Novel approaches to high-throughput measurements of replicative lifespan in yeast by microfluidic size sorting and genetic engineering

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Saccharomyces cerevisiae **has been well established as a model system in aging research for more than 50 years. Whereas the chronological life span (CLS) is defined as the length of time a non-dividing yeast cell survives, replicative lifespan (RLS) refers to the number of times an individual cell divides before it senesces. Substances influencing the RLS are of particular interest for the development of therapeutics that counteract early aging and cancer. The standard RLS assay in yeast is highly laborious, requiring separation of mother and daughter cells by micromanipulation after every division.**

In cooperation with an SME in Berlin we are developing a high-throughput screen in a microfluidic device that enables to simultaneously test the influence of various drugs on the RLS of yeast. For this purpose, a drug sensitive yeast strain was established in the genetic background of the *S. cerevisiae* W303 strain via knockout of three main multidrug transporter. Cell size of mother and daughter cells was measured on a Zeiss fluorescence microscope, and data were evaluated with the prism software. Cells will be fixed via mechanical forces on a chip, and daughter cells will be monitored by a novel detection system.

In a second approach, a linearly growing yeast strain is generated via a daughter-specific selective knockout of cell cycle genes. Failure of the daughter cells to complete cell division should result in an accumulation of aging mother cells in liquid culture, providing a direct correlation between RLS and increase in optical density.

Replicative lifespan in yeast

Aging in yeast is assayed by measuring replicative or chronological lifespan. Replicative lifespan is defined by the number of times an individual cell divides before it senesces and dies. *S. cerevisiae* as a budding yeast undergoing asymmetric cell division is used. The standard replicative lifespan assay is performed by manual separation of daughter cells from mother cells every 2-3 h using a micromanipulator, making this standard RLS highly laborious. We are developing a microfluidic RLS-assay which is suitable for parallel, continuous measurements and therefore appropriate for high-throughput screening of compounds having potential effects on aging. Mother cells shall be retained in a microfluidic chamber, whereas daughter cells will peel away and be detected physically with light.

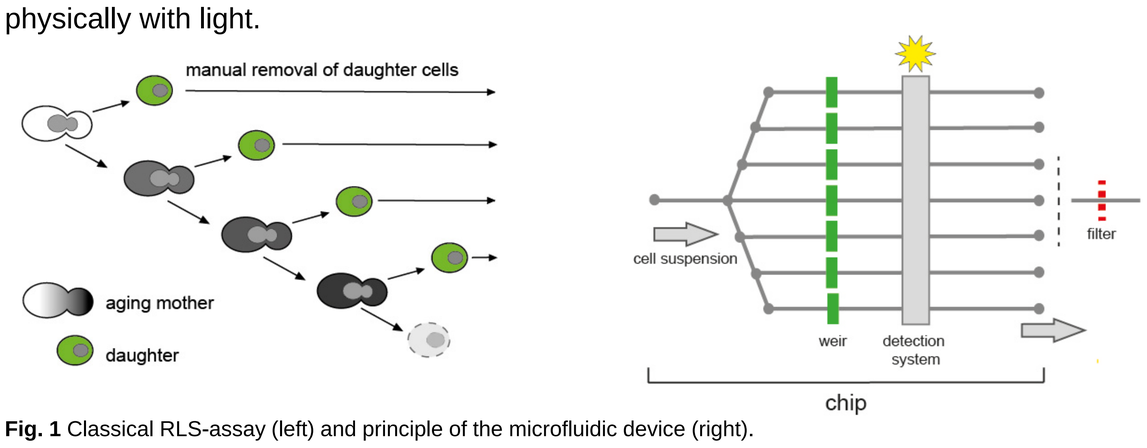

Fig. 1 Classical RLS-assay (left) and principle of the microfluidic device (right).

Development of a drug-sensitive strain

To ensure that tested compounds are not immediately exported from the cells, the genes of the three main ABC-transporter PDR5, SNQ2 and YOR1 were sequentially deleted. Afterwards, cell size was measured and evaluated with the prism software. Cells grown in rich YPD medium show bigger cell sizes, especially for the daughter cells. Therefore the size difference between mother and daughter cells is smaller than from cells grown in minimal SD medium. The size difference between mother and daughter cells is approximately the same for the drug-sensitive strain in SD medium and for the commonly used wildtype strain in YPD medium.

	YPD medium		
strain	mother [µm]	daughter [µm]	difference [µm]
W303 wildtype	8,82	6,41	2,41
W303 ΔABC	8,42	6,26	2,17

	SD medium		
strain	mother [µm]	daughter [µm]	difference [µm]
W303 wildtype	8,53	4,99	3,54
W303 ΔABC	8,06	5,54	2,52

Fig. 3 a Median size of 1000 cells, grown in minimal or rich media.

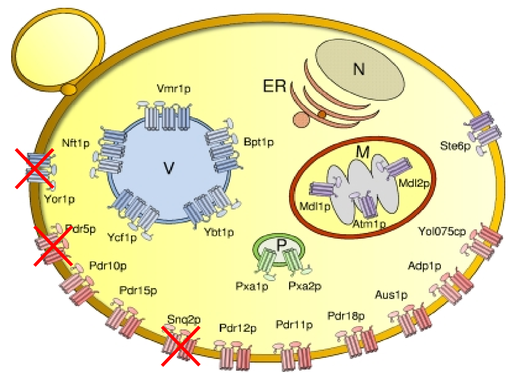

Fig. 3b Three knocked out membrane transporter.
source: mmbr.asm.org

Comparison of cell sizes

Cell size differences between mother and daughter cells are crucial for using yeast cells in this chip.

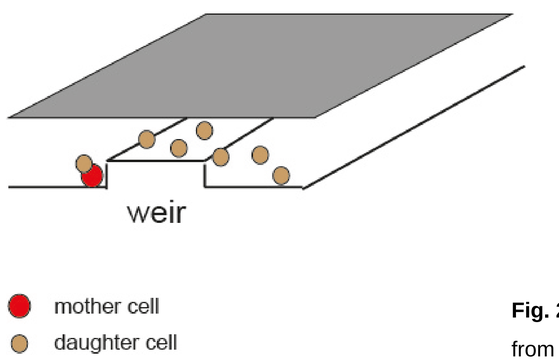

Fig. 2a Chip for separating mother from daughter cells.

Cell sizes of different yeast strains were measured with a Zeiss Axio imager fluorescence microscope. Mother and daughter cells were scored manually as shown.

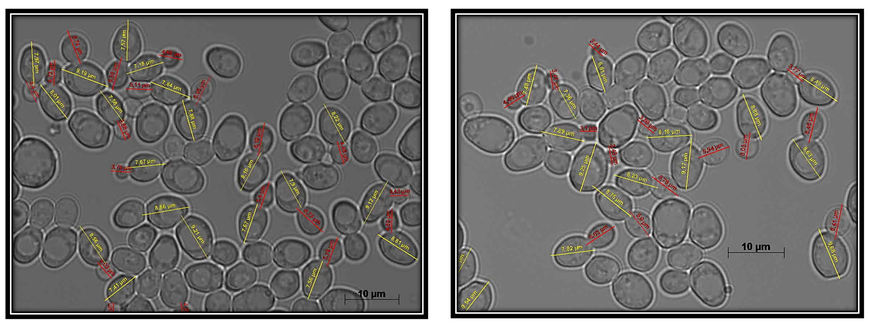

Fig. 2b Wildtype strain W303 and the drug-sensitive strain W303 ΔABC in SD-medium, measuring of mother (yellow) and daughter cells (red) by Zeiss Axiovision Software. Magnification 1000x.

Generation of a linearly growing yeast strain

A selective knockout of cell cycle genes will be achieved by utilization of daughter-specific promoters and translational repression mechanisms. Failure of the daughter cells to divide should result in a linear growth behavior and accumulation of aged mother cells in liquid culture. Thus, RLS can be assayed directly via the increase in optical density. Usage of the strain in microfluidic approaches further minimizes measuring errors that could occur in the case of high cell division rates or a delayed release of daughter cells from their mother.

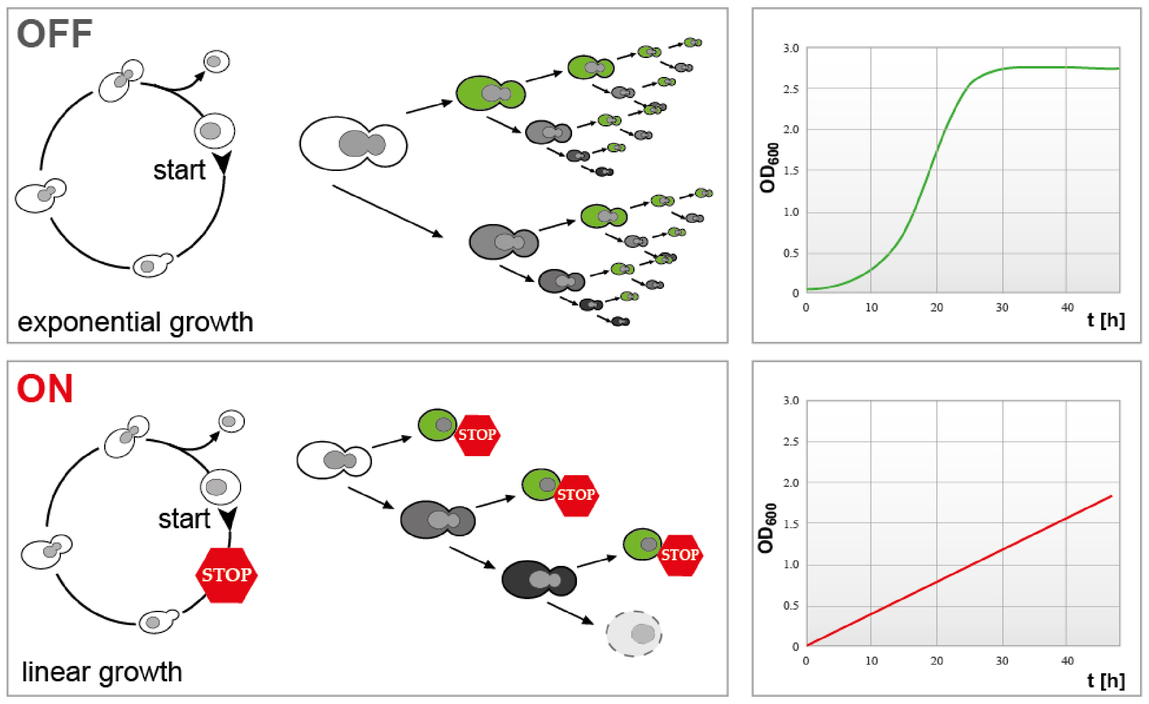

Fig. 4 Generation of a linear growing yeast strain. While wildtype *S. cerevisae* show an exponential growth behavior (OFF), selective knockout of cell cycle genes in daughter cells (ON) results in linear growth.

Perspectives

Additional knockouts for known genes affecting lifespan will be generated as positive and negative controls for the compound screen.
The usability of different chip models is currently tested with latex beads of defined sizes by our cooperation partner M2 Automation.
As a long-term perspective the microfluidic device and the linearly dividing strain are aimed to serve as high-throughput measuring systems for replicative lifespan that can be used as a time-saving alternative to the traditional micromanipulation assay.

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