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Models proposed for the growth of rod-shaped bacteria generally assume cell volume to increase exponentially and cell surface to extend linearly during the cycle. As a result one of the two parameters, cell censity or cell width, will vary during the cell cycle, while the other remains constant. Although there is some experimental[1] (Poole, 1977; van Eden et al., this conference) and theoretical[2], evidence for cell density to change during the cycle, the idea that cell width remains constant is only supported by limited observations made by Marr et al.[3]

Extensive measurements of steady state populations of various *Escherichia coli* strains have consistently indicated an exponential decrease of cell width with increasing cell length, both after preparation of the cells for electron microscopy by the method of agar filtration and after phase-contrast microscopy of living cells. The analysis was refined by considering separately the unconstricted cells and three classes (slight, medium and deep) of constricted cells. Unconstricted cells with twice the average length of newborn cells ($2\,\bar{L}_o$) are about 5% thinner than the cells of average newborn length. The tendency to become thinner with increasing length also held for constricted cells, but here average cell width increased drastically with increasing degree of constriction. No such correlation between cell width and cell length was detected for the gram-positive *Bacillus subtilis*.

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DEVELOPMENT AND BREAKDOWN OF PEROXISOMES IN THE YEAST *HANSENULA POLYMORPHA* IN RESPONSE TO A CHANGING ENVIRONMENT

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Cells of *Hansenula polymorpha* growing exponentially on glucose, generally contained a single peroxisome with dimensions of 0.1 - 0.2 µm, irregular of shape and located close to the cell wall. The organelles were generally closely associated with one or more strands of

ER and contained catalase together with D-amino acid oxidase, hydroxy acid oxydase and urate oxidase.

After transfer of cells growing exponentially on glucose into methanol-containing media, large peroxisomes with dimensions up to 1.5 μm with a partly crystalline matrix developed in these cells within 6 hours. These organelles originated from the small peroxisomes in the glucose cells by means of growth and division; *de novo* synthesis was not observed. Prolonged cultivation in methanol media resulted in an increased number of peroxisomes while their growth was associated with an increase in size of the crystalline nucleus. Completely crystalline organelles, generally rectangular in shape, were observed in stationary phase cells and cells grown in continuous cultures under methanol limitation. In old cells from these cultures up to 18 peroxisomal profiles have been observed, which together made up 80% of the cytoplasmic volume.[1]

The increase in volume density of peroxisomes in cells grown on methanol was associated with the synthesis of high amounts of alcohol oxidase and catalase, the two enzymes which catalyze the oxidation of methanol to formaldehyde[2]. However, the development of the crystalloid in the peroxisomes was only dependent on the synthesis of alcohol oxidase in the cells. It was therefore not surprising to find that the periodicity of the crystal structure of recrystallized alcohol oxidase, purified from methanol-grown *H. polymorpha*, was identical to that of the crystalloid in peroxisomes in methanol-grown cells.

Addition of glucose to a suspension of methanol-grown cells of *H. polymorpha* leads to a rapid loss of both alcohol oxidase and catalase activity[3]. In addition other typical peroxisomal enzymes like hydroxy acid oxidase are rapidly inactivated. Electron microscopical observations have shown a rapid degradation of peroxisomes in these cells. This process is probably initiated by fusion of the peroxisomes with the vacuole or part of the vacuole. Morphometrical analysis showed that 4 hours after the addition of glucose the volume density of the peroxisomes had decreased to approximately 10% of the original value. The molecular mechanisms involved in the degradation of peroxisomes and the decrease of peroxisomal enzyme activities associated with it are unknown. Preliminary experiments suggested that the process is irreversible and independent of protein synthesis in the cells.

It seems that the above kinetics of the development and breakdown of peroxisomes in *H. polymorpha* in response to changes in its environment makes this organism a most suitable model system for studies on the regulation of the synthesis and turnover of these organelles and their enzymes.

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ELECTRON MICROSCOPE ANALYSIS OF THE MODE OF ELONGATION IN *E. COLI* CELLS, AFTER INHIBITION OF DNA REPLICATION AND/OR CELL DIVISION

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The shape of the distribution of cell sizes in a steady state population reflects the mode of growth of the individual cells[1]. However, models for exponential or linear growth predict only small differences in the shape of the distributions[2]. This makes it difficult to eliminate models by comparison of experimental and theoretical size distributions.

In the present analysis cells were allowed to grow into small filaments by inhibiting (i) DNA replication with nalidixic acid and (ii) cell division with ampicillin. Size distributions were obtained by measuring the lengths and widths of cells prepared for electron microscopy by the method of agar filtration[3]. Assuming different growth models and the existence of a coupling between DNA replication and the mode of surface synthesis, predictions can be made on the behavior of the size distribution during a period of balanced growth in the presence of the inhibitor. For instance, the distribution is expected to become bimodal after inhibition with nalidixic acid if a direct coupling between DNA replication and doubling in the constant rate of surface synthesis occurs in the middle part of the cycle.

The results show that nalidixic acid affects immediately surface synthesis in all three strains examined (B/r F 26, B/r H 266, K 12 CR 34). Only in the case of *E. coli* B/r F 26 a bimodal size distribution was obtained in one of two experiments. In the three to four