The hexokinase phosphorylation landscape: new regulation of an "old" enzyme

1- State of the art

Glucose is the preferred carbon and energy source for most organisms and largely impact many regulatory pathways in living cells. Although it is the most abundant monosaccharide on Earth, extracellular glucose concentrations fluctuate, and most cells must adapt to these variations to survive. The remarkable capacity of eukaryotic cells to optimize their metabolism in response to glucose availability requires glucose sensing systems and sophisticated signal transduction pathways. Glucose catabolism involves multiple cellular enzymatic processes starting with glycolysis which is one of the most conserved biological pathways in life kingdom. The first step of glycolysis is the irreversible phosphorylation of intracellular glucose in glucose-6-phosphate by the hexokinases/glucokinases enzymes. This step is essential for cells and the hexokinase activity is submitted to multiple regulations to efficiently metabolize glucose according to its extracellular availability. In mammals, hexokinase disfunctions cause several pathologies such as hereditary diabetes, hyper-insulinemia and the Warburg effect in cancer cells. In addition to their well-known role as glucose phosphorylating enzymes, hexokinases are also involved in many other cellular functions such as insulin secretion, apoptosis, and longevity.

Similarly to the Warburg effect in cancer cells, the yeast *Saccharomyces cerevisiae* produces energy through glycolysis and fermentation rather than oxidative phosphorylation, even in the presence of oxygen. This metabolic treat depends on a molecular process known as glucose repression. The hexokinase Hxk2 plays a pivotal role during glucose repression by feeding glycolysis with glucose-6-P and as a nuclear transcriptional regulator inhibiting the expression of genes involved in alternative carbon source metabolism. Understanding what are the molecular mechanisms that control hexokinases functions is then crucial to better appreciate their cellular role in physiological and pathological situation.

Among the molecular mechanisms known to control its activity, the phosphorylation of hexokinase is particularly important. In *S. cerevisiae*, glucose dependent phosphorylation of Hxk2 at serine 15 controls its transcriptional function during glucose repression through the regulation of its nucleo-cytoplasmic distribution and its interaction with different transcriptional factors. Recent phosphoproteomic studies in yeast have shown that Hxk2 is phosphorylated on several other residues suggesting a high degree of regulation by phosphorylation. **However, the physiological consequences of Hxk2 phosphorylation on its functions and on yeast cellular physiology remains largely unknown.**

We propose to characterize the role of hexokinase phosphorylation on its functions and on cell adaption to glucose variation. Yeasts will be used as experimental models since they display a high degree of conservation, and efficient genetic, molecular, and genomic tools to study glucose signaling and metabolism.

2- Strategy

Unbiased identification of Hxk2 phosphorylation sites: to identified Hxk2 phosphorylation sites a Hxk2 phospho-mapping strategy will be followed. After GFP-trap purification followed by proteolytic cleavage, Hxk2 phospho-peptides will be enriched by iMAC and/or TiO2 and sequenced by MS/MS analysis. The effect of carbon source on Hxk2 phosphorylation will be then addressed via the same strategy after glucose starvation of the cells. This might allow to identify differentially phosphorylated residues in Hxk2 in response to carbon source.

Functional characterization of Hxk2 phosphorylation sites: Each Hxk2 phosphorylated serine, threonine or tyrosine identified will then be mutated to alanine (non-phosphorylatable) an aspartic/glutamic acid (phosphomimetic residues) via a CRISPR/Cas9. Each mutant will be then analyzed for hexose-kinase activity by enzymatic assays and glucose dependent growth. The influence of these phosphorylation events on Hxk2 regulatory function will be addressed by analyzing in each mutant 1/ the expression of

glucose repressed genes (transcriptional reporter systems) and 2/ the ability of mutated Hxk2 to interact with the transcriptional repressor Mig1 (Co-IP) in response to carbon sources. This might allow to understand if Hxk2 phosphorylation at these different residues promotes or inhibits hexokinase functions. These mutants would be further characterized by a structural approach to correlate the effect of Hxk2 phosphorylation on its structure and on its activities.

Hxk2 phosphorylation and signaling pathways: It is important to identify the different kinases phosphorylating Hxk2 at the identified residues and what are the signals controlling their activity toward Hxk2. To isolate the kinases phosphorylating Hxk2 a targeted proximity-dependent biotynylation labelling strategy is currently developed in the lab. Our preliminary results indicate that several protein kinases might interact with Hxk2. Theses identified kinases will be tested for Hxk2 phosphorylation by kinases assays and by analyzing the effect of their belonging signaling networks on Hxk2 *in vivo* functions during glucose repression.

3 - Expected Results

By identifying Hxk2 phosphorylation sites, the belonging kinases and the consequence on hexokinase functions this project will help to better understand the complex mechanisms of Hxk2 regulation necessary for yeast cells to adapt to carbon sources. Regarding the high degree of identity between yeast and mammalian hexokinases, the conservation of theses regulatory mechanisms might be later addressed in mammals, helping to better understand the role of hexokinase in physiological and pathological situation such as diabetes or during the Warburg effect in cancer cells.

Supervisor(s) name(s), Affiliation(s), eMail address(es) for contact:

Dr. Alexandre Soulard (HDR)
UCBL1 – UMR5240 – MAP – équipe SMAL
alexandre.soulard@univ-lyon1.fr

Proposed collaboration within ArchiFun network (not mandatory at this stage):

Proposed list of secondments (not mandatory, but recommended if known already):

Main ArchiFun theme involved:

Mechanisms of cancer onset

Physiology