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SPOTLIGHT

Glial SIK3: A central player in ion and volume homeostasis in *Drosophila* peripheral nerves

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The electrical properties of neuronal cells rely on gradients of ions across their membranes and the extracellular fluid (ECF) in which they are bathed. Little is known regarding how the ECF volume and content is maintained. In this issue, Li et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201907138) identify the kinase SIK3 in glia as a key signal transduction regulator in ion and volume homeostasis in *Drosophila* peripheral nerves.

Evidence suggests that glial cells, long considered the support cells of neurons, contribute to extracellular fluid (ECF) regulation. In ECF, the regulation of extracellular K^+ ions, $[K^+]_o$, is thought to be particularly critical. On the downswing of the action potential, central and peripheral neurons release K^+ into the ECF. Due to the tortuously small extracellular space volume (ECV) and the relatively low concentration of K^+ ions outside the cell relative to inside, K^+ can accumulate, leading to neuronal depolarization and alterations in neuron action potential propagation. The extrusion of ions leads also to subsequent flow of water due to osmotic pressure. Central and peripheral glia express a compliment of transporters, pumps, water, and ion channels used to regulate ECV, water, and K^+ homeostasis. Emerging evidence suggests that *Drosophila melanogaster* may be an ideal model system to garner insight into how the ECF ion content and volume is maintained (1).

In this issue, Li et al. use the power of *Drosophila* genetics to investigate the regulation of ECF in larvae nerves (2). Using a GAL4/UAS system with a glial-specific GAL4 driver in 500 different RNAi UAS lines, these authors identify a single protein whose disruption by RNAi causes nerve swelling—the salt-inducible kinase 3 (SIK3; Fig. 1). By reinstating SIK3 expression in SIK3-deficient larvae, specifically in the wrapping glial of the peripheral nerve, the authors were able to completely rescue the phenotype. Reexpression in subperineurial glia partially rescued the nerve swelling phenotype. No rescue of the phenotype was observed when SIK3 was restored in neurons or perineurial glia. Using a complementary approach, specifically in wrapping glia, RNAi of SIK3 recapitulated the nerve swelling phenotype in the global knockout (KO). Intriguingly, despite the massive swelling observed, the axons themselves were grossly morphologically intact.

As K^+ homeostasis is a key component of the ECV, the authors next investigated the role of K^+ in the nerve-swelling phenomenon of the SIK3 mutant larvae. WT larva fed a high-K^+ diet demonstrated normal peripheral nerves. In contrast, the swelling phenotype was exacerbated in SIK3 mutant larvae fed a high K^+ diet. A high Na^+ diet had no effect on WT or glia SIK3-KO larvae. This dysregulation in K^+ homeostasis led to Na_v-dependent hyperexcitability in the axons of motor neurons innervating *Drosophilia* larval muscles, culminating in increased susceptibility to seizures by mechanical stimulation in the SIK3-mutated adult flies.

What is the pathway that leads from SIK3 dysfunction to nerve swelling? A primary target of SIK3 is histone deacetylase 4 (HDAC4). Li et al. demonstrate that when phosphorylated by SIK3, HDAC4 is localized in the cytoplasm (2). In the absence of SIK3, unphosphorylated HDAC4 enters the nucleus to regulate expression of genes that regulate ion and volume homeostasis. Supporting this, KO of HDAC4, specifically from glia, prevented the nerve swelling of the SIK3-mutated larvae. In contrast, overexpression of HDAC4 in glia exacerbated nerve swelling, an effect that was abolished when cooverexpressing SIK3. Finally, the authors were able to reverse the neuronal hyperexcitability and seizure susceptibility of the SIK3 mutant flies by treating them with HDAC4 inhibitor trichostatin A. Thus, HDAC4 is a key downstream signaling element in the SIK3 signal transduction cascade.

What genes are downstream of this novel SIK3-HDAC4 signal transduction cascade? Screening for various transcription factors, Li et al. identified that myocyte enhancer factor 2 (Mef2) RNAi in gliarecapitulated the nerve swelling phenotype (2). Continuing to chase the pathway, the authors demonstrate two critical Mef2-regulated genes long implicated in volume and $[K^+]_o$ regulation: *fray*, a protein kinase that activates *ncc69* (the fly orthologue for the human Na-K-Cl transporter NKCC1), and *drip*, the fly equivalent for the mammalian glial Aquaporin 4 (AQP4). *fray* has been previously implicated in the nerve swelling phenotype (3).

What are the roles of these genes in mammals? SIK3 is a constitutive kinase that is involved in skeletal development, glucose regulation, and sleep (4). Mechanistically, SIK3 phosphorylates synaptic regulatory proteins associated with sleep (4). Interestingly, specific SNPs in nonneuronal SIK3 are associated with hearing ability (5). HDAC4 is a histone deacetylase, which represses gene expression and is involved in many physiological and pathophysiological processes (6). Drugs that treat both bipolar disorder and epilepsy such as valproic acid

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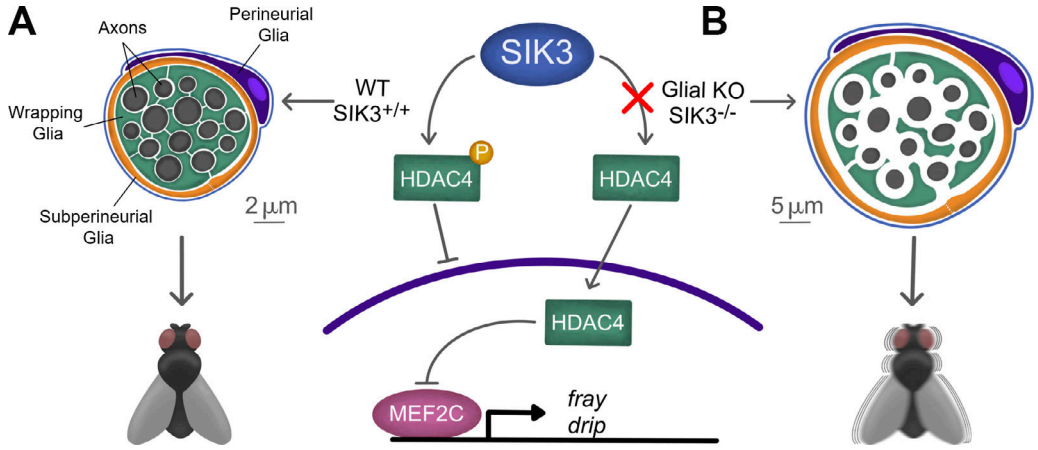

Figure 1. **SIK3, a central regulator of ion and volume homeostasis in the *Drosophila* peripheral nerve. (A)** WT levels of SIK3 are required to maintain basal cytoplasmic phosphorylated HDAC4, healthy peripheral nerves, and normal fly behavior. **(B)** Loss of SIK3, specifically in wrapping glia, leads to unphosphoryalted HDAC4, translocation of HDAC4 to the nucleus, relief of inhibition of MEF2-dependent transcription of *fray* (a protein kinase that activates *ncc69*, the fly orthologue for the human Na-K-Cl transporter NKCC1), and *drip* (fly orthologue of mammalian AQP4). Glial-specific KO of SIK3 also leads to suceptibility to mechanically induced seizures in the fly. Image of the *Drosophila* nerve is adapted from Altenhein et al. (13)

and carbamazepine also have inhibitory effects on HDACs (7, 8). HDAC phosphorylation by SIK3 in fly circadian neurons affected male sex drive rhythm (9). Remarkably, HDAC4 is involved in the physiology of Schwann cells—the enwrapping glia in the peripheral nervous system (10). Thus, the actuality of this pathway in enwrapping glia and the physiological role it asserts fits well with previous research.

fray has been previously implicated in axonal ensheathment in the *Drosophila* larva (3) and in regulation of ECV (11). Its deletion causes symptoms similar to the SIK3 mutation (3). AQP4, the mammalian homologue of *Drosophila drip*, is a well-known glial protein that is involved in ECV regulation and potassium regulation (12). The study by Li et al. shows transcriptional regulation of these proteins by the SIK3-HDAC4 pathway and the importance of this regulation on ECV, peripheral nerve integrity, and seizures (2). This exemplifies the strength of the *Drosophila* model: the ability to screen hundreds of genes and discover pathways that affect physiology. These findings raise additional questions regarding this pathway. Does this pathway occur in mammals and does it persist in the adult? Are other transcription factors besides Mef2 affected by this pathway? What other genes are downregulated by SIK3-HDAC4–dependent Mef2 repression? What are the upstream factors that regulate SIK3? Can $[K^+]_o$ alterations be measured on control and SIK3 KO peripheral nerves? Given the critical role of K^+ ions and volume homeostasis in maintenance of a healthy central and peripheral nervous system, answers to these questions may identify druggable targets to treat various pathophysiologies.

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