A Crystallized View of AMPK Activation

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AMP-activated protein kinase (AMPK) is a key metabolic regulator. Recent work (Chen et al., 2009) elucidates the structural interaction between the autoinhibitory sequence and the kinase domain of the AMPK catalytic subunit. Enhanced understanding of the molecular mechanics of AMPK activation might lead to novel therapeutic approaches.

AMP-activated protein kinase (AMPK) has emerged as an important regulator of diverse metabolic pathways, mitochondrial biogenesis, cell polarity, and cell growth. Highly regulated by the cellular energetic state, AMPK activity is increased by AMP and antagonized by ATP. When energy demand outstrips ATP production, net ATP breakdown ensues, increasing intracellular AMP concentration and activating AMPK. Thus, AMPK functions as a cellular fuel gauge that detects energetic stress, such as occurs during ischemia, hypoxia, and hypoglycemia, or during exercise in skeletal muscle and heart (Young, 2008). AMPK also receives cues from the extracellular environment and is activated by hormones (adiponectin and leptin) and cytokines (IL-6 and MIF) (Miller et al., 2008). Beyond its internal cell-centric function, AMPK in the hypothalamus modulates signals that regulate whole-body energy homeostasis.

Once activated, AMPK stimulates energy-generating catabolic pathways and inhibits energy-requiring anabolic processes. Contemporary interest in AMPK was launched by observations that activated AMPK accelerates glucose transport, glycolysis, and fatty acid oxidation, while also inhibiting protein and glycogen synthesis. Because AMPK also interacts with intracellular signaling pathways and modulates gene expression, it has a broad range of physiologic actions in most cells.

Research on AMPK structure has the potential to elucidate the specific mechanisms regulating the kinase and could lead to novel therapeutic strategies for AMPK activation. However, understanding the molecular structure of the intact AMPK molecule has proved challenging, because AMPK is a heterotrimeric complex of α, β, and γ subunits. The α subunit contains the kinase domain and an adjacent autoinhibitory sequence, the β subunit includes a glycogen binding domain and bridges the α and γ subunits, and the γ subunit contains nucleotide-binding (Bateman) domains that are essential for AMPK activation by AMP (Figure 1). AMPK α subunit activity is critically dependent on the phosphorylation of Thr172 within its activation loop. Thr172 is phosphorylated by upstream LKB1 and Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK), and dephosphorylated by protein phosphatases (PP2C). AMP binding to the γ subunit has the dual effect of inducing allosteric activation and inhibiting Thr172 dephosphorylation (Davies et al., 1995). This latter effect is critical in the regulation of AMPK activity.

Previous crystal structures have provided insight into the configuration of AMPK's critical functional domains (Scott et al., 2009). The AMPK equivalent kinase in yeast (SNF1) has been highly utilized as a model system for structural analysis. The SNF1 complex includes the proteins Snf1, Sip2, and Snf4, orthologs of the AMPK α, β, and γ subunits, respectively. Core fragments of the heterotrimeric SNF1 complex have also been crystallized (Amodeo et al., 2007). However, one important caveat to these structures is that the yeast SNF1 complex is not actually activated by AMP, the cardinal feature of mammalian AMPK.

The holy grail of AMPK structural biology, crystallization of the intact mammalian heterotrimeric AMPK complex, has proved elusive. However, the full-length mammalian AMPK γ subunit and fragments of the β and γ subunits have recently been crystallized, with analysis yielding a core structure (Xiao et al., 2007). This revealed the unanticipated discovery that γ subunits contain three AMP binding sites, one of which is nonexchangeable, challenging the existing paradigm of only 2 AMP binding sites. Thus, despite a substantial amount of recent research on the structure and function of AMPK, the "molecular gymnastics" that actually transduce AMPK catalytic activation remain a puzzle.

In a recent issue of *Nature,* Chen et al. take some of the mystery out of this puzzle (Chen et al., 2009). They have successfully crystallized a fragment of *S. pombe* Snf1, containing both the kinase and autoinhibitory domains. Their novel crystal structure indicates that the Snf1 autoinhibitory sequence interacts with the "backside" of the bilobar kinase domain, constraining it in an open and inactive configuration (Figure 1). They have also crystallized a novel *S. cerevisiae* Snf1 kinase domain fragment that is phosphorylated in the activation loop. Lacking the autoinhibitory domain, this structure has a more closed configuration with higher activity than previously identified nonphosphorylated kinase domain fragments from *S. pombe*. Taken together, these data suggest that the autoinhibitory domain might exert a molecular "Heimlich maneuver" on the kinase domain, forcing it open from the backside and preventing it from closing into its active configuration.

Based on their initial findings, Chen et al. identified candidate amino acids that mediate the interaction between the autoinhibitory sequence and the kinase domain (Chen et al., 2009). Point mutations of these amino acids increased the catalytic activity of both the *S. pombe* Snf1 fragment and rat AMPK α1 subunits. Notably, these same interface mutations increased the activity of the intact rat AMPK $\alpha\beta\gamma$ complex, independent of the

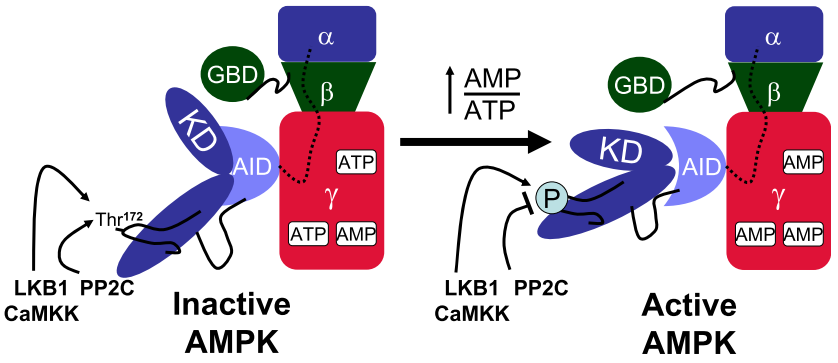

Figure 1. AMPK Activation Model

The AMPK complex is comprised of three subunits: the α catalytic subunit (blue) including the bilobar kinase domain (KD) that contains Thr172 in the activation loop of the catalytic cleft and an autoinhibitory domain (AID) (light blue) sandwiched between the backside of the kinase domain and the γ subunit; the β subunit (dark green) containing a glycogen binding domain (GBD) and binding sites for both the α and γ subunits; the γ subunit, which contains the nucleotide binding sites (Bateman domains). When the AMP/ATP ratio is low, little AMP is bound and AMPK is in its inactive form, with the autoinhibitory AID tethering the backside of the kinase domain in its inactive open configuration. This open cleft allows both upstream kinases (LKB1 and CaMKK) and protein phosphatases (PP2C) to interact with Thr172, maintaining low phosphorylation of this critical activating site. When the AMP/ATP ratio increases with energetic stress, more AMP is bound to the γ subunit and the autoinhibitory subunit releases from the hinge of the kinase domain, which then assumes its active more closed configuration. The closed cleft also inhibits access of protein phosphatase to Thr 172, favoring the action of the upstream kinases to maintain a high phosphorylation state of the activating site. Adapted from Pang et al., 2007; Xiao et al., 2007; Riek et al., 2008; Chen et al., 2009.

AMP concentration. These mutations also slowed dephosphorylation of α subunit Thr172 by PP2Cα and effectively abolished the AMP dependence of this dephosphorylation. Because the phosphorylation state of the Thr172 site is such a critical determinant of AMPK activity, these latter findings hold perhaps the greatest physiologic significance. They specifically indicate that the autoinhibitory sequence has an important role in modulating Thr172 phosphorylation.

These observations build on a legacy of prior investigations into the molecular regulation of AMPK, including recent studies that suggested a backside interaction between the autoinhibitory and kinase domains (Pang et al., 2007; Riek et al., 2008). The model proposed by Chen et al. sandwiches the autoinhibitory sequence between the α subunit kinase domain and the γ subunit, potentially enabling it to transduce AMP binding into activation of the kinase domain (Figure 1). Their results also argue that the autoinhibitory sequence of the kinase domain does not function as a pseudosubstrate. This contrasts with other well-known protein kinases, such as protein kinase C, that are inhibited by pseudosubstrate domains.

While the findings of Chen et al. enhance our understanding of the molecular mechanics of AMPK regulation, key questions remain. Most important mechanistically, how does AMP binding actually alter the interaction between the autoinhibitory and kinase domains? In particular, how does AMP binding to the γ subunit block dephosphoryation of the α subunit Thr172, whose phosphorylation state is so critical to the activity of AMPK? In terms of translating these findings, can the autoinhibitory domain be targeted as a novel strategy for activating AMPK? Prior pharmacologic agents have activated AMPK indirectly through increasing AMP concentration (metformin), serving as AMP mimetics (AICAR), or through binding to the β subunit (A769662). Recent results already provide an initial answer to this question with proof-of-principle findings that small molecules targeted at the autoinhibitory domain might activate AMPK (Pang et al., 2008).

Thus, the authors are to be congratulated on their contribution, which helps to unravel the molecular twists and turns that mediate AMPK activation. Further investigation in this field hopefully will advance the treatment of conditions in which AMPK is likely to have an important role, including diabetes, cancer, and heart disease.

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