INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄR BIOLOGI

Dynamic regulation of the Mig1 transcriptional repressor under glucose de/repression

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Abstract

The budding yeast *Saccharomyces cerevisiae* AMP-activated protein kinase/SNF1 is a member of a highly conserved protein family present in all eukaryotes. Snf1 regulates energy homeostasis; in yeast, it is best-known for its role in cellular adaptation to glucose limitation. Utilisation of carbon and energy sources other than glucose is controlled, among others, via regulation of gene expression. Expression of genes essential for metabolism of alternative sources, such as maltose, galactose and sucrose is regulated by the transcriptional repressor Mig1, which in turn is controlled by Snf1. Mig1 shuttles in and out of the nucleus in response to glucose availability, which makes it a convenient read-out for Snf1 pathway activity in single cell analysis. The overall goal of this thesis is to achieve a better understanding of the dynamic control of an AMPK/SNF1 signal transduction pathway using the budding yeast *S. cerevisiae* as a model system. We combined classic biochemical, molecular and microbiology approaches with cutting-edge biophysical and imaging methods to fill gaps in our understanding of signal transduction mechanisms. Being the first ones to use millisecond imaging to monitor signal transduction, thus, the first ones to observe single Mig1 molecules in live cells, we found that regardless of glucose availability Mig1 is present in the cytoplasm and the nucleus as monomer and oligomers. We observed similar clusters of the transcription activator Msn2. Thus, we suggest that eukaryotic gene regulation is mediated through transcription factors which act as multimeric clusters. The structure of those clusters is stabilised by depletion forces that mediate interactions between intrinsically disordered regions of transcription factors. Classic biochemical approaches revealed a dual mechanism of Mig1 dephosphorylation which includes glucose-dependent and glucose-independent events. We also found evidence for a novel step of Mig1 regulation which includes tyrosine phosphorylation. We show that the expression of Mig1 is itself glucose-regulated in a Snf1-dependent manner. Taken together, this work provides novel concepts in understanding of the AMPK/Snf1 signal transduction pathway with specific emphasis on Mig1 regulation.

Keywords: cell signalling, glucose repression, Mig1, phosphorylation, *Saccharomyces cerevisiae*