Mechanisms of autoregulation of Snf1/AMPK in budding yeast and its nucleocytoplasmic dynamics

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Abstract: Mechanisms that control the balance between nutrient availability and metabolism are well conserved in eukaryotic organisms. In *Saccharomyces cerevisiae*, the SNF1 complex regulates cellular metabolism according to the supply of glucose, alternatively supporting fermentation or mitochondrial respiration. The mammalian homolog of SNF1, the Ser/Thr kinase AMPK, has a key role in the metabolic rewiring in response to nutrient depletion, stimulating catabolic pathways while inhibiting growth and proliferation.

The activation state of SNF1 is determined by the modulation of its phosphorylation on a conserved Thr, which is accompanied by a change in its nucleo-cytoplasmic distribution. Snf1 phosphorylation is regulated by Sak1, one of the main activating kinases of the SNF1 complex along with Tos3 and Elm1, and by the Reg1/Glc7 phosphatase complex (Coccetti et al., 2018). We recently identified several Snf1-dependent phosphosites on Sak1 and Reg1, leading to the intriguing hypothesis of an autoregulation mechanism of SNF1 (Caligaris et al., 2023). The aim of this study is to unravel the mechanisms underlying SNF1 regulation, by characterizing the loss of Snf1-dependent phosphosites on these two proteins.

We therefore decided to study the effects of 10 Snf1-dependent phosphosites on Sak1 and 16 Snf1-dependent phosphosites on Reg1 by mutating all of them to Ala (not-phosphorylatable residues). By using these engineered strains, we were able to observe a loss of SNF1 autoinhibition following a nutritional shift down (from high to low glucose condition).

To further investigate the spatial dynamics of Snf1 and their control by nutrient variation, we set up a new single Cell imaging live method, to study the nucleocytoplasmic dynamics of Snf1 upon glucose reduction using microfluidics systems. Time-lapse imaging experiment at different time-points before and after the glucose reduction revealed that a significant proportion of Snf1 goes into the nucleus rapidly after the shift down, but then exited from the nucleus at later time points, again supporting the notion of a negative feedback regulation. Altogether, our findings provide evidence indicating that the SNF1 complex is capable of negatively regulating its own long-term activation state, by phosphorylating its activating kinase Sak1 and phosphorylating its phosphatase Reg1. Experiment on the dynamic localization of SNF1 in Sak1 and Reg1 mutant strains are ongoing to better elucidate this feedback regulation.