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Actin'g against the Ball and Chain

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Spinocerebellar ataxia type 13 is a rare autosomal-dominant neurodegenerative disease induced by mutations in the voltage-dependent Kv3.3 potassium channel. Recently in *Cell*, Zhang et al. (2016) provide new insights into how Arp2/3-dependent actin polymerization modulates both Kv3.3 activity and its ability to stimulate actin polymerization via Hax-1.

Actin polymerization is essential for a multitude of fundamental cellular processes, from the regulation of endocytosis in yeast to the coordination of cell migration during development and throughout the lifetime of multicellular organisms. For the actin cytoskeleton to function effectively, it is vital that its polymerization is regulated in time and space. Failure to do so can have disastrous consequences for an organism, including the loss of cell polarity and tissue integrity to the promotion of tumor cell metastasis. In non-pathogenic situations, the tight control of actin polymerization is achieved through a variety of signaling networks that regulate the localization and activity of cellular factors, such as the Arp2/3 complex, which stimulates the formation of new actin filaments. The Arp2/3 complex consists of seven proteins and is conserved in virtually all eukaryotes (Abella et al., 2016; Campellone and Welch, 2010). It plays a central role in many different cellular processes by virtue of its ability to generate branched actin filament networks. In the vast majority of cases, the most upstream event in signaling networks regulating actin polymerization is the activation of a membrane-bound receptor or channel, and regulating their activity is at the heart of controlling actin polymerization. Given this, it is interesting that the activity of many ion channels is also regulated by components of the actin cytoskeleton or actin itself (Sasaki et al., 2014; Smani et al., 2014; Williams et al., 2007).

In their recent paper in *Cell*, Zhang et al. (2016) demonstrate how actin polymerization regulates the activity of the voltage-dependent Kv3.3 potassium channel that is mutated in Spinocerebellar ataxia type 13 (SCA13). SCA13 is a rare human autosomal-dominant neurodegenerative disease, which, depending on the mutation, results in early- or late-onset cerebellar atrophy (Zhang and Kaczmarek, 2015). The Kv3.3 channel is widely expressed in the nervous system and is particularly abundant in the auditory brainstem and cerebellum. As with other members of the Kv3 family, Kv3.3 functions as a delayed rectifier-type channel that contributes to action potential repolarization. However, during situations of prolonged membrane depolarization, Kv3.3 channels inactivate slowly over several hundred milliseconds by a "ball-and-chain" or N-type inactivation mechanism involving the association of its N terminus with the channel pore. The precise molecular details of this interaction remain to be established, but PKC-mediated phosphorylation of two serine residues in the N terminus of Kv3.3 or its complete removal abolish channel inactivation (Desai et al., 2008).

In contrast to other Kv3 family members, Kv3.3 also has an extensive cytoplasmic C-terminal domain. Moreover, mutations in this region of the molecule can lead to SCA13, raising the likelihood that additional regulatory mechanisms exist. Zhang et al. realized that the late-onset SCA13-inducing G592R mutation is embedded in a proline-rich sequence that is reminiscent of those found in WASP and WAVE family members, which are responsible for activating the Arp2/3 complex. When expressed in CHO cells, the authors found that Kv3.3 but not its G592R mutant interacts and co-localizes with the Arp2/3 complex at the plasma membrane. More importantly, the presence of Kv3.3 induces cell rounding and significantly enhances the association of the Arp2/3 complex and actin with the plasma membrane. Pharmacologically mediated inhibition of the Arp2/3 complex, actin polymerization, or the small GTPase Rac, which can activate WAVE, also suppressed the impact of Kv3.3 on cell morphology and redistribution of actin to the plasma membrane. Moreover, patch-clamping experiments demonstrate that loss of an Arp2/3-nucleated actin network increases the rate of Kv3.3 inactivation. Using a combination of approaches, Zhang et al. (2016) went on to demonstrate that actin exerts its effects on Kv3.3 by modulating the ability of its N terminus to inactivate the channel.

Based on their observations, the authors propose that the C-terminal region of Kv3.3 promotes Arp2/3-induced actin polymerization at the plasma membrane, which in turn reduces the rate of channel inactivation. It is therefore curious that the G592R mutant, which does not interact with the Arp2/3 complex, does not undergo accelerated deactivation. Indeed, its inactivation is significantly slower than the wild-type channel, even in the absence of actin polymerization in Latrunculin B-treated cells. It was also resistant to the accelerated inactivation induced by the inhibition of the Arp2/3 complex or Rac. The behavior of the G592R mutant, however, did resemble a channel that has lost its "ball-and-chain" inactivation. This suggests that channel inactivation is likely to involve interactions between the N- and C-terminal domains that are disrupted by the G592R mutation and/or modulated by the presence of an actin network nucleated by the Arp2/3 complex. The actin network will also undoubtedly influence clustering of the channel. Further analysis, including super-resolution imaging, will shed more

light on the organization of actin associated with Kv3.3 and its role in regulating the channel's activation state and clustering. What is already clear is that the G592R mutant is substantially less effective at inducing changes in cell morphology as well as the accumulation of Arp2/3 and actin at the plasma membrane.

To explore whether Kv3.3 is capable of stimulating actin polymerization in a more physiologically relevant situation, Zhang et al. (2016) generated cerebral organoids from induced pluripotent stem cells derived from a patient with the G592R mutation. Analysis of the growth cones from neurons harboring the G592R mutation revealed a striking difference in actin organization compared to matched controls. Actin-rich filopodia were still present, but the characteristic Arp2/3-dependent actin veil structures usually seen between these structures were largely absent and the normal morphology of the growth cone was disrupted. Another striking feature of the G592R mutation is the reduced amount of Kv3.3 present in the growth cone compared to the wild-type situation. This reduced targeting, which parallels observations in CHO cells, may reflect loss of stabilizing interactions with actin at the plasma membrane and/or defects in Arp2/3 and actin-dependent channel trafficking from the Golgi.

In their efforts to understand how Kv3.3 stimulates Arp2/3-dependent actin polymerization, the authors found that the C-terminal region interacts with Hax-1. Hax-1, which is overexpressed in many tumors, is an anti-apoptotic protein that promotes cell migration by interacting with cortactin and Rac, two Arp2/3 complex regulators (Gomathinayagam et al., 2014; Radhika et al., 2004). Loss of Hax-1 suppresses the ability of the Kv3.3 channel to promote a rounded cell morphology or induce accumulation of Arp2/3 and actin on the plasma membrane. Depletion of Hax-1 also increased the rate of channel inactivation. These phenotypes mirror those seen with the G592R mutant, although this mutation does not impact the association of Hax-1 with the channel in pull-downs from cell lysates. The most straightforward explanation is that there are additional interactions of the Kv3.3 channel with cortactin and/or Rac. Indeed, cortactin can interact with the wild-type channel but not the G592R variant. It is also recruited to the plasma membrane together with Rac and its downstream effector WAVE3 in cells expressing Kv3.3. Moreover, enrichment of WAVE3 on the plasma membrane is abolished in the absence of Hax-1. Although the precise molecular details of the interactions remain to be established, a simple working model would have the C terminus of Kv3.3 interacting directly with Hax-1, leading to Rac-mediated activation of WAVE to promote Arp2/3-driven actin polymerization. The presence of cortactin in the same Hax-1 complex would facilitate stabilization of the branched actin network formed by Arp2/3 and suppress the rate of channel activation. Such a model would explain why Rac inhibition results in loss of Arp2/3, actin accumulation on the membrane of Kv3.3-expressing cells, and the increased rate of channel inactivation. It also begs the question, what is actually activating Rac? Intriguingly, Hax-1 contains a binding motif for Rac and Cdc42 activating PIX family GEFs (Radhika et al., 2004). The observations of Zhang et al. (2016) now suggest new avenues of research into understanding how the actin cytoskeleton regulates potassium channels. It also suggests that Hax-1 may be playing a more important role in regulating Arp2/3-driven actin polymerization during cell migration and tumor metastasis than previously thought.

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