Development of the monosynaptic stretch reflex circuit
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Significant advances have been made during the past few years in our understanding of how the spinal monosynaptic reflex develops. Transcription factors in the Neurogenin, Runx, ETS, and LIM families control sequential steps of the specification of various subtypes of dorsal root ganglia sensory neurons. The initiation of muscle spindle differentiation requires neuregulin 1, derived from Ia afferent sensory neurons, and signaling through ErbB receptors in intramuscular muscle fibers. Several retrograde signals from the periphery are important for the establishment of late connectivity in the reflex circuit. Finally, neurotrophin 3 released from muscle spindles regulates the strength of sensory-motor connections within the spinal cord postnatally.

Introduction
The monosynaptic stretch reflex circuit is a unique model system for studying the development of a neuronal circuit. Synaptic connections in this circuit are highly precise, yet all of its components are easily accessible for anatomical, functional and genetic investigation. The reflex circuit comprises two distinct functional units: a sensory unit and an effector unit. The sensory system relays information about the length of a muscle to the CNS. It is composed of relatively few, stretch-sensitive, muscle-embedded mechanoreceptors, known as ‘muscle spindles’, and specific subpopulations of proprioceptive neurons (Ia afferents) that innervate the muscle spindles peripherally and make excitatory monosynaptic connections to α-motoneurons in the spinal cord. By contrast, the effector system controls muscle contraction. Its activity is regulated by inputs from the peripheral sensory system, the spinal cord and higher brain centers. It consists of α-motoneurons, each of which projects to a specific muscle and innervates many extrafusal muscle fibers at neuromuscular junctions.

The precise coordination of sensory and effector systems controls the contraction or relaxation of a given muscle: when a muscle is stretched, the activation of Ia afferents at muscle spindles specifically excites the motoneurons projecting to the same or related (homonymous or synergistic) muscles, thereby increasing the tension in the muscle and counteracting the initial stretch. In contrast, motoneurons innervating antagonistic or functionally unrelated muscles receive little or no excitatory input from these Ia afferents. Thus, although the elements of the spinal monosynaptic reflex are interconnected in perhaps one of the simplest and most accessible neuronal circuits known, the selectivity of connections in this circuit makes it an excellent system with which to study the principles underlying the formation of specific synaptic connections.

Recently, significant advances have been made in our understanding of the molecular events that underlie the differentiation of all elements of this circuit. In this review, we focus on those studies and the major steps in this process are shown schematically in Figure 1. References to earlier work can be found in a recent review [1].

Molecular specification of Ia afferents during development
Ia afferents comprise only a minor fraction of all neurons in the dorsal root ganglion (DRG). A key issue is the nature of the molecular events that control their specification and thus their distinction from other subpopulations of DRG neurons. Although no genes expressed exclusively by Ia afferents have been identified so far, progress has been made in elucidating the early events that control the generation and survival of the proprioceptive neuronal lineage, which includes Ia afferents (Figure 1a).

Proprioceptive afferents express the receptor tyrosine kinase TrkC. Both TrkC and its ligand, neurotrophin 3 (NT3), are required for the survival of proprioceptive sensory neurons [2,3]. In contrast, nerve growth factor is required for the survival of TrkA-expressing cutaneous neurons. NT3 is expressed by mesenchyme surrounding
the DRG, by motoneurons and by developing embryonic muscles. Much evidence suggests that only muscle-derived NT3 is essential for the survival of Ia afferents. The injection of antibodies against NT3 into peripheral tissues causes a decrease in the number of proprioceptive neurons [4], and supplementation of exogenous NT3 rescues the number of Ia afferents in limb-ablated chick embryos [5]. In addition, in transgenic mice overexpressing NT3 under the control of a muscle-specific promoter, the number of proprioceptive afferents and muscle spindles increases, and a selective rescue of proprioceptive neurons is achieved in mice lacking endogenous NT3 [6,7]. These findings suggest that the normal amounts of NT3 made by muscle are insufficient to rescue all of the Ia afferents generated.

Several classes of transcription factors have been implicated in the specification of different classes of sensory neurons, although the extent of our understanding of the transcriptional cascades controlling sensory neuron specification lags behind our understanding of motoneuron specification [8,9].

First, the combinatorial expression of the basic helix–loop–helix proteins neurogenin 1 (Ngn1) and neurogenin 2 (Ngn2) is essential for the generation of all DRG sensory neurons in mice [10]. Most proprioceptive neurons are derived from an early Ngn2-dependent precursor population. In contrast, the generation of most cutaneous sensory neurons depends completely on Ngn1. In Ngn2-deficient mice, there is a delay in the generation of proprioceptive neurons [10], suggesting that, in the absence of Ngn2, Ngn1-dependent precursors (which are born later) can generate this neuronal population. The issue of how this rescue is achieved has not been addressed as yet.

Second, recent studies suggest that members of the heterodimeric core-binding factor/Runx family of transcription factors are involved in controlling the survival and/or specification of proprioceptive afferents [11*,12*]. These studies show that Runx3 is essential for proprioceptive afferent development and that mice deficient in Runx3 show uncoordinated, ataxic movements, similar to TrkC-, NT3- or Er81-deficient mice ([2,3]; and see below). Nevertheless, the mechanism of how Runx3 is involved in proprioceptive afferent specification has not been resolved. Depending on the mutant strain, the interpretation points either to a role in the survival of proprioceptive afferent neurons through transcriptional control of TrkC.
[11*,13] or to a function controlling the development of axonal projections of proprioceptive DRG neurons [12*].

Last, the Ets transcription factor Er81 has been implicated in a late step of Ia afferent differentiation. In the absence of Er81, Ia afferents terminate prematurely in the intermediate spinal cord, leading to an almost complete absence of monosynaptic connections between Ia afferents and α-motoneurons [14]. This study thus provides genetic evidence that a transcription factor induced by peripheral cues controls a late aspect of connectivity of proprioceptive afferents. In future studies, it will be interesting to learn more about the interplay between the different transcription factors and their role in controlling the differentiation of proprioceptive afferent neurons.

Differentiation of muscle spindles

Many studies have suggested that the differentiation of muscle spindles depends on inductive signals provided by Ia afferents. A series of elegant experiments based on surgical elimination has suggested that sensory, but not motor, innervation is crucial for the maturation of muscle spindles (reviewed in [15]). In addition, analyses of TrkC- or NT3-deficient mice, in which proprioceptive neurons die before their axons invade the muscle, have revealed a complete absence of muscle spindles, whereas half the complement of muscle spindles is observed in mice heterozygous for NT3 [2,3]. The TrkC/NT3 signaling system is not required, however, for the initiation of muscle spindle differentiation. Proprioceptive afferents in the mesencephalic trigeminal nucleus, unlike those in the DRG, do not require NT3 for their survival, and the muscle spindles supplied by these afferents still form normally in NT3- and TrkC-deficient mice [16,17].

Recent studies have shown that neuregulin 1 (Nrg1) and the ErB receptor system have an important role in this early inductive interaction between Ia afferents and nascent muscle spindles (Figure 1c; [18*,19*]). The identification of three transcription factors — the zinc-finger transcription factor Egr3 [20] and the Ets transcription factors Pea3 and Erm [14], which are expressed selectively by intrafusal muscle fibers even at early developmental stages — has made it possible to monitor early muscle spindle differentiation. The expression of these transcription factors is regulated by Nrg1–ErB signaling in other biological processes [21], making Nrg1 and ErB receptors good candidates for controlling the initiation of muscle spindle differentiation.

Two main isoforms of Nrg1 can be distinguished: cysteine-rich-domain isoforms are expressed broadly by most or all DRG neurons, whereas immunoglobulin-like isoforms are expressed selectively in the TrkC-expressing proprioceptive afferent population [19*]. A receptor for Nrg1, ErB2, is expressed in intrafusal muscle fibers and the connective tissue surrounding muscle spindles [18*]. Conditional genetic elimination of all Nrg1 isoforms from sensory and motor neurons blocks both the initiation of spindle differentiation, including the expression of Egr3, Pea3 and Erm, and the elaboration of Ia afferent terminals at nascent muscle spindles. In contrast, elimination of only the cysteine-rich-domain isoforms does not cause defects in muscle spindle differentiation, suggesting that the immunoglobulin-like isoforms of Nrg1 provided by proprioceptive afferents are sufficient for initiating the differentiation of muscle spindles [19*].

Similarly, deletion of ErB2 expression in muscle results in severe ataxic behavior and an absence of muscle spindles in the adult, suggesting that this Nrg1 receptor is also required for muscle spindle formation [18*]. Despite the fact that Ia afferents do not initiate muscle spindle differentiation in Nrg1-deficient mice, they nevertheless contact individual myotubes [19*]. In future work, it will be interesting to determine whether distinct intrafusal precursors are generated that can be selectively recognized by Ia afferents but not by α-motoneurons, an issue that has been debated for many years.

Development of peripheral and central projections of Ia afferents

To establish a functional stretch reflex, both motor and sensory axons must project to their correct target muscles and the central branches of Ia afferents must terminate in the ventral horn of the spinal cord in the proximity of motoneuron dendrites. Because motoneuron cell bodies supplying a particular muscle are grouped together in the spinal cord, it has been possible using anatomical methods to show that motoneurons are already pre-specified to innervate a specific target region at the time that their axons emerge from the spinal cord (reviewed in [22]).

In contrast, sensory neurons that supply a particular muscle are not clustered together in the DRG, and therefore it has been difficult to demonstrate whether Ia afferents are pre-specified to supply specific muscles and to project into the correct lamina in the spinal cord before they enter their target region. As molecular markers for subsets of proprioceptive sensory neurons have become available [23,24*,25**], it will be possible to determine whether these genes are expressed before or after sensory axons contact their peripheral targets, and how these genes control the development of peripheral and central projections.

The Ets genes Er81 and Pea3, as well as several members of the cadherin family of genes, fail to be induced after limb ablation [23,25**], demonstrating that their expression depends on peripheral signals. Genetic evidence indicates that some of these peripherally regulated genes may be instrumental in controlling late aspects of connectivity [14,26]. Support for this idea is also provided by
the localized expression of glial cell line-derived neurotrophic factor — a factor that is required for the induction of Pea3 expression in motoneurons [27].

In addition, several surgical experiments suggest that during development the particular target region encountered by Ia afferents not only may be involved in the development of their projections but also may determine the selectivity of their central connections with motoneurons ([28]; reviewed in [29]). To resolve the issue of the role of peripheral targets in controlling reflex connectivity, further studies should be directed towards identifying the peripheral signals that regulate Ia-specific gene expression and the molecular mechanisms that underlie this regulation.

Recent studies have proposed the involvement of two cell-adhesion molecules from the immunoglobulin superfamily in the projection of sensory axons in the spinal cord. In chick embryos, F11/F3/contactin is expressed by proprioceptive DRG neurons, whereas expression of axonin-1/TAG-1 is restricted to nociceptive DRG neurons, whose axons terminate in superficial layers of the spinal cord (Figure 1a; [30]). The injection of a function-blocking antibody against F11 selectively perturbs the development of Ia projections to the ventral spinal cord. The blockade of NrCAM, which is a binding partner of F11, also disrupts Ia afferent projections, suggesting that interactions between F11 and NrCAM are required for Ia axons to establish their specific projections to the ventral horn.

Once Ia afferents have reached the vicinity of their target region in the ventral spinal cord, they branch extensively as they innervate motoneurons. This branching activity may be mediated, at least in part, by Wnt3 expressed by motoneurons in the lateral motor column (Figure 1c; [31]). When DRG neurons are cultured in the presence of Wnts on Ia afferent branching in vivo and should identify the factors involved in the branching of Ia afferents at thoracic levels of the spinal cord.

**Selective synaptic connections between Ia afferents and motoneurons**

Synaptic connections between Ia afferents and motoneurons are highly selective. Afferents supplying a single muscle provide a characteristic pattern of inputs to particular subsets of motoneurons at a given segmental level of the spinal cord. Although synaptic connections in some sensory systems are refined by electrical activity during development, the correct pattern of connections in the spinal reflex circuit is apparent from the earliest times that monosynaptic sensory inputs to motoneurons can be recorded [32–34] and is independent of the normal pattern of electrical activity [35]. It is therefore likely that these connections are specified by molecular cues that are expressed differentially by Ia afferents and motoneurons that supply different muscles. Recent experiments have identified several gene families whose members are expressed differentially in subsets of sensory and motoneurons, and these genes are therefore potential determinants for synaptic specificity in this system.

Two members of the Ets family of transcription factors, Er81 and Pea3, are expressed selectively in distinct motor pools — that is, in groups of motoneurons supplying individual muscles (Figure 1b,c; [14,23]). In chickens, a high degree of coincidence in the expression of Er81 and Pea3 in sensory and motoneurons supplying the same muscle has been described [23]. Because Ets genes have been proposed to regulate, either directly or indirectly, the expression of homophilic cell-adhesion molecules, this coincidence of Ets gene expression might lead to selective adhesion between homonymous sensory and motor neurons, thereby providing a molecular mechanism for selective synapse formation.

Whereas the expression of Er81 and Pea3 by distinct motoneuron pools is conserved between chicken and mouse, the expression of these factors in DRG sensory neurons seems to be more divergent between the two species [14]. In mouse, Er81 expression coincides with all proprioceptive afferents and, as a consequence, Er81-deficient mice show connectivity defects in all Ia afferent neurons. The precise identity of the subpopulation of Pea3-expressing DRG neurons is not yet clear, although the expression of Pea3 at late embryonic stages is not as widespread as that of Er81 among the TrkC-expressing afferents. Resolving whether the expression of Er81 and Pea3 in motoneurons contributes to the selectivity of connections between Ia afferents and motoneurons awaits the generation of conditional mutants.

A recent search for genes that are expressed differentially in sensory neurons supplying different muscles in chick embryos has identified a regulator of the LIM family of transcription factors — the LIM-only homeodomain 4 (Lmo4) protein (Figure 1b; [24*]). Although Lmo4 is expressed in most (~85%) TrkC-expressing sensory neurons in the period when sensory–motor connections are forming, Ia afferents that supply a few specific muscles are largely devoid of this protein. Lmo4 is known to inhibit the activity of LIM homeodomain transcription factors by competing with them for binding to the cofactor NLI, which is required for LIM-dependent gene expression. By regulating the transcriptional ability of LIM homeodomain proteins, Lmo4 might provide diversity in the phenotypes of sensory neurons that share common expression patterns of LIM homeodomain.
factors, including their choice of motoneuronal targets in the spinal cord.

Finally, the actual proteins mediating the selective recognition process between Ia afferents and motoneurons are likely to be cell-surface proteins. Cadherins are prevalent cell-surface homophilic recognition molecules, and analysis of their expression pattern in the brain has led to the proposal that the matching of cadherin expression by presynaptic and postsynaptic neurons may participate in the establishment of regional-specific neuronal connections (Figure 1b; [36]). Motoneuron pools of the chick lumbar spinal cord express distinct combinations of type II cadherins, and perturbation of the combinatorial expression pattern by ectopic expression of one of these cadherins, MN-cad, or by dominant-negative approaches has led to the conclusion that a specific pattern of cadherin expression may be required for segregating distinct motor pools during development [25**].

Type II cadherins are also expressed by distinct subpopulations of DRG sensory neurons and for at least two of them, T-Cad and MN-Cad, this expression is correlated with motoneurons supplying the same muscle [25**], suggesting that cadherin expression may contribute to the formation of specific connections between Ia afferents and motoneurons. Recent evidence also suggests that members of the cadherin gene family may be regulated either directly or indirectly by Ets transcription factors [25**,26,37]. Ectopic expression of Er81 in chick spinal cord induces the expression of MN-Cad [25**] and brachial motoneurons in Pea3 mutant mice show deregulation of Cad7 and absence of Cad8 expression [26].

Another class of cadherin-related neuronal receptors (CNR) is also expressed in subsets of sensory and motor neurons during development [38]. The presence of more than 20 spliced variants of CNR genes and their synaptic localization suggests that CNR might participate in the selection of specific sensory-motor synapses [39]. Future experiments in which cadherin expression in the sensory–motor system is perturbed should determine how cadherin expression contributes to the formation of specific connections between Ia afferents and motoneurons.

**Regulation of synaptic strength by muscle spindles**

Once the correct pattern of synaptic connections between Ia afferents and motoneurons has been established, the strength of these connections must be maintained and perhaps even modulated throughout life. Recent experiments indicate that NT3 produced by intrafusal muscle fibers in spindles is essential for maintaining functional synaptic connections between Ia afferents and motoneurons (Figure 1d). Although muscle spindles are initially generated in Egr3-deficient mice [20], they do not produce NT3 [40*] and eventually most of them degenerate [41]. In these mice, Ia afferents still project into the ventral horn of the spinal cord, but the excitatory postsynaptic potentials (EPSPs) that they evoke in motoneurons are small. If NT3 is provided for several days after birth by intramuscular injection, normal EPSPs are restored [40*], showing that NT3 normally provided by intrafusal muscle fibers is required for Ia synaptic function at early postnatal stages.

This requirement for trophic support continues into adulthood. When a muscle nerve is cut in adult cats or rats, depriving sensory neurons of their connection with muscle, the conduction velocity of Ia afferents and their synaptic connections with motoneurons are reduced. Application of NT3 to the central end of the nerve prevents this loss in conduction velocity and synaptic connectivity [42]. A possible mechanism for this potentiation is suggested by the observation that the direct application of NT3 to isolated spinal cords of neonatal rats causes a rapid (~20 min) potentiation of the short-latency, AMPA/kainate-receptor-mediated sensory input to motoneurons — an action that requires the presence of functional NMDA receptors [43]. But after the first postnatal week, this rapid potentiation can no longer be induced, even though Ia-motoneuronal connectivity still requires NT3 after this time. Restoration of synaptic connections in Egr3-deficient mice by peripheral injections of NT3 also requires several days to develop [40*], implying that NT3 is not acting directly on the central connections. These observations suggest that NT3 may regulate synaptic strength by more than one mechanism.

Finally, the strength of synaptic connections between Ia afferents and motoneurons may be actively regulated throughout life. The amount of NT3 released by intrafusal muscle fibers is insufficient to potentiate these synapses maximally, because supplemental intramuscular injections of NT3 during the first postnatal week increase synaptic strength to above normal levels, both in wild-type rats and in Egr3-deficient mice [40*,44]. Chronic application of NT3 to the cut central ends of muscle nerves in adult cats also potentiates Ia EPSPs above their normal amplitude [42]. NT3 release from embryonic muscle has been shown to be dependent on electrical activity in the muscle [45]. If release of NT3 from intrafusal muscle fibers is similarly dependent on the activity of muscle spindles, this would provide a feedback mechanism for controlling the strength of reflex connections throughout life, depending on the degree of activity of a particular muscle.

**Conclusions**

Much progress has been made in the past three years in our understanding of the molecular mechanisms underlying the development of the spinal monosynaptic reflex
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circuit. Distinct subpopulations of motor and sensory neurons seem to be specified by the expression of different families of transcription factors and by the differential expression of cell-adhesion molecules; however, our understanding of these molecular cascades in sensory neurons is only just emerging. Important progress has come also from the identification of neuronally derived signals that trigger the differentiation of muscle spindles, and from the insight that signals from target regions themselves not only are necessary for neuronal survival but also control important aspects of axonal trajectory, target recognition, synaptogenesis and efficacy of synaptic connectivity.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


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12. Inoue K, Ozaki S, Shiga T, Ito K, Masuda T, Okado N, Iseda T, • Kawaguchi S, Ogasawa M, Bae SC et al.: Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. Nat Neurosci 2002, 5:946-954. The strain of Runx3-deficient mice in this study shows severe limb ataxia, but unlike in the strain studied by Levanon et al. [11], TrkC-expressing afferents survive. Instead, these afferents fail to project to their targets in the spinal cord and the periphery. The cellular phenotype caused by deletion of Runx3 may therefore vary in different genetic backgrounds. Together, these two studies [11,12] identify Runx3 as an important factor in controlling the specification of proprioceptive sensory neurons.


Combinatorial expression of type II cadherins defines specific motoneuron pools. A functional role in establishing the segregation and clustering of motoneurons into distinct pools is likely, because misexpression of type II cadherins affects motor pool sorting. Type II cadherins are also expressed differentially by proprioceptive sensory neurons, raising the possibility that they regulate additional steps in the development of sensory–motor circuits.


Deletion of the muscle spindle-specific transcription factor Egr3 results in loss of NT3 production from early postnatal muscle spindles and loss of monosynaptic connections between Ia afferents and motoneurons. Postnatal intramuscular injections of NT3 in Egr3-deficient mice restores sensory–motor connections. Thus, NT3 derived from muscle spindles regulates the synaptic connectivity between proprioceptive afferents and motoneurons.


