Signal flow between CWI/TOR and CWI/RAS in budding yeast under conditions of oxidative stress and glucose starvation

Mima Ivanova Petkova, Nuria Pujol-Carrion and Maria Angeles de la Torre-Ruiz*
Departament de Ciències Mèdiques Bàsiques-IRBLleida; Facultad de Medicina; Universidad de Lleida; Lleida, Spain

The CWI pathway cross-talks with TOR and RAS in both the oxidative and glucose starvation responses. Mtl1 is the cell-wall protein in charge of sensing and regulating this response. Rom2 and Rho1, which are the upper elements in the pathway, mediate this signal. Several outputs are involved and required for this response, one of which, ribosomal gene expression, seems to be regulated by Sfp1, amongst other possible transcription factors. Moreover, cross-talk also occurs in a reverse flow from TOR and RAS to the CWI pathway. Thus Tor1 and Ras2 inhibition also activates Slt2 in the absence of the Mtl1 protein and assures the proper adaptive response to oxidation and glucose deprivation.

Mtl1 is a cell-surface protein of Saccharomyces cerevisiae with a very high similarity to Mid2.1 Mtl1 localization has not been described to date, however its sequence contains a transmembrane domain and a very long domain rich in serine-threonine residues.2 This domain could be a suitable region of O-mannosylation, as it is similar to that described for Mid2.3 Mtl1 has been described as a molecule required for survival under oxidative stress conditions.4 Mtl1 is a putative sensor responsible for transmitting the oxidative signal to the CWI (Cell Wall Integrity pathway) and thereby inducing the activation of Slt2: the last kinase in the cascade; it is also responsible for the depolarization of the actin cytoskeleton.4 We have recently demonstrated that Mtl1 is required to inactivate both the Tor1 and Ras2 functions in response to glucose depletion and oxidative stress provoked by hydrogen peroxide.5 This is how Mtl1 transmits these signals to Rom2, the GAP (GTPase Activating Protein) of Rho1,5 which then activates the Rho1 GTPase. From Rho1, the signal follows two simultaneous routes: (A) One leads to a cascade of activation Pkc1-Bck1-Mkk1/Mkk2, ending in Slt2 dual phosphorylation and the downstream events concomitantly with the activation of the pathway.5 (B) The second signal, which is, in fact, a signal of repression, is transmitted to Tor1 and Ras2. This repression eventually has several outputs: ribosomal gene repression (an ATP housekeeping mechanism), cAMP descent and the activation of a wide subset of genes which are potentially regulated by the dual transcription factor Msn2/Msn4.6 At this point, we do not know the exact nature of the connection between Rho1-Tor1-Ras2. We can speculate, however, that two models can be proposed: Rho1 first inactivates Tor1 protein from the TORC1 complex and Tor1 transmits the signal to Ras2 inactivation in response to glucose deprivation and hydrogen peroxide treatment. In the second model, Rho1 signals simultaneously, but independently, to Tor1 and Ras2 inactivation. According to our study, both models suit the results that we present in the paper. Saccharomyces cerevisiae is a model that facilitates study of the various cross-talks regulating the different signal transduction pathways. The relationship between Tor-Ras and the PKC1 pathway suggests that the three pathways are essential for survival in response to specific stresses. The repression of ribosomal gene transcription is a general defence mechanism in stress response.
One question which arose from our study was whether the transcription factor that regulates ribosomal gene expression in the signal was mediated by Mtl1. Preliminary results from our lab suggest that Sfp1 could be involved in this process and in oxidative response. Sfp1 is a transcription factor whose nuclear localization determines the transcriptional induction of both ribosomal and RiBi genes. Sfp1 is positively regulated by TOR and RAS activity. In response to several types of stress, including oxidative treatment, Sfp1 translocates to the cytoplasm, which provokes the repression of ribosomal gene transcription. We observed that Sfp1 overexpression reduced cell viability upon hydrogen peroxide treatment, especially in mtl1 mutants. As previously reported, ribosomal gene repression did not occur under these conditions. The observation that Sfp1 overexpression severely impaired mtl1 cell viability upon hydrogen peroxide treatment suggests that Sfp1 could be negatively regulated by Mtl1 in response to specific types of stress. An excess of ribosomal gene transcription under glucose starvation and oxidative stress conditions would constitute a waste of ATP and would be detrimental for cell viability. This result did not exclude the existence of other regulatory proteins in our system, but more studies of this mechanism are required before we can draw any further conclusions.

There is an information flow between different signal transduction pathways and the mechanisms that integrate information from different signal pathways in a common response. However, how the different pathways talk is not completely understood. In a recent review, other authors have commented on CWI cross-talk with other pathways. We described a process that connects the PKC1-MAPK pathway with TOR and RAS. In our study, the signal flowed from Mtl1, an element on the pathway to each of the other two routes: TOR and RAS. We also have data that suggest the existence of cross-talk in the opposite direction: from RAS2 and TOR1 to CWI. In both double mutants ras2mt1 and tor1mt1, we therefore observed an example of Slt2 phosphorylation in response to peroxide treatment and glucose depletion, which contrasted with the absence of Slt2 activation determined in the single mutant mtl1 (Fig. 2). The CWI activation observed in these mutants when stressed, correlated with an increase in cell viability that was similar to that determined in wild type cells. These results demonstrate that both oxidative and nutritional stress provoked a simultaneous signalling flow from Mtl1 to several different pathways: CWI, TOR and RAS. RAS2 and TOR1 inhibition in the absence of Mtl1 also induced the activation of Slt2, the last kinase on the CWI pathway. These results indicate that the connection between the three pathways occurred at several different levels. On one hand, Mtl1 seemed to be the key regulator in response to external insults, as in the cases of oxidative and glucose depletion stress. In the absence of Mtl1, CWI was not activated and TOR1 and RAS2 were not inactivated; this severely impaired cell viability. However, according to the data shown here, another signal flowed from the inactivated RAS2 and TOR1 to activate CWI; this was independent of the cell wall receptor Mtl1 and assured that in response to certain specific types of stress, the downregulation of RAS2 and/or TOR1 guaranteed the activation of the CWI pathway. These mechanisms were necessary for cell survival and adaptation in the budding yeast system.

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