CNR Short-term mobility programme 2013
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Scientific report on research activity performed at University of Kent, School of Biosciences, Canterbury in Prof. Campbell W. Gourlay's laboratory
Project title "Mitochondria-cytoplasm-nucleus cross-talk in cell stress response in yeast as a model organism"
Duration of stay: October 6th - October 26th 2013

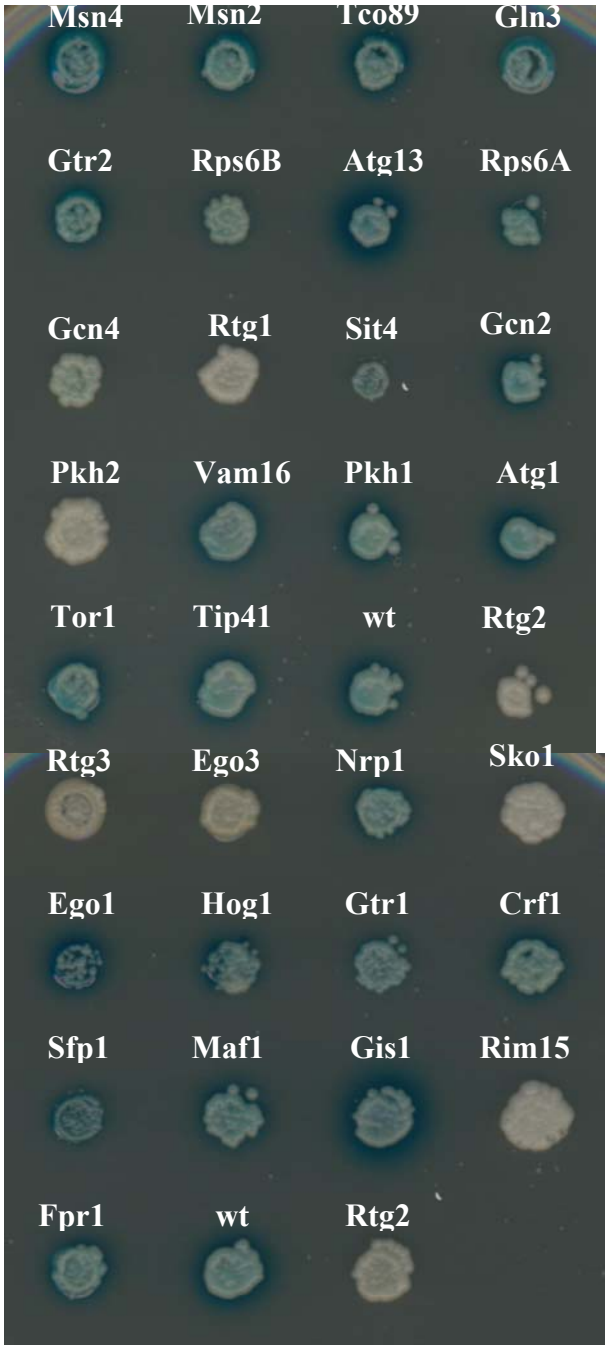
Mitochondria play a critical role in many cell regulatory and signaling events, well beyond their established role as a cell powerhouse (Goldenthal & Marin-Garcia, 2004). When faced with stressful conditions, mitochondria are found to be important determinants of cell destiny. The role of mitochondria in apoptosis has been well studied, and it has been shown that complex mitochondrial features, such as release of pro-apoptotic proteins, mitochondrial fragmentation and loss of membrane potential, crucial for programmed cell death (PCD) execution in mammals are present and functional in yeast (Eisenberg*, et al.*, 2007) (Guaragnella*, et al.*, 2012). In our model system, consisting of glucose-grown yeast cells, in which PCD is induced with acetic acid (AA), mitochondria are shown to gradually lose its function *en route* to AA-PCD, as shown by respiratory control ratio (RCR) decrease and loss of cytochrome *c* oxidase activity (Guaragnella*, et al.*, 2011). However, it is not known whether and how a modification of mitochondrial metabolism could somehow influence AA-PCD occurrence and/or change cell sensitivity to an apoptotic stimulus.

Glucose is a fermentable carbon source responsible for down-regulation of respiration, while raffinose is a poorly fermentative carbon source in which respiration is derepressed (Randez-Gil*, et al.*, 1998). We found that, when raffinose is used as a sole carbon source, yeast cells become resistant to the same acetic acid treatment that causes PCD in glucose grown cells, in a manner mostly dependent on the retrograde (RTG) pathway activation (Guaragnella*, et al.*, 2013). RTG signaling pathway is mitochondria to nucleus communication pathway that enables cells to adapt to mitochondrial dysfunction by reprogramming a set of nuclear genes involved in anaplerotic pathways and concomitant metabolic reprogramming. Yeast RTG signaling has been characterized in details (Liu & Butow, 2006) and it has been shown to be linked to aging, mtDNA maintenance, TOR signaling and nutrient sensing pathways. Ras/cAMP/PKA is nutrient signaling pathway involved in the control of cell growth and proliferation and the induction of stress responses (Reinders*, et al.*, 1998), and the links between Ras and mitochondrial function have been shown in yeast, thus making this pathway a good candidate to integrate environmental signaling with mitochondrial regulation (Leadsham & Gourlay, 2010).
In Prof. Gourlay's laboratory it has been demonstrated that dysfunctional mitochondria activate Ras upon their outer membrane, which triggers a series of pro-cell-death signaling events (Leadsham*, et al.*, 2013). Active GTP-bound Ras visualization was made using an RBD-GFP reporter in live cells. Ras2 is known to be a positive regulator of the RTG pathway (Kirchman*, et al.*, 1999), but the interaction between these two pathways is largely unexplored (Jazwinski & Kriete, 2012). During Dr Ždraleviæ stay at the University of Kent the same reporter plasmid pYX212 RBDx3-GFP was used to transform W303-1B wt and mutant strains lacking *RTG2*, in order to study the Ras dynamics in our experimental model. We found that active Ras protein is found predominantly in nucleus and plasma membrane in glucose- and raffinose-grown wt cells, both at pH 7 and pH 3, but, interestingly, it is found in mitochondria in Δrtg2 cells grown in raffinose.

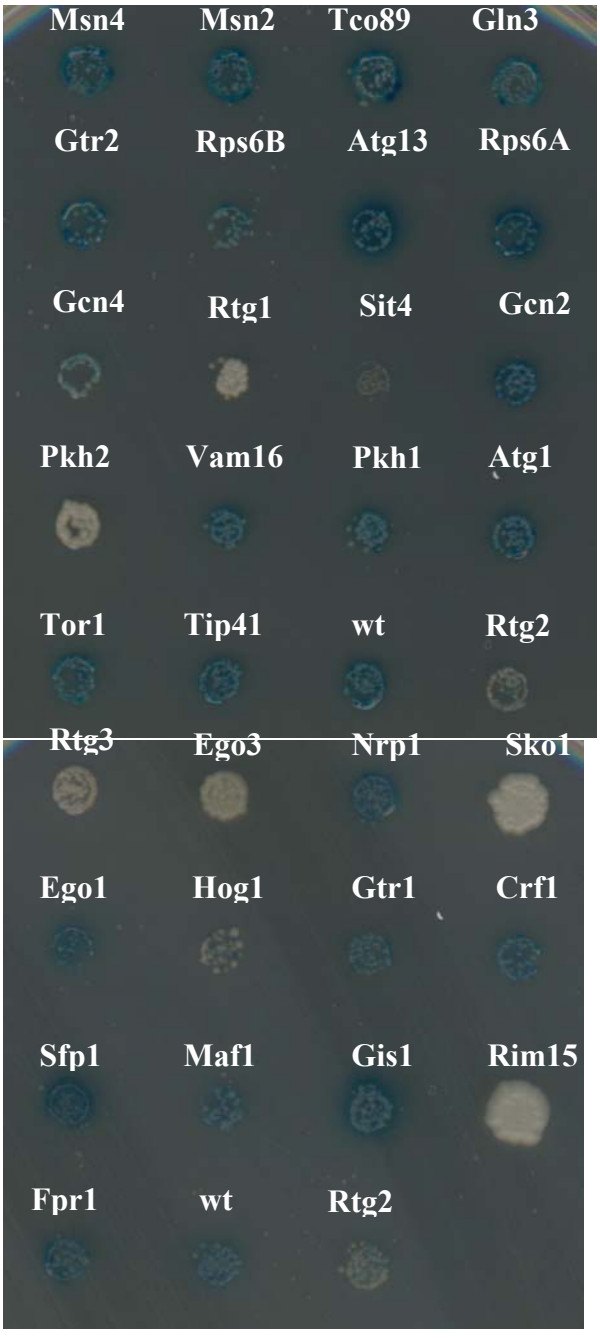
To study the interaction between RTG and TOR signaling pathways in mitochondrial stress response, a preliminary screening of a mini-library of TOR deletion strains for the expression of *CIT2*, a prototypical retrograde target gene, was performed. An *in vivo* β-galactosidase agar-plate assay was used to screen a mini-library of 32 selected BY4741 yeast deletion strains lacking genes

involved in TOR signaling pathway for *CIT2* expression. To this aim, the library was transformed with pBL101 p*CIT2*-lacZ reporter plasmid, in which expression of the lacZ reporter gene is under control of the *CIT2* promoter. Results of TOR deletions screening both on SC glucose and SC raffinose plates are given in the following figure:

SC Glucose + X-Gal



SC Raffinose + X-Gal



Blue colonies indicate positive reaction, i.e. those are the strains in which RTG-dependent *CIT2* expression is detected. White colonies, such as Δego3, Δsko1, Δsit4, Δhog1 and Δrim1 indicate that these genes are involved in the regulation of *CIT2* expression. Further investigation is needed to better understand the involvement of these genes in retrograde response to mitochondrial dysfunction. In a case of strains like Δatg13, which show more intense staining than wt, quantitative analysis of β-galactosidase activity is needed to assess hyperactivation of *CIT2* expression.

With the aim of studying the involvement of mitochondrial function in yeast cell response to acetic acid treatment, oxygen consumption measurements on intact cells grown in different carbon sources and at different pH were performed. By using Oxygraph-2 k system (Oroboros, Innsbruck, Austria) for high-resolution respirometry it was found that W303-1B wt cells, grown in glucose or in raffinose, show active mitochondrial respiration, both at pH 7, and at pH 3, a condition in which we have observed an activation of retrograde response in raffinose-grown cells. As expected, due to their glycolytic metabolism, glucose-grown cells take up oxygen less effectively than raffinose-grown cells, with RCR ratios being 2 and 1,8, respectively. After shift to pH 3, RCRs were 1,8 and 2, in glucose- and raffinose-grown cells, respectively. In any case, FCCP addition resulted in an increase of the oxygen consumption, and Antimycin A inhibited the respiration completely. Technical and operational skills acquired during Dr Ždralević stay in Caterbury will be of a benefit in the performing these bioenergetic studies at IBBE which has recently acquired the Oxygraph-2 k system.

Data obtained during Dr Ždralević stay at the University of Kent will form a basis for future collaboration projects between the IBBE and the University of Kent aimed at studying the interaction between different signaling pathways involved in yeast AA-PCD resistance mechanism and in stress response.

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