smaller neurite arbors than wild-type neurons. The density of sympathetic innervation of the iris *in vivo* was reduced in CaSR-deficient mice, demonstrating the importance of this signaling for normal development. Notably, these perturbations did not lead to a reduction in neuronal survival, which could have accounted for the results.

The effects of the CaSR on neurite extension and branching are not restricted to the peripheral nervous system. Perinatal mouse hippocampal pyramidal neurons, which also express the CaSR, had shorter and less complex dendrites when transfected with the dominant-negative CaSR in organotypic cultures. These findings suggest that the CaSR may also be involved in the normal development of neurons in the brain.

These results arise from the coincidence of substantial CaSR expression and fetal elevation of extracellular calcium levels that occurs during development. The fetus has relatively high levels of free ionized extracellular calcium, both in the blood and in the cerebrospinal and brain interstitial fluid. This results in constitutive activation of the CaSR in both the peripheral and central nervous system. The CaSR regulates sympathetic neuron axon outgrowth during a limited developmental window when it is expressed at high levels, a system that may have evolved to take advantage of fetal elevation of extracellular calcium.

CaSR signaling may be modulated in several ways. The CaSR belongs to family C of the G protein–coupled receptors, along with metabotropic GABA receptors ($GABA_BR$), several metabotropic glutamate receptors (mGluR) and a group of taste receptors. It associates and coprecipitates with mGluR and with $GABA_BR$[4,5]. This dimerization raises the possibility that CaSR homodimers and CaSR:mGluR and CaSR:$GABA_BR$ heterodimers have different functions. On the other hand, heterodimerization may promote specific subcellular localization of CaSR without causing a change in receptor function. Inactivating mutations of the CaSR cause hypercalcemia, whereas activating mutations lead to hypocalcemia. However, the range of symptoms of these disorders has made it difficult to discern the primary effects on the nervous system. Conditional knockouts in specific tissues will allow investigation of neurological consequences of mutations in CaSR in neurons.

These findings provide the impetus to address several interesting questions. How is CaSR activation coupled to changes in neuronal morphology? It will be important to determine the mechanisms linking CaSR activation to the extension of axons and dendrites. The CaSR is a G protein–coupled receptor, and its stimulation leads to the activation of phospholipase C and the inhibition of adenylate cyclase. Identification of downstream targets of CaSR signaling is now a priority. What other functions does the CaSR have in the developing nervous system? It seems improbable that its regulatory role is restricted to neurite outgrowth. How many calcium sensors are there? Several mGluRs act as calcium sensors, megalin is a calcium sensor that is a member of the low-density lipoprotein superfamily, and genetic lines of evidence suggest the existence of still other calcium sensors[6]. The functions of these CaSRs remain to be determined.

The most provocative question concerns the function of the CaSR in the adult nervous system. The effect of external calcium on neurite outgrowth from sympathetic neurons described in Vizard *et al.*[2] is restricted to a brief period during development. However, the CaSR is also expressed in the adult brain, albeit at lower levels[6], in the basal ganglia, cerebellum and hippocampus, among other structures. Determination of its subcellular localization should provide clues as to its function.

Vizard *et al.*[2] suggest that the CaSR may function in the adult brain by modifying the architecture of dendrites in response to changes in synaptic activity. Calcium mobilization in cultured human embryonic kidney cells produces an extracellular signal that can be detected in nearby cells expressing the CaSR, suggesting that there is a form of intercellular communication that allows cells to determine the calcium signaling status of their neighbors[7]. These results suggest a scenario in which the concentration of extracellular calcium is increased locally at active synapses as a result of extrusion from presynaptic terminals by pumps and exchangers. Elevated extracellular calcium then stimulates the growth of presynaptic and postsynaptic processes, bringing them in closer apposition and contributing to increased synaptic strength. Alternatively, a reduction in extracellular calcium concentration following synaptic activity[8] could reduce neurite extension and weaken nearby synapses.

This study demonstrates that calcium has an expanded repertoire of signaling roles in neuronal development, acting extracellularly as well as intracellularly, now as a first, as well as a second, messenger. These findings are an important step toward revision of the traditional view of calcium signaling only intracellularly in the nervous system.

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β-catenin in reverse action

Amy K Y Fu, Zelda H Cheung & Nancy Y Ip

Motor neurons and muscle fibers interact in complex ways to build the neuromuscular synapse. A new study shows that β-catenin is required in muscle to provide an unknown retrograde signal that is necessary for presynaptic transmitter release.

Precise apposition of pre- and postsynaptic terminals is essential for the development of synapses. At the neuromuscular junction (NMJ), for example, reciprocal signaling between the motor nerve and the target muscle is required for the differentiation of presynaptic motor neuron terminals and postsynaptic muscle membrane[1]. Although factors from motor terminals regulate clustering of postsynaptic proteins and acetylcholine receptors (AChRs) on muscle membrane, muscle-derived signals act retrogradely to control the differentiation of presynaptic terminals on motor nerve. The identity of these retrograde factors remains largely enigmatic. Several candidates have been proposed[2], but their *in vivo* role in NMJ formation remains unclear. In this issue, through the generation of β-catenin conditional-knockout mice, Li *et al.*[3] elegantly demonstrate that muscle β-catenin is involved in NMJ formation.

The authors are at the Department of Biochemistry, Biotechnology Research Institute and Molecular Neuroscience Center, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China.
e-mail: boip@ust.hk

In particular, it seems to control a retrograde signal that is required for correct presynaptic differentiation and NMJ development (**Fig. 1**).

β-catenin is a cytosolic protein that has been implicated in many cellular functions, including cell adhesion and cell mobility. The adhesion of cells depends on a homophilic interaction between cadherins, a family of cell adhesion proteins. β-catenin acts as a bridge that links cadherins to the actin cytoskeleton and is critical for stable adhesion of cadherins. The cadherin-catenin complexes are localized to both pre- and postsynaptic compartments of CNS synapses[4], and β-catenin is important for presynaptic assembly at CNS synapses[5,6]. *In vitro* studies have shown that β-catenin is also involved in neurotransmitter receptor clustering[7]. However, no role for β-catenin in NMJ development has been demonstrated. In addition, it remains unknown whether β-catenin acts in a presynaptic or postsynaptic manner, or in both, in synapse development. Constitutive β-catenin knockout mice could not provide answers to these questions, as these mice are embryonic lethal, and β-catenin is missing in both pre- and postsynaptic compartments.

To dissect the roles of β-catenin in NMJ formation, Li *et al.*[3] used the Cre/loxP system to selectively ablate β-catenin in either motoneurons or skeletal muscle. β-catenin–loxP–flanked mice were crossed with two mouse lines that expressed Cre recombinase under the control of HB9 (HB9-Cre) or human skeletal α-actin (HSA-Cre). HB9 is a motoneuron-specific transcription factor, and its promoter allows selective Cre ablation of β-catenin in developing motoneurons. On the other hand, HSA-Cre mice show Cre expression in somites throughout development, starting from E9.5, thus enabling the selective ablation of β-catenin in developing muscle. Surprisingly, the HB9–β-catenin conditional-knockout mice are viable, with no obvious behavioral defects and grossly normal NMJ morphology and function. In contrast, the HSA–β-catenin conditional-knockout mice die soon after birth and show defects in the initial formation of AChR clusters and motoneuron branching. These findings suggest that muscle β-catenin, instead of motoneuron β-catenin, is pivotal for the assembly of the pre- and postsynaptic apparatus at the NMJ.

Detailed analysis of the synaptic morphology of HSA–β-catenin conditional-knockout mice revealed that, although the band of AChR clusters remained centralized in the diaphragm, the band became wider, with increased size of individual AChR clusters. Defects in band width and cluster size were observed as early as E14.5, a time when AChR clusters are initially formed. These observations are consistent with an earlier report by the same group on the ability of β-catenin to regulate AChR clustering in myotubes *in vitro*[7]. Presynaptically, the primary phrenic nerves were mislocated from the central region of the diaphragm in the HSA–β-catenin conditional-knockout mice, moving to the tendon region of the diaphragm. In addition, the secondary intramuscular nerves became fewer and longer. These defects suggest that β-catenin is necessary for a retrograde signal from muscle to remodel presynaptic axons, possibly by restricting the outgrowth of axons, and thereby affecting presynaptic differentiation.

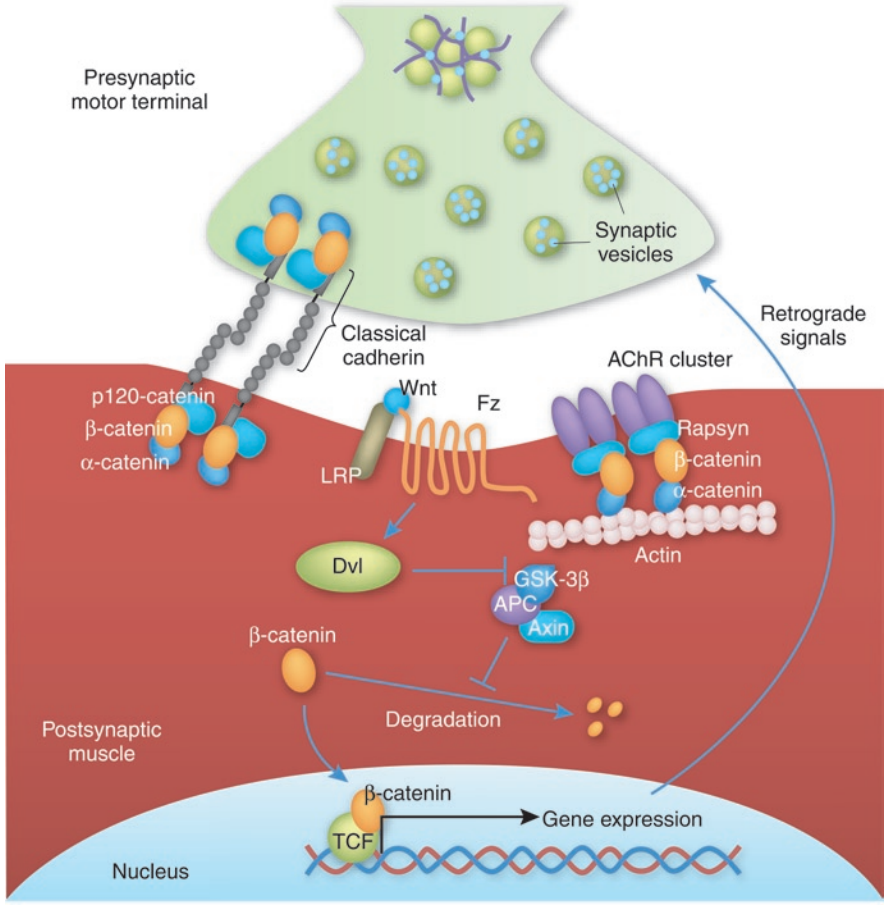

Figure 1 β-catenin in muscle regulates pre- and postsynaptic differentiation of developing NMJs. Muscle β-catenin seems to be necessary for the differentiation and function of motor neuron terminals. This is probably mediated by retrograde signals generated by β-catenin–dependent gene transcription. In addition, β-catenin affects the distribution and size of AChRs at the postsynaptic muscle membrane. Although this effect is independent of β-catenin's interaction with cadherin, it is possible that β-catenin acts as a bridge between AChRs and β-catenin–associated cytoskeleton to regulate AChR clustering.

Despite the presynaptic defect in HSA–β-catenin conditional-knockout mice, every AChR cluster remained innervated by a presynaptic terminal and responded to cholinergic stimulation, suggesting that the NMJs are functional. Nonetheless, neurotransmission was impaired at the NMJs of the HSA–β-catenin mice. Furthermore, basal and depolarization-induced neurotransmitter release was inhibited in these mice, raising the intriguing possibility that muscle β-catenin is essential for spontaneous and nerve-evoked neurotransmitter release and that the impaired neurotransmission is a result of abnormal presynaptic differentiation. Collectively, these findings provide strong evidence that β-catenin controls a target-derived cue in muscle that contributes to presynaptic organization.

The mechanisms by which muscle β-catenin regulates presynaptic differentiation remain largely unknown. Interaction between catenin and cadherin at CNS synapses seems to enhance the adhesion of cadherins across synapses, resulting in modification of synaptic structure and efficacy[5,6]. However, as ablation of β-catenin in motoneurons has no obvious effects on NMJ morphology[3], it is unlikely that β-catenin–cadherin–mediated interactions at pre- and postsynaptic terminals regulate NMJ differentiation. On the other hand, β-catenin is also a transcriptional coactivator for gene regulation through activation of the canonical Wnt signaling pathway[8]. β-catenin forms a multicomponent complex with various

signaling components of the Wnt pathway, including dishevelled (Dvl) and adenomatous polyposis coli (APC). β-catenin is degraded by the proteasome pathway in the absence of Wnt signals, and activation of Wnt signaling inhibits the β-catenin destruction complex. This leads to stabilization of cytosolic β-catenin and promotes translocation of β-catenin to the nucleus to initiate target gene transcription through its interaction with transcription factor T cell factor/LEF1. Intriguingly, some of the signaling proteins of the canonical Wnt pathway, including Dvl and APC, regulate AChR clustering in muscle *in vitro*[9,10]. These observations, together with those reported in this paper[3], suggest that Dvl, APC and β-catenin may have noncanonical functions in mammalian NMJ formation. Whether mammalian NMJ formation is regulated by Wnt signaling, as at the *Drosophila* glutamatergic NMJ[11], awaits further study.

In light of the ability of muscle β-catenin to affect presynaptic differentiation and its function as a transcriptional coactivator, it is tempting to speculate that β-catenin may induce expression of target-derived retrograde factors in muscle. Candidate retrograde signals at NMJ include trophic factors, protease inhibitors and adhesion molecules acting individually or in combination[12]. β-laminin, collagen chains and fibroblast growth factors act as presynaptic organizers in muscle[2]. However, our current understanding of these retrograde signals is largely derived from *in vitro* studies. Whether these candidate proteins act *in vivo* and whether they are regulated by β-catenin requires further examination. On the other hand, there is a close relationship between AChR dispersal and axon retraction. The potential ability of β-catenin to anchor AChRs on postsynaptic muscle membrane[7] raises the possibility that the abnormal postsynaptic activity in the muscle of HSA–β-catenin mutant mice may contribute to the presynaptic defects observed. Consistent with this notion, regulation of AChR clustering by various signals in muscle might control the projection of motor nerves[13,14]. It will thus be interesting to examine whether the β-catenin–dependent regulation of AChR cluster size and density in muscle contributes to presynaptic differentiation at the NMJ.

In conclusion, the significance of the current study is several-fold. Despite the many proteins that have been implicated in the regulation of synapse development, delineating their precise sites of action has been difficult because of the reciprocal influence of the nerve terminal and the postsynaptic regions on their respective differentiation. Furthermore, numerous proteins act both pre- and postsynaptically, with the mechanisms that govern the expression of these signals remaining largely unknown. The elegant new study by Li *et al.*[3] not only demonstrates that muscle β-catenin, and not motoneuron β-catenin, is required for NMJ development, but also reveals that muscle β-catenin directs a retrograde signal that is required for presynaptic differentiation. In addition, the use of transgenic animals has established the importance of muscle β-catenin in NMJ formation *in vivo*. These findings also underscore the emerging role of Wnt signaling proteins in the regulation of synapse development. Furthermore, given that some key proteins that are crucial for CNS synaptic assembly are also localized at the NMJ[15], the findings by Li *et al.*[3] provide the ground work for subsequent characterization of the mechanisms by which β-catenin regulates CNS synapses. The identification of muscle β-catenin–dependent signals for motoneurons may also contribute to our understanding of neuromuscular disorders, including muscular dystrophy and amyotrophic lateral sclerosis. Some of the major tasks that remain include understanding how β-catenin cooperates with other retrograde signals in muscle to regulate NMJ development and identifying these mechanisms that underlie the actions of these retrograde signals.

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A commanding control of behavior

Vatsala Thirumalai & Hollis T Cline

Descending projection neurons to the spinal cord carry important information for movement initiation and control. A new study shows that relatively few projection neurons may be needed to generate certain visually guided movements.

Ever get the feeling that you must move quickly just to keep up with this fast-paced world? If it is any consolation, you are not alone. When challenged with a constantly moving visual scene, zebrafish try to keep up by swimming in the direction of the perceived motion. An article in the current issue examines how this behavior may be encoded in the brain[1].

Zebrafish respond to drifting gratings by turning their body toward and swimming in the direction of perceived motion. Such a behavioral response to large-field visual motion, called the optomotor response, presumably keeps the fish in position in a moving water column[2]. In the new study, Orger *et al.*[1] used this behavior to study the pattern of activation of spinally projecting neurons when fish swim straight ahead or when they turn to the left or to the right.

To consistently generate either forward swimming or lateral turns, the authors coupled the direction of grating drift to the movements of the fish, so that the fish always perceived the same stimulus. Thus, if a grating drifting from left to right caused the fish to turn right by 90°, then the grating was shifted by 90° so that it drifted from left to right relative to the fish once again. This approach enabled the authors to reliably evoke forward swimming and turns in response to gratings drifting in 24 different directions. Swimming direction was tuned to the direction of perceived visual motion (except when the gratings drifted from head to tail, in which case left and right turns were equally probable). Because the direction of swimming is robustly tuned to the direction of perceived visual motion, Orger *et al.*[1] were then able to test which spinal projection neurons were active during directional swimming in immobilized fish. They used calcium imaging of spinal projection neurons while presenting gratings drifting in different directions. The underlying assumption was that, in freely moving fish, active spinal projection neurons would encode the directional swimming response.

The authors are at the Cold Spring Harbor Laboratory, Beckman Building, One Bungtown Rd., Cold Spring Harbor, New York 11724, USA.
e-mail: cline@cshl.edu