Scientific report
Dr Nicoletta Guaragnella, CNR-Short-term mobility programme 2013 performed at University of Southern California, Longevity Institute, Los Angeles in Prof. Valter Longo's laboratory.
Project proposal "The role of sirtuins in programmed cell death and cell stress response"
Duration of stay april 29th-may 20th 2013

Background

Sirtuins are a class of highly conserved proteins that possess either mono-ribosyltransferase or deacylase activity. The name Sir comes from the yeast gene 'silent mating-type information regulation 2 '. They are involved in the regulation of a variety of cellular processes, including aging, apoptosis and stress resistance.
In particular, Sir2 is the highest conserved deacetylase from yeast to mammals that modulates life span in yeast, worms, and flies and stress response in mammals.
Beyond the role of Sir2 in the maintenance of the silent chromatin at the mating-type loci, telomeres, and rRNA-encoding DNA repeats, in Prof. Longo's laboratory has been demonstrated that Sir2 inactivation extends yeast chronological life span, defined as the length of time stationary yeast cells remain viable in a quiescent state, causes ethanol depletion in the extracellular medium and higher stress resistance compared to wild type cells (Fabrizio et al. 2005). Acetic acid, which is a well known by-product of yeast glucose fermentation, is a typical trigger of yeast apoptosis (Guaragnella et al. 2012). Moreover, it has been identified as a major cell-extrinsic mediator of chronological aging cells (Burtner et al. 2009). The biological reasons underlying sir2 longevity and its related stress resistant phenotype are still largely unexplored and the relations between sir2 and apoptosis in yeast aging cells require further investigations.

Objective and research activity

Dr Nicoletta Guaragnella, researcher at CNR-Institute of Biomembrane and Bioenergetics in Bari, Italy, performed research activity in Prof. Longo's laboratory at University of Southern California Longevity Institute from april 29th to may 20th, 2013 within the CNR Short-Term Mobility Programme 2013. In particular, within her project proposal "The role of sirtuins in programmed cell death and cell stress response", she investigated the effect of sir2 deletion and the role of carbon source metabolism on cell viability, oxidative stress resistance and acetic acid release in the extracellular medium in a model of yeast aging cells. The role of carbon source metabolism was analyzed by testing the effects of short and long-term exposure to acetic acid, which is a typical non-fermentable carbon source, on cell viability and stress resistance. The experiments were performed in two different yeast genetic backgrounds, DBY746 and BY4741, in order to have major representative results.

Cell viability assay

DBY746 and BY4741 wild type and sir2 mutant cells were grown up to stationary phase (72 hours) and cell viability was monitored every two-three days up to day 9 from the beginning of the experiment. Cells were properly diluted in water, plated on rich medium and incubated at 30°C for 2 days. The number of colony forming units at day 3 is considered 100% viability.

Stress resistance assay

DBY746 and BY4741 wild type and sir2 mutant cells treated or not with acetic acid at physiological concentrations (20-50 mM) were incubated with two different concentrations (100 and 200mM) of hydrogen peroxide for 30 minutes. Then, cells were properly diluted, spotted on rich medium plates and incubated at 30°C for two days. Cell growth was then evaluated.

pH mesurements

pH was measured in extracellular supernatants collected from DBY746 and BY4741 wild type and mutant cells by means of specific stripes working in the pH range of 0-6.

Acetic acid measurements

Acetic acid was measured in extracellular supernatants collected from DBY746 and BY4741 wild type and mutant cells by means of a spectrophotometric determination in a microplate reader.

Results

It was observed that:

In all tested conditions BY4741 strains show an increased lifespan compared to DBY746 and this is true either for wild type or sir2 cells.

As expected, sir2 cells live longer than wild type cells.

Sir2 cells treated with 20 mM acetic acid show higher viability compared to wild type cells in both strains backgrounds analyzed.

Sir2 cells treated with 50 mM acetic acid are more sensitive than wild type cells in both strains backgrounds analyzed.

Sir2 cells are more resistant to oxidative stress than wild type cells after short and long-term exposure to acetic acid. With the exception of 50mM acetic acid long-term exposure, which resulted to be toxic for sir2 cells.

No significant changes were observed for the extracellular pH, which was measured in all tested conditions.

In sir2 cells a complete depletion in the extracellular level of acetic acid was observed at day 7 compared to wild type cells.

Conclusions and perspectives

These results confirm the role of sir2 in extending lifespan of yeast cells and aim to gain insight into the mechanisms underlying this effect, that are largely unexplored. Since metabolism has a major impact on cell fate determination and longevity, studying whether and how carbon source metabolism plays a role in lifespan extension represents a valuable approach to address this issue.

The effects of acetic acid exposure on cell viability and oxidative stress resistance were evaluated in this work. Having observed that physiological concentration of acetic acid in the range of 0-20mM do not affect sir2 life span extension, but 50mM acetic acid resulted to be toxic for the cells, it is possible to conclude that exposure to certain concentrations of acetic acid can influence life span of sir2 aging cells and therefore abrogate their higher viability and stress-resistant phenotype. Whether this is specifically due to acetic acid has to be verified by testing the effect of other carbon sources, such as glucose or ethanol. Also whether high concentration of acetic acid causes apoptotic cell death in sir2 cells and this is due to a defect in acetic acid assimilation remain to be clarified. Surely, the complete depletion of extracellular acetic acid observed in sir2 cells compared to wild type suggests a possible correlation between higher cell survival and capacity to assimilate/metabolize acetic acid. This is in agreement with the evidence that acetic acid is a major cell-extrinsic mediator of chronological aging cells (Burtner et al. 2009).

To understand the upstream signaling pathways regulating intracellular acetate metabolism and extracellular acetic acid excretion in sir2 lacking cells will help to clarify the mechanisms underlying longevity and stress resistance. A recent work by Casatta et al. proposes that sir2 inactivation influences positively the acetate utilization by way of an increased flux of the glyoxylate/gluconeogenic pathway. In the chronological aging paradigm, this implies low levels of toxic extracellular factors (ethanol and acetic acid) and an increase of protective intracellular factors (trehalose) in the sir2Δ cultures which all together may favor a better long-term survival and extension of chronological life span (Casatta et al. 2013).

References

-Fabrizio P, Gattazzo C, Battistella L, Wei M, Cheng C, McGrew K, Longo VD. Sir2 blocks extreme life-span extension. Cell. 2005, 123(4):655-67.
-Guaragnella N, Antonacci L, Passarella S, Marra E, Giannattasio S. Achievements and perspectives in yeast acetic acid-induced programmed cell death pathways. Biochem Soc Trans. 2011, 39(5):1538-43.
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-Casatta N, Porro A, Orlandi I, Brambilla L and Vai M. Lack of Sir2 increases acetate consumption and decreases extracellular pro-aging factors. Biochimica et Biophysica Acta 2013, 1833(3):593-601.

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