# Identification of AMP-activated protein kinase (AMPK) in the shark rectal gland

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The activity of AMP-activated protein kinase (AMPK) increases during metabolic stress as AMP levels rise and ATP levels fall. Once activated, AMPK acts to restore energy homeostasis by phosphorylating multiple substrates that act both to stimulate energy production and minimize energy consumption. AMPK has been linked to cystic fibrosis transmembrane conductance regulator (CFTR) mediated chloride secretion in several different tissues. This study sought to detect the AMPK protein by Western blot and cloning and to begin to define its interaction with CFTR in the dogfish shark rectal gland.

The ultrasensitive energy sensor AMP-activated protein kinase (AMPK) orchestrates the regulation of energy-generating and energy-consuming pathways[1]. The activity of AMPK increases during conditions of cellular and environmental stress such as heat shock, hypoxia and ischemia in response to elevated intracellular AMP/ATP ratios. AMPK is a heterotrimer consisting of a catalytic α-subunit and regulatory β- and γ-subunits[2]. Each of these subunits exists as multiple isoforms (α1, α2, β1, β2, γ1, γ2, γ3)[1]. The α1 isoform appears to be expressed in most tissues, while the α2 isoform is expressed predominantly in skeletal and cardiac muscle and liver[2]. Substrate specificity may be influenced by differences in the subcellular compartmentalization of AMPK complexes, depending on whether they contain α1 or α2 catalytic subunits[1].

AMPK has been linked to cystic fibrosis transmembrane conductance regulator (CFTR) mediated chloride secretion in several mammalian tissues[1,2]. AMPKα binds to the CFTR COOH-terminal tail at residues 1420-1457, phosphorylates CFTR at residue $Ser^{768}$ in the regulatory domain and inhibits PKA-stimulated gating of CFTR by decreasing its open probability. The relationship between AMPK and CFTR was initially identified by a yeast two-hybrid screen[1].

The rectal gland of the spiny dogfish shark (SRG) (*Squalus acanthias*) is an ideal model for studying epithelial chloride secretion through CFTR, as its apical membrane contains record amounts of the protein[3]. We hypothesize that under hypoxic conditions AMPK activity is increased in the SRG leading to an inactivation of CFTR by AMPKα. To test our hypothesis, immunoblot nalysese, $I_{sc}$ studies and cloning were performed. Each confirmed the presence of AMPK in the gland.

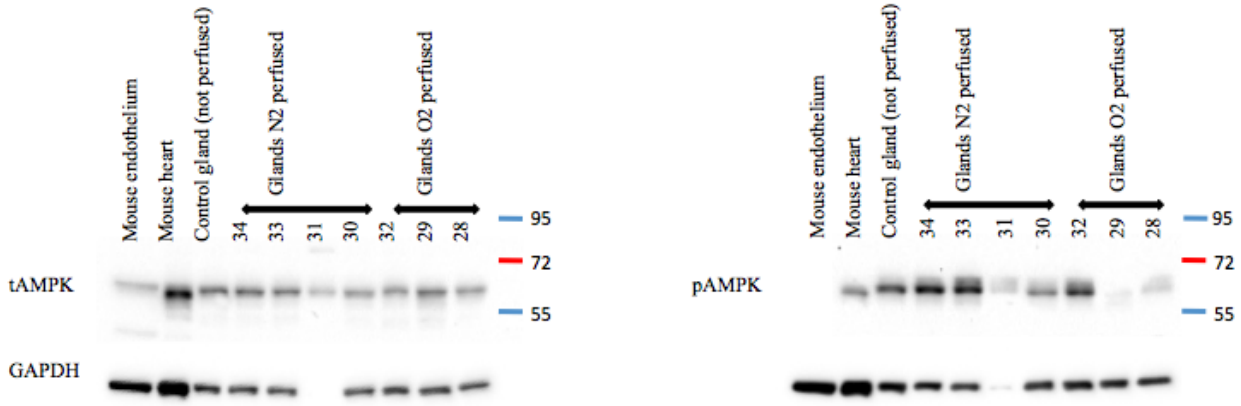



**Figure 1.** Western blot analysis detecting immunoreactivity of shark AMPK, including t (total) and p (phosphorylated) AMPK of the expected MW of 63 kDa. Antibodies used were Cell Signaling rabbit anti AMPKα (t) and rabbit anti-phospho-AMPKα (p). The rabbit anti phospho-AMPKα (p) detects AMPKα only when phosphorylated at threonine172 in the catalytic domain and the cloned shark 389 bp sequence (see below) contains threonine172. Positive controls are mouse endothelium and mouse heart lysate.

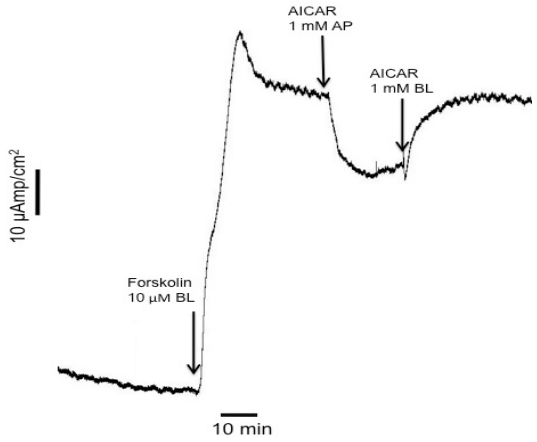
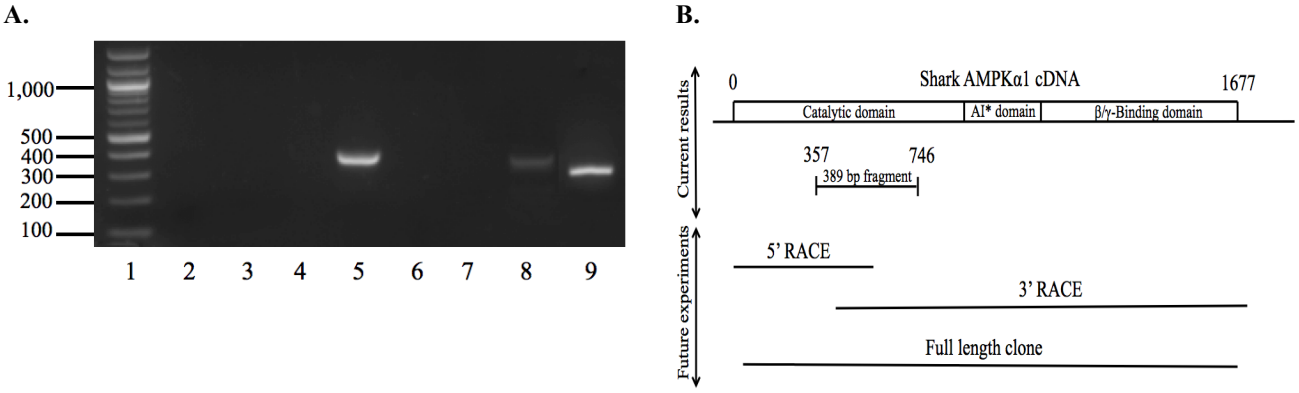



**Figure 2.** Representative experiment with AICAR, a pharmacological AMPK activator, inhibits chloride secretion when applied to the apical side of polarized monolayers of cultured shark rectal tubules. Transepithelial chloride transport was measured as short-circuit current ($I_{SC}$) in Ussing chambers. When applied to the basolateral side of the cultured monolayers, AICAR stimulates chloride secretion. Mean inhibition after apical addition of 1 mM AICAR (n=5) was 27 ± 5.0%, *P < 0.05 compared to $I_{SC}$ before AICAR.

**Figure 3**. (Below) Panel A. Cloning of shark the AMPKα1 gene. PCR of shark rectal gland cDNA yielded a 389-bp product (lane 5) containing a portion of the catalytic domain of AMPKα1 using degenerate primers from conserved regions of human, cow, rat, pig, chicken and frog AMPKα1. Lane 1, 100-bp DNA ladder; lane 2-7 PCR with different pairs of AMPKα1 degenerate primers; lane 8 control using TASK-1 potassium channel degenerate primers (394-bp PCR product); lane 9 control for cDNA synthesis using human HeLa RNA and β-actin specific primers (353-bp PCR product). Panel B: Strategy for obtaining full-length sequence of shark AMPKα1. A BLAST search of the 389-bp product against the human genome shows 93% similarity with human AMPK α1. Within this sequence were the active site, the ATP-binding site, the substrate binding site and the activation loop of the catalytic AMPKα domain.

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3. **Forrest JN Jr.** Cellular and molecular biology of chloride secretion in the shark rectal gland: regulation by adenosine receptors. *Kidney Int* 49:1557-1562, 1996.