Unraveling the ribbon synapse

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A new study identifies pikachurin, a previously-unknown dystroglycan-binding protein that is critical for the apposition of photoreceptor and bipolar cell dendrites at the ribbon synapse. This work could explain some of the visual defects seen in several muscular dystrophies.

Synaptogenesis requires the precise targeting of axons, the apposition of presynaptic and postsynaptic termini, as well as the proper differentiation and maturation of synaptic termini. In retinal photoreceptor ribbon synapses, bipolar cells and horizontal cells contact rod or cone photoreceptors through invaginations of the photoreceptor termini. Although the mechanisms that orchestrate the development of the photoreceptor ribbon synapse have not been precisely defined, defects in photoreceptor and bipolar cell coupling have been implicated in the abnormal retinal physiology seen in Duchenne and Becker muscular dystrophies (DMD and BMD, respectively). In this issue of *Nature Neuroscience*, Sato *et al.*[1] identify pikachurin, a previously unknown dystroglycan-binding protein, which is critical for the apposition of bipolar cell dendrites to the presynaptic termini of photoreceptor ribbon synapses. This study highlights the importance of the extracellular matrix to synaptic development and may also help to explain the molecular underpinnings of the visual defects that are present in several muscular dystrophies.

Muscular dystrophies are a heterogeneous group of diseases that cause skeletal muscle weakness and wasting. Several muscular dystrophies are associated with mutations that affect the function of the dystrophin-glycoprotein complex (DGC). The core components of the DGC in skeletal muscle include dystrophin, dystroglycan, the sarcoglycan/sarcospan complex, syntrophin and dystrobrevin. In skeletal muscle, the interactions of dystroglycan with the extracellular matrix and the intracellular cytoskeleton are critical for the formation of a transmembrane link that confers structural integrity to skeletal muscle cells. Along with the skeletal muscle defects, several mutations that affect the expression or post-translational processing of DGC components are also associated with brain and eye defects. DMD and BMD, the most prevalent types of muscular dystrophy, are commonly associated with learning impairments and abnormal electroretinograms, although visual function is preserved. In contrast, brain and eye malformations (glaucoma, retinal dysgenesis and detachment) are prominent in muscle-eye-brain disease (MEB), a severe form of congenital muscular dystrophy.

Many components of the DGC are broadly expressed in the CNS and may have important roles at the synapse. In the brain, dystroglycan and dystrophin are expressed at a subset of GABAergic synapses[2], and long-term potentiation, a proposed mechanism by which information is stored at synapses, is blunted in mice with a brain-specific deletion of dystroglycan[3]. The expression of dystroglycan and dystrophin coincides with the first expression of synaptic markers in the outer plexiform layer of the retina[4], although its synaptic function is not known. The study by Sato *et al.*[1] now suggests that dystroglycan is important in the development of the ribbon synapses because of its ability to act as a receptor for pikachurin, an extracellular matrix–like protein.

Sato *et al.*[1] identified pikachurin by comparing the retinal gene-expression profiles of mice with a photoreceptor-specific deletion of *Otx2* with that of wild-type mice. Otx2 is a homeodomain transcription factor that is important for the cell-fate determination of both rod and cone photoreceptors[5]. *Otx2* conditional null mice are characterized by a total loss of retinal photoreceptors and an increase in the number of amacrine-like cells[5]. Pikachurin transcript was undetectable in the *Otx2* conditional null retina, suggesting that the loss of pikachurin expression may contribute to the phenotype of the *Otx2* conditional null mice. By monitoring pikachurin expression, the authors found that pikachurin was expressed in prospective photoreceptors of the neuroblast layer as early as embryonic day 14.5, during the peak of cone genesis and the beginning of rod genesis. Furthermore, the authors observed that the subcellular localization of pikachurin in the adult retina was restricted to the synaptic cleft of the ribbon synapse, near the postsynaptic termini of bipolar cells.

To characterize the effect of pikachurin function on retinal development, Sato *et al.*[1] generated *pikachurin*$^{-/-}$ mice by the targeted disruption of the *pikachurin* gene. Notably, bipolar cell dendrites in ribbon synapses of both rod and cone photoreceptors were completely absent in these mice. In contrast, the numbers of horizontal cell dendrites in the *pikachurin*$^{-/-}$ ribbon synapses were normal. Additionally, the tips of ON bipolar cells were positive for the postsynaptic marker mGluR6, even though they failed to form appropriate contacts with the photoreceptor termini. The authors examined the effect of pikachurin function on retinal physiology by recording electroretinogram responses under both scotopic (dark-adapted) and photopic (light-adapted) conditions, which elicit responses from the rods and cones, respectively. In *pikachurin*$^{-/-}$ mice, the amplitude of the b-wave, generated in part by the activity of ON bipolar cells, was attenuated under both scotopic and photopic conditions. Together, these results indicate that the ultrastructural impairment of the *pikachurin*$^{-/-}$ ribbon synapses has a marked physiological importance, affecting both the timing and the amplitude of the bipolar cell response to the electrical activity of rod or cone photoreceptors.

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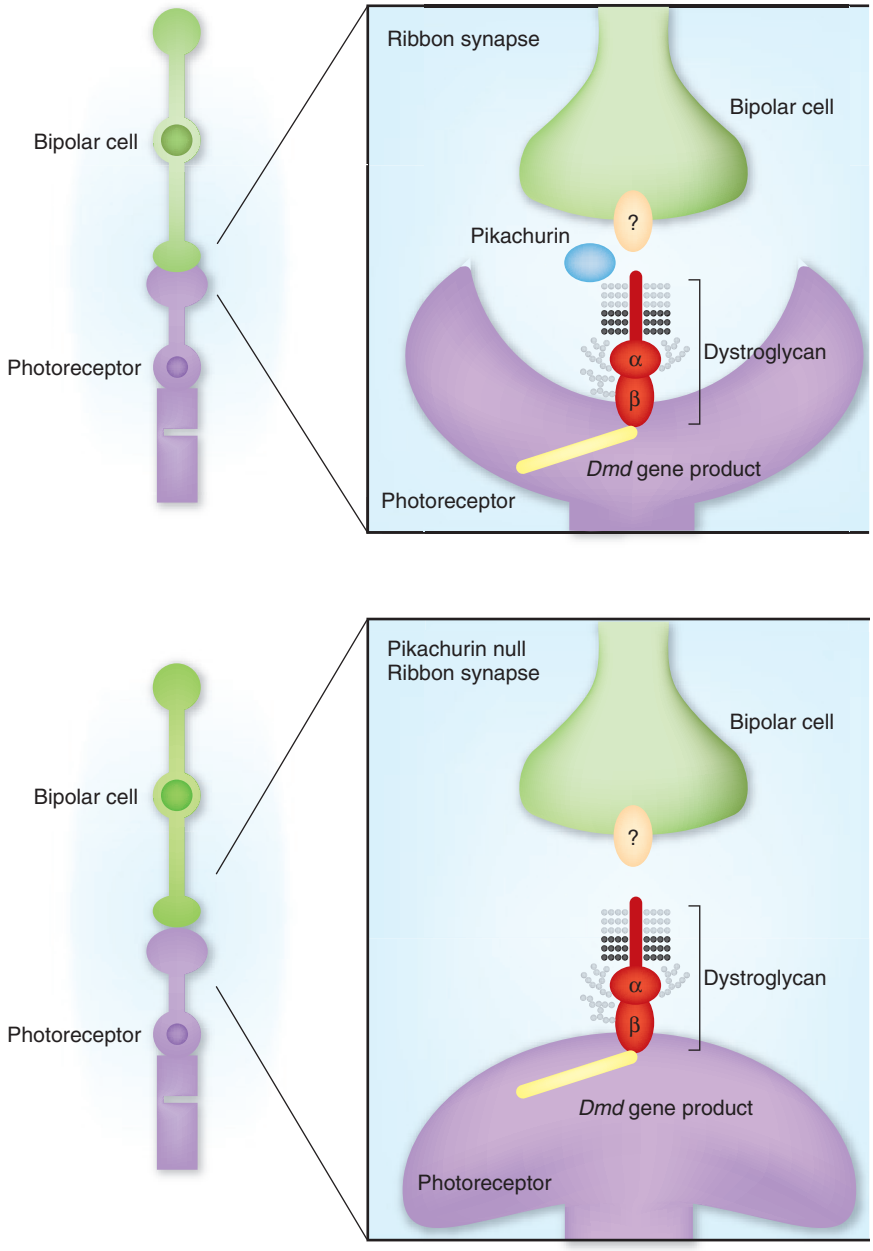

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Figure 1 Pikachurin is necessary for the apposition of photoreceptor and bipolar cell termini. (**a**) Bipolar cell processes contact photoreceptors through invaginations of the photoreceptor termini. Pikachurin binds to α-dystroglycan in the synaptic cleft. Sato *et al.*[1] propose that pikachurin mediates the apposition of the presynaptic and postsynaptic termini by inducing a structural change in the photoreceptor terminus or by attracting the postsynaptic terminus through an unknown factor. (**b**) In *pikachurin*$^{-/-}$ mice, bipolar cell processes fail to extend into the photoreceptor ribbon synapses. The alteration of synapse ultrastructure impairs synaptic physiology. ?, unknown factor.

Interestingly, an attenuation of the electroretinogram b-wave similar to that described by Sato *et al.*[1] is also present in patients with DMD and BMD[6], which are caused by mutations in dystrophin. It has been proposed that an uncoupling of the bipolar cells and the photoreceptors may be responsible for the disturbance of retinal physiology in DMD[7]. Furthermore, the structure of pikachurin suggested that it may interact directly with dystroglycan, which anchors dystrophin to the plasma membrane. The α-subunit of dystroglycan (α-dystroglycan) binds to laminin G modules of laminin-1 and laminin-2 and to the laminin G–like domains of perlecan, agrin and neurexin[8,9]. Pikachurin contains similar laminin G–like domains and Sato *et al.*[1] confirmed that pikachurin colocalized and biochemically interacted with α-dystroglycan. As in the case of the interaction of α-dystroglycan with agrin or perlecan, the interaction of pikachurin and α-dystroglycan was dependent on the availability of a glycoepitope recognized by the IIH6 antibody.

In humans and mice, the post-translational addition of glycans to α-dystroglycan is essential for α-dystroglycan ligand binding. For example, MEB, which is caused by mutations of the POMGnT1, one of the glycosyltransferases involved in the synthesis of functional α-dystorglycan glycosylation, is associated with a loss of the IIH6 glycoepitope on α-dystroglycan and a loss of dystroglycan/laminin binding in skeletal muscle[10]. Although the precise molecular mechanism is unclear, the abnormal electroretinograms observed in MEB patients[11] and mouse models of the disease[12] suggest that a disruption of dystroglycan-ligand interactions may also underlie the visual impairment in MEB. Mutations in the genes encoding other putative glycosyltransferases (that is, *Large*[13] and *Fukutin*[14]) in mice are also associated with hypoglycosylation of dystroglycan and abnormal electroretinograms, supporting a role for dystroglycan-ligand interactions in the physiology of the retina. Further analyses of the cellular function of pikachurin may provide important clues as to the mechanism of MEB and the rapidly expanding group of congenital muscular dystrophies that are associated with defects in the post-translational processing of dystroglycan.

Sato *et al.*[1] suggest that pikachurin may control the apposition of photoreceptor and bipolar cell dendrites, either as an attractant or as a structural component, during the development of the photoreceptor ribbon synapse (**Fig. 1**). In the first scenario, pikachurin might attract the bipolar cell dendrites through an unknown factor on the bipolar cell postsynaptic termini. Alternatively, binding of pikachurin may cause a structural change in the photoreceptor presynaptic terminus that is necessary for the proper apposition of the bipolar cell dendrites. Such a structural change may involve a rearrangement of the intracellular cytoskeleton or the extracellular matrix. In support of this hypothesis, the extracellular matrix has previously been shown to make important contributions to synapse ultrastructure. Notably, mutating laminin β2 causes ultrastructural and physiological defects[15] that closely resemble those described by Sato *et al.*[1].

The identification and characterization of pikachurin by Sato *et al.*[1] provides new insight into the development of the photoreceptor ribbon synapse and the synaptic function of dystroglycan. This work, however, raises several new questions. Although it is clear that

pikachurin is critical for the normal development of the ribbon synapse, the challenge now is to determine the precise mechanism by which pikachurin orchestrates the apposition of photoreceptor and bipolar cell termini. It also remains to be determined whether pikachurin/dystroglycan binding is necessary for pikachurin function and whether pikachurin function is affected in human muscular dystrophies. Biochemical and ultrastructural analyses of patient tissue or more readily available tissue from mouse models of muscular dystrophy are necessary to address some of these questions. With its intriguing parallels to the deficits seen in muscular dystrophies, pikachurin provides a useful tool for probing the defects associated with mutations of dystrophin or abnormal dystroglycan processing.

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Fresh air is good for nerves: hypoxia disturbs axon guidance

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The transcription factor hypoxia-inducible factor 1 (HIF-1) triggers multiple cellular responses to cope with hypoxia. A study in this issue suggests that elevated HIF-1 also causes axon guidance defects under hypoxic conditions.

Oxygen is critical for the function and survival of all eukaryotic cells; consequently, cells have developed various protective mechanisms for coping with hypoxia. Neurons are the cells most sensitive to hypoxic insults and hypoxia causes a number of human neurological diseases, such as perinatal brain injury, ischemic stroke and hypoxic encephalopathy as a result of circulatory collapse. Conserved HIF-1, a transcription factor of the basic helix-loop-helix family, protects cells from hypoxia by regulating a wide variety of target genes to increase anaerobic metabolism or enhance vascularization of hypoxic tissues[1]. Previous studies had shown that HIF-1 is required for neuronal survival in hypoxic conditions, and HIF-1–deficient mice show severe nervous system anomalies[2]. In this issue, however, Pocock and Hobert[3] add a surprising twist to the story by showing that increased HIF-1 levels are responsible for hypoxia-related axon defects in the nematode *C. elegans*.

Under physiological normoxic conditions, the oxygen-dependent prolyl 4-hydroxylase EGL-9 hydroxylates a specific proline residue of HIF-1. The modified proline is recognized by the E3 ubiquitin ligase von Hippel-Lindau protein 1 (VHL-1), and the ubiquitinated HIF-1 is then targeted for proteasomal degradation[1]. Hypoxia inhibits the initial hydroxylation of HIF-1, allowing it to accumulate, which in turn activates various anti-hypoxic molecular pathways.

Pocock and Hobert[3], however, found that hypoxia caused cell migration and axon-guidance defects in specific types of neurons of *C. elegans*. The authors focused on two classes of neurons: the HSN motor neurons and the PVQ interneurons. When reproductive young *C. elegans* hermaphrodites were subjected to hypoxia, substantial defects in HSN neuronal migration and PVQ axon guidance, which are normally completed during embryogenesis before the animals hatch, were found in the progeny that they produced. Moreover, extension of the HSN axons, which occurs post-embryonically, was also affected. These defects were completely suppressed by the loss of HIF-1, suggesting that HIF-1–regulated genes are responsible for the defects induced by hypoxia. This conclusion was supported by two additional observations. First, loss-of-function mutations in the genes *egl-9* and *vhl-1* caused cell migration and axon guidance defects that were similar to those induced by hypoxia, and these defects were also suppressed by a *hif-1* mutation. Second, expression of a stable form of HIF-1 that cannot be hydroxylated by EGL-9 also caused similar axon defects. Expression of this stable HIF-1 in midline motor neurons, which serve as guideposts for the axons of the HSN neurons, induced the HSN axon defects, but expression of HIF-1 in the HSN neurons themselves did not. These results indicate that increased levels of HIF-1 induce axon defects in a non–cell-autonomous fashion.

What are the intracellular signals that stabilize HIF-1 in hypoxic cells? In vertebrates, reactive oxygen species (ROS) produced by mitochondria stabilize HIF-1 under hypoxic conditions[4]. Mutations in several *C. elegans* superoxide dismutases and catalases that remove ROS also induced axon defects that looked similar to those caused by hypoxia. These defects were also suppressed by the loss of HIF-1. Insulin signaling is known to increase ROS levels. Thus, consistent with the hypothesis that ROS can mediate the effects of hypoxia on axon guidance, the loss of DAF-2, the *C. elegans* insulin/IGF receptor, suppressed hypoxia-induced axon defects. Loss of DAF-2 failed to suppress the axon defects caused by expression of stable HIF-1, indicating that *daf-2* acts upstream of *hif-1*.

What are the downstream targets of HIF-1 that affect axon guidance in hypoxia? Answers to this question may provide insights to the mechanisms of axon guidance in hypoxic situations and might also shed light on the design of potential treatments for human neurological disorders caused by hypoxia. To address this question, Pocock and Hobert[3] took advantage of work done by other labs using microarray analysis to identify potential HIF-1 targets. One HIF-1 target is *vab-1*, which encodes the sole *C. elegans* Eph receptor tyrosine kinase[5]. Eph receptors and their ephrin ligands regulate axon guidance in metozoans[6].

Pocock and Hobert[3] confirmed that *vab-1* transcription was increased under hypoxic conditions and in *vhl-1* mutant animals.

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