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Crystal Structure of the Heterotrimer Core of *Saccharomyces cerevisiae* AMPK Homolog SNF1

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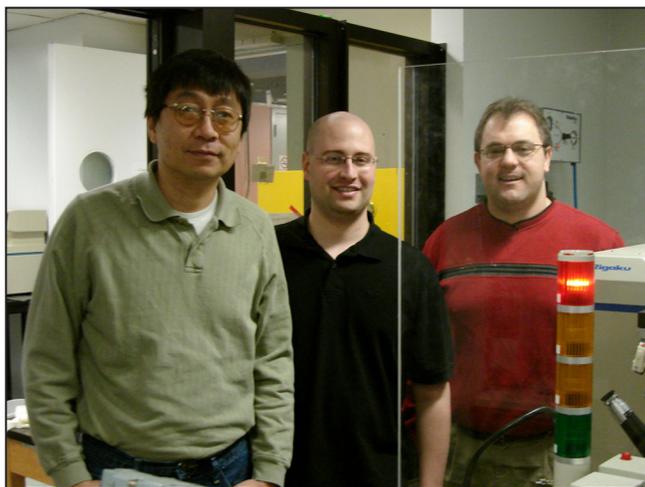
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*AMP-activated protein kinase (AMPK) is a crucial enzyme for maintaining energy homeostasis in eukaryotes. It is regulated by the intracellular ratio of AMP:ATP. When this ratio is high, indicating a low energy state, AMPK is activated, whereas, it is inhibited when the ratio is low. Upon activation, AMPK upregulates a number of catabolic processes while simultaneously downregulating energy-consuming processes. AMPK is an attractive drug discovery target for obesity and type II diabetes, among other diseases. We report here the structure of the heterotrimer core of the *S. cerevisiae* homolog of AMPK, SNF1, at 2.6 Å resolution.*

AMPK is a critical enzyme for regulating energy homeostasis in all eukaryotic organisms. It is a heterotrimeric protein consisting of a catalytic α subunit and two regulatory (β and γ) subunits. The α subunit contains an N-terminal kinase domain, followed by a regulatory sequence and a region important for heterotrimer formation. The β subunit has a glycogen binding domain (GBD) and a C-terminal region responsible for heterotrimer association. The γ subunit contains two Bateman domains, each of which can bind one or two molecules of nucleotide, and this subunit is responsible for regulation of the protein by competitive AMP or ATP binding. When the energy charge of the cell is low (high AMP:ATP ratio), AMPK is activated through AMP binding, while ATP binding inhibits the protein. We present here the structure of the heterotrimer core of SNF1, the *S. cerevisiae* homolog of AMPK, at 2.6 Å resolution.

nantly in *E. coli* using a tricistronic expression construct containing the α subunit Snf1, β subunit Sip2, and γ subunit Snf4 (**Figure 1**). The structure of SNF1 was solved by molecular replacement using the structures of the *S. pombe* AMPK core and the GBD of the rat $\beta 1$ subunit as models. The heterotrimer is held together by a hydrophobic core formed by an eight-stranded mostly antiparallel β -sheet with four strands from Snf1, three from Sip2, and one from Snf4 (**Figure 2**). Our structure of SNF1 contains two unique features compared

to those of mammalian and *S. pombe* AMPK. The first of these is a regulatory sequence (RS) in Snf1, from residues 460 to 495, which interacts with Snf4. This is the first structural evidence of direct interactions between Snf1 and Snf4. The interface is quite extensive, measuring approximately 1150 Å²/molecule, and contains an antiparallel β -sheet between the two subunits. Of particular interest is that this region has been linked by biochemical and genetic data to autoinhibition of AMPK, and provides a possible mechanism by which the protein can be regulated. We speculate that our structure represents the active conformation of the SNF1 heterotrimer core, with the RS of Snf1 sequestered away from the kinase domain, thus removing autoinhibition. Another unique feature of our structure is the presence of the glycogen binding domain in the β subunit (Sip2). This domain interacts primarily with Snf4, and is located such that the carbohydrate binding site is exposed to the



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The SNF1 heterotrimer was expressed recombi-

solvent, indicating its potential for ligand interaction.

An interesting difference between SNF1 and mammalian AMPK is that, based on biochemical evidence, SNF1 does not appear to be activated by AMP *in vivo*. Interest-

ingly, however, our data suggest that SNF1 still has the capacity to bind AMP, although possibly with lower affinity. Our structure contains several differences in the γ subunit (Snf4), which binds the nucleotide, but further studies are required to fully elucidate the mo-

lecular mechanism for AMPK/SNF1 activation.

In conclusion, our structure of the heterotrimer core of SNF1 provides new insights into potential mechanisms of regulation for this crucial enzyme in energy homeostasis.

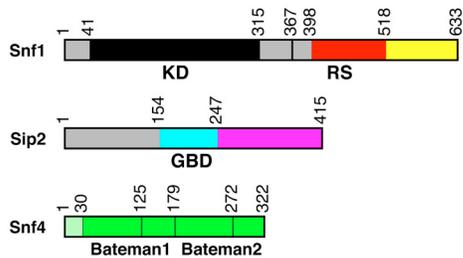


Figure 1. Domain architecture of the SNF1 heterotrimer. Those regions corresponding to residues found in the structure are represented in color; other regions are in black or gray. KD, protein kinase domain; RS, regulatory sequence; GBD, glycogen binding domain.

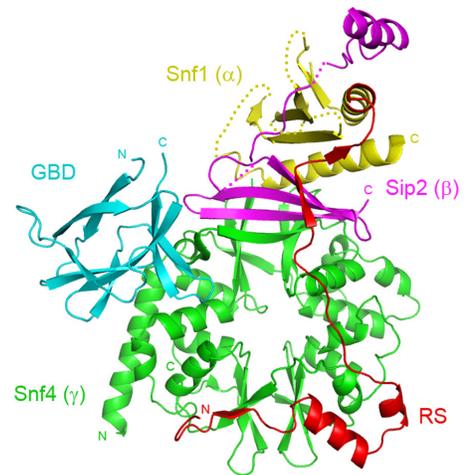


Figure 2. Structure of the SNF1 heterotrimer core. Regions are colored corresponding to Figure 1.