AMPK links cellular bioenergy status to the decision making of axon initiation in neurons

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Neuronal polarization begins by the selection of a single minor neurite that subsequently undergoes rapid extension until reaching a formidable length. To ensure that the highly active growth can be sustained by a sufficient energy supply, neurons are supposed to sense their energy status prior to initiating polarization. Our recent work shows that the bioenergy sensor, AMPK, plays a crucial role in the regulation of axon initiation. Activation of AMPK to mimic energy-lacking conditions results in a halt in axon selection and growth, leading to a loss of neuronal polarization.

AMP-activated protein kinase (AMPK) is the master regulator for cellular energy homeostasis. Although originally identified as an AMP responsive protein¹ capable of phosphorylating enzymes involved in lipid synthesis,² AMPK has more recently emerged as a major regulator of energy metabolism in all eukaryotic cells, capable of affecting an ever growing number of cellular processes. Existing as a heterotrimeric protein, AMPK consists of an α , β and γ subunit in equal stoichiometry. While the α subunit possesses serine-threonine kinase activity, the γ subunit, through two AMP/ATP binding Bateman domains, confers sensitivity to the cellular AMP:ATP ratio and enables AMPK to function as an energy sensor.³ Increases in this ratio, resulting from various biosynthetic pathways and pathological states such as ischemia and hypoxia^{4,5} causes the direct binding of AMP to the respective binding sites at the γ subunit. AMP binding results in a conformational change within the α subunit, leading to phosphorylation at Thr172 and activation of AMPK with the participation of upstream kinases, referred to as AMPK kinases (AMPKKs).

Upon activation, AMPK works to maintain the balance of energy by promoting ATP-producing catabolic processes such as glucose metabolism⁶ and fatty acid oxidation⁷ and inhibiting ATP-consuming anabolic processes including the synthesis of proteins^{8,9} and lipids.¹⁰ In addition to regulating the activity of enzymes involved in the metabolism and production of ATP, AMPK also possesses the capability to regulate transcription factors through phosphorylation to achieve persistent changes through gene expression.^{11,12} As a consequence of AMPK-activated metabolic adjustment, an increase in ATP levels and

thus a reduction of AMP:ATP ratio occurs, which will allow dephosphorylation of Thr172 and inactivation of the kinase.¹³

From membrane transport, receptor trafficking and turnover to action potential formation and synaptic transmission, the large number of diverse cellular processes occurring simultaneously within the neuron requires vast amounts of energy in order to reach completion. Despite the numerous studies performed on understanding energy requirements to sustain brain functions, much less is known about the role of energy-sensing signaling cascades in early brain development. Evidence has emerged to suggest that while AMPK certainly plays a significant role in whole body metabolic regulation, especially in the context of metabolic disorders and strenuous exercise,¹⁴⁻¹⁶ the kinase also demonstrates a critical function in the establishment of cell morphology. For instance, it was shown that AMPK activity is required for the formation of epithelial cell polarity in *Drosophila*.¹⁷ Given that cell growth and morphological specialization must be supported by a sufficient energy supply, a link between AMPK activity and cell development should be expected. This is especially true in the case of neuron, where its polarization process requires the differentiation and extension of minor neurites into multiple dendrites and a single axon, which eventually grow into a structure of impressive size and complex geometry.¹⁸ During the early stage of neuronal growth, a single neurite is chosen as an axon and this selected neurite will grow extremely rapidly until reaching a length of roughly 40,000 times that of the cell body.¹⁹ Given that the dramatic scale in axon growth entails a tremendous amount of energy-consuming activities, including protein and lipid synthesis and assembly, axonal delivery and membrane targeting, as well as bidirectional signaling, it is a major commitment for a neuron to ensure the efficiency and sufficiency in energy supply. Therefore, the decision making in axon initiation must be energy conscious. Supporting this notion, our recent work indicates an essential role of the energy-sensing AMPK pathway in neuronal polarization.

We find that when AMPK activity is pharmacologically enhanced to mimic energy-lacking conditions during the transition from neuronal growth stage 2 to stage 3, a time point when axon initiation and polarization begins to occur,²⁰ axon initiation is effectively repressed, resulting in non-polarized neurons with no axon.²¹ This effect exists on both the morphological and molecular levels, indicated by the lack of specific localization of Tau-1 and MAP2, marker molecules that are normally expressed exclusively in the axon and dendrites, respectively.

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Figure 1. A schematic illustration of bioenergy status-dependent regulation of neuronal polarity. (A) Under normal energy conditions, PI3K is transported to the tip of a single neurite via KIF5/Kinesin Light chain (KLC) complex interaction. Once at the tip, PI3K promotes downstream signaling that results in an increase in actin dynamics and microtubule stability, ultimately enabling neurite extension and axon specification. (B) Periods of energetic stress increase intracellular AMP levels, resulting in the phospho-activation of AMPK. Active AMPK phosphorylates KLC, leading to dissociation of PI3K from the motor complex. The loss of PI3K enrichment at the neurite tip prevents the signaling required for axon specification and neuronal polarization.

Mechanistically, we find that a dislocation of PI 3-Kinase (PI3K) is responsible for the AMPK effect, as enrichment of PI3K at the neurite tip is believed to have a key role for axonal initiation and polarization.²⁰ Furthermore, AMPK directly phosphorylates the kinesin light chain (KLC) of the Kif5 motor protein, leading to a dissociation of the motor from its cargo PI3K. Thus, a failure in PI3K delivery to the axonal tip during AMPK activation, presumably occurring under energy-lacking circumstances, prevents the establishment of neuronal morphology,²¹ as illustrated in Figure 1.

In addition to the capability of AMPK to prevent the enrichment of PI3K at the neurite tip, AMPK activation also leads to a robust increase in general activity of the PI3K-Akt system. While this phenomenon occurs during the timeframe of neuronal polarization, Akt activation seems to be less involved with polarity inhibition, but instead exists as a rescue mechanism to increase the cellular energy supply. As opposed to peripheral cells, neurons utilize glucose almost exclusively as their energy source.²² As stated previously, the central dogma of AMPK function is to increase ATP production and decrease its consumption in an effort to increase the availability of cellular energy. To this end, it has already been shown that AMPK activity causes translocation of the glucose transporter GLUT3 to the cell membrane to facilitate glucose uptake in neurons.²³ Furthermore, insulin-like growth factor-1 (IGF-1), a proven upstream activator of the PI3K and Akt pathway,²⁴ has also been shown to regulate glucose metabolism in the developing brain,²⁵ thereby identifying a common goal of AMPK and PI3K signaling. The relationship between AMPK and PI3K/Akt may also have evolved to ensure neuron survival during energetically

stressful conditions, given the neuroprotective roles of both the PI3K²⁶ and AMPK.²⁷⁻²⁹

Paradoxically, LKB1, a major AMPKK in non-neuronal cells,^{30,31} has been shown to be necessary for axon initiation.^{32,33} However, this finding also suggests that LKB1 is likely to achieve its effect via a distinct signaling cascade, rather than through activation of AMPK. In support of this notion, LKB1 deficient cortical neurons display no decrease in total or phosphorylated AMPK under basal conditions.³² It is possible that the action of LKB1 in AMPK activation is dominant only during energetic stress, when the activation loop of AMPK has been exposed through AMP binding. If this scenario holds true it will be interesting to know whether loss of LKB1 prevents AMPK phosphorylation and polarity inhibition during periods of energetic stress. Secondly, it is possible that, unlike non-neuronal cell types, LKB1 is not the main upstream kinase of AMPK in neurons. In fact, other proteins have been shown to activate neuronal AMPK, including calmodulin-dependent protein kinase- β (CaMKK β).³⁴⁻³⁶ Given the requirement of Ca²⁺ in CaMKK β activation,³⁷ it is intriguing to hypothesize that Ca²⁺ influx, occurring during cell firing and synaptic transmission or excitotoxicity, serves as a mediator bridging neuron activity or neurotraumatic insults to AMPK activation. In fact, AMPK is capable of phosphorylating GABA_B receptor to inhibit postsynaptic depolarization.³⁸ This might represent a protective measure that transits calcium influx during hyperactivity to subsequent synaptic inhibition.

Despite the dramatic inhibitory effect of elevated AMPK activity on neuronal polarization, it is surprising that expression of the kinase dead version of AMPK in either cultured hippocampal

neurons or cortical brain slices has no effect on axon growth and neuronal polarization.²¹ In line with this, AMPK α 1/ α 2 knockout also reveals that basal AMPK activity is not required for cortical neurogenesis, neuronal migration or polarization.³⁹ Together, these findings imply a negligible function for basal AMPK activity during normal polarization, suggesting that AMPK may play a key role under pathological energy-lacking conditions. Consistently, we have demonstrated that a brief ischemia challenge during early development induces a dramatic suppression in axon initiation and neuron polarization. It is not clear whether axon growth can resume after a long recovery following AMPK activation, but at least short periods of time do not allow for an obvious recovery, as neurons remain unpolarized three days after the removal of AMPK activators.²¹

As a direct long term consequence of early suppression in axon differentiation and growth, synaptic formation and the establishment of neuronal networks should be compromised. It is intriguing to know whether a reduction in neural connectivity, caused by pathological challenges during early development, such as neonatal ischemic stroke and hypoxic-ischemic encephalopathy, can be the cellular abnormality that underlies the clinical behavior in motor control and cognitive deficiencies associated with these pathologies.⁴⁰ With the identification of an increasing number of AMPK downstream targets, and deeper understanding of the cellular function of the AMPK signaling network, we hope that novel implications and therapeutic strategies will be discovered for bioenergy-associated metabolic and developmental disorders.

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