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Dynamic control of the yeast AMPK/SNF1 pathway in response to glucose signals

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Abstract

The SNF1/AMP-activated protein kinase (AMPK) belongs to a family of energy sensors that is conserved in all eukaryotes. Activated by ATP depletion, AMPK plays a vital role in restoring the energy balance by enhancing energy-generating and damping energy-requiring processes. Yeast SNF1 is activated by depletion of glucose in the growth medium but is also affected by other environmental stresses such as salt, oxidative and alkaline stresses. Currently the regulatory mechanism by which glucose controls the activity of SNF1 is incompletely understood. The aim of this thesis was to achieve a better understanding of the glucose regulation of the SNF1/AMPK pathway in the yeast *Saccharomyces cerevisiae*. By employing time-lapse imaging of the nucleo-cytoplasmic shuttling of the transcription factor Mig1, which is directly controlled by Snf1, we revealed the ability of the Snf1-Mig1 system to monitor not only the changes in glucose concentrations but also the absolute levels of glucose. It was also found that this system is highly flexible and rapidly adapts to glucose changes. Monitoring Mig1 migration in cells expressing different glucose uptake systems indicated that the profile of Snf1-Mig1 activity parallels the characteristics of the expressed hexose transporter, suggesting a firm link between glucose uptake and the regulation of the SNF1 pathway. Single cell studies of Mig1 nuclear/cytoplasmic shuttling revealed a significant cell-to-cell variability, which was studied using nonlinear mixed effects modelling. Our model was able to quantify characteristics of Mig1 translocation which cannot be directly measured experimentally such as the time, amplitude and duration of Mig1 transient response. SNF1 shares a number of structural and functional similarities with its mammalian ortholog AMPK. We show that different AMPK isoforms confer growth of the *snfΔ* mutant on SNF1-dependend carbon sources indicating functional complementation. Moreover, mammalian AMPK expressed in yeast showed proper regulation by glucose suggesting a conserved mode of regulation. Our data also showed that compound 991, an AMPK activating drug candidate, was able to enhance the activity of yeast-expressed AMPK providing scope for employing yeast for the screening of drugs affecting AMPK activity.

Keywords: AMPK, SNF1, Mig1, glucose signalling, dynamic control, mechanism, *Saccharomyces cerevisiae*