

ABSTRACT

Translational control and selective translation of some mRNAs represents a regulatory mechanism of cell to adapt to various physiological and environmental stresses. In *Saccharomyces cerevisiae*, activation of the translational control pathway GCN, whose major transducer is Gcn2p kinase, favours adapt to stress by nutrient starvation. Gcn2p is activated by uncharged tRNA in amino acid starvation conditions. Gcn2p phosphorylated to eIF2 α (Sui2p) at ser-51 and this inhibits mRNA general translation, while enabling selective translation of some mRNA that are necessary for cellular survival. One is *GCN4* mRNA, a transcription factor that regulated biosynthesis gene activation between others.

Intracellular pH modulates many cellular systems, but mechanism of regulation and perception are mostly unknown. Previously in the group has identified two genes of *S. cerevisiae* important for tolerance to intracellular acidification by permeable weak acid: *LEU2* and *GCN2*. In this thesis, it was found that *LEU2* works by removing the dependence of extracellular leucine uptake in strains with leucine auxotrophy. Also, it was done on the molecular mechanisms by which Gcn2p respond to acid intracellular pH. Gcn2p intracellular acidification activates probably by inhibition of aminoacyl-tRNA synthetase because we observe the accumulation of uncharged tRNA^{leu} without leucine starvation. Gcn2p is required for leucine transport and knockout mutant *gcn2 Δ* is sensitive to acid stress if auxotrophy for leucine and Gcn4p isn't required for acid tolerance. Also, at ser51>ala eIF2 α mutant is acid sensitive; this suggests that Gcn2p, by phosphorylation of eIF2 α , can activate translation of unknown regulator of amino acid transporter different to Gcn4p.

In relation to genotoxic stress, previous evidence showed that Gcn2p is involved in cell cycle control in response to DNA damage by regulating the G1-S transition. But, how Gcn2p response happens and what effectors are involved? We have discovered that different DNA-damaging agents activated Gcn2p kinase, including the alkylating agent MMS. All of them somehow generate replication stress. The genetic characterization of the mutants of GCN pathway shows that Gcn1p and Gcn20p are involved in the phosphorylation of eIF2 α by Gcn2p in response to MMS. Furthermore Gcn2p and Gcn1p may have a role associated with MMS toxicity independent of translational control.

Screening of various checkpoint and/or DNA repair proteins showed that Xrs2p, Tel1p and Mag1p are required for activation of Gcn2p induced by MMS. Xrs2p and Tel1p participate in signalling and checkpoint DNA damage pathway, where the MRX complex is independent of translational control. Gcn2p activation is dependent of repair protein encoded by *MAG1* (3-methyladenine DNA glycosylase), which is required for DNA damage repair due to alkylating agents such as MMS. In response to MMS-mediated translational control appears to be implicated RAD52 epistatic complex. Therefore Gcn2p is functionally connected with the DNA damage repair machinery and/or checkpoint. Gcn2p activation by MMS is mediated by inhibition of some aminoacyl-tRNA synthetases. Between this appear to have an important role Frs2p, α subunit of cytosolic phenylalanyl-tRNA synthetase.