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The specification and wiring of mammalian cutaneous low-threshold mechanoreceptors

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Abstract

The mammalian cutaneous low-threshold mechanoreceptors (LTMRs) are a diverse set of primary somatosensory neurons that function to sense external mechanical force. Generally, LTMRs are composed of A β -LTMRs, A δ -LTMRs, and C-LTMRs, which have distinct molecular, physiological, anatomical, and functional features. The specification and wiring of each type of mammalian cutaneous LTMRs is established during development by the interplay of transcription factors with trophic factor signalling. In this review, we summarize the cohort of extrinsic and intrinsic factors generating the complex mammalian cutaneous LTMR circuits that mediate our tactile sensations and behaviors.

INTRODUCTION

The sensation of mechanical force from the external world is an integral part of our everyday experience. It helps us move through space, allows us to identify objects and textures, and facilitates social behaviors such as shaking hands, hugging, and kissing. Mechanical force causes movement of the skin or hair, which in turn activates mechanosensitive neuronal fibers (mechanoreceptors) innervating the skin or hair follicles. While the high-threshold mechanoreceptors (i.e., the nociceptive neurons that only respond to intense mechanical stimuli) mediate mechanical pain, the cutaneous low-threshold mechanoreceptors (LTMRs) mediate innocuous touch.¹ Mammalian cutaneous LTMRs are a subpopulation of dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons. Their cell bodies lie in the DRGs or TGs and elaborate a single axonal process that bifurcates to give rise to a peripheral branch (innervating the skin/hair) and a central branch (innervating the spinal cord or brainstem).

Classically, the mammalian cutaneous LTMRs have been categorized based on their conduction velocity, cell body and axon diameter, and myelination thickness. A β -LTMRs are fast conducting (ranging from 30 to 100 m/second, averaging ~40–60 m/ second), have large soma size/axon diameters, and are highly myelinated. A δ -LTMRs conduct more slowly (ranging from 4 to 30 m/second, averaging ~20 m/second), have intermediate cell body/axon diameters, and are thinly myelinated. C-LTMRs are slow conducting (<2.5 m/second, averaging ~0.6–1 m/second), have small cell body/axon diameters, and are unmyelinated.¹

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Each LTMR can be further classified based on its response to sustained mechanical stimuli. ‘Rapidly adapting’ (RA) mechanoreceptors fire at the onset, and sometimes at the offset, of a stimulus, but only fire sparingly or not at all during the stimulus. ‘Slowly adapting’ (SA) mechanoreceptors not only respond with a burst of firing at the stimulus onset, but sustain firing throughout the stimulus. In between these response types, ‘intermediate adapting’ LTMRs show a burst of firing at the stimulus onset followed by maintained firing throughout the stimulus at a rate lower than SA-LTMRs.¹ As described above and further below, A β -, A δ -, and C-LTMRs display great diversity with regard to their physiological, molecular, anatomical, and functional properties. In this review, we will discuss recent progress in understanding the specific molecular mechanisms that control the development of each type of mammalian cutaneous LTMR.

EARLY DEVELOPMENT OF MAMMALIAN DRG NEURONS

All DRG neurons are derived from neural crest cell progenitors that delaminate from the neural tube between embryonic Day 9 (E9) and E10.5 in mice and migrate ventrolaterally.² These cells coalesce into ganglia beginning around E9.5^{2,3} and then give rise to postmitotic neurons. Those exiting the cell cycle differentiate quickly, with the last DRG neurons exiting the cell cycle around E13.5.⁴ DRG neurogenesis and early differentiation depend on the expression of basic helix-loop-helix (bHLH) neurogenin transcription factors (Figure 1). An initial wave of neurogenin 2 (*Neurog2*) expression generates a group of NTRK3⁺, NTRK2⁺, or RET⁺ large-diameter neurons that will become the majority (~70%) of A β / δ -LTMRs and proprioceptors (somatosensory neurons sensing muscle length and tension) (Box 1). A second wave of neurogenin 1 (*Neurog1*) expression generates the remaining ~30% of the A β / δ -LTMRs/proprioceptors as well as NTRK1⁺ small diameter neurons that will become C-LTMRs, nociceptors, and pruriceptors (somatosensory neurons mediating itch sensation).^{3,5}

BOX 1

NEUROTROPHIC FACTOR RECEPTORS AND THEIR LIGANDS

NTRK1 (formerly known as TRKA) is the receptor for nerve growth factor (NGF). NTRK2 (formerly known as TRKB) is the receptor for brain-derived growth factor (BDNF) and neurotrophin 5 (NTF5, formerly known as neurotrophin 4). NTRK3 (formerly known as TRKC) is the receptor for neurotrophin 3 (NTF3). NGF, BDNF, NTF5, and NTF3 also bind to the low-affinity neurotrophin receptor NGFR (formerly known as p75). RET and GFRA3 are the receptor and coreceptors, respectively, for the glial cell line-derived neurotrophic factor (GDNF) family of ligands.⁶

Neurog1 and *Neurog2* promote the expression of other bHLH transcription factors including *Neurod1* and *Neurod4* (formerly known as *Math3*),⁷ which are characteristic of early neuronal differentiation. Shortly before these neurons exit the cell cycle, they start to express the homeodomain transcription factors *Pou4f1* (formerly known as *Brn3a*) and *Isl1*.^{8,9} (Figure 1). *Pou4f1* and *Isl1* each regulate a diverse set of genes with a significant amount of overlap. Together, *Pou4f1* and *Isl1* facilitate the exit out of the neurogenic phase by

repressing genes important for early development (including *Neurog1*, *Neurod1*, and *Neurod4*) and by promoting the expression of genes involved in sensory neuron differentiation. These include other transcription factors, neurotrophic receptors, axon growth/targeting genes, and neuronal function genes.^{10–14} DRGs lacking both *Pou4f1* and *Isl1* fail to express *Ntrk1*, *Ntrk2*, *Ntrk3*, and *Ret*, demonstrating that specification of all DRG sensory neurons is dependent on *Pou4f1/Isl1* activity.¹⁴ In addition, deletion of either *Pou4f1* or *Isl1* results in major sensory axon growth defects and failure to innervate the skin due to the misregulation of axon growth/targeting genes in these mutants.^{11,15} Moreover, expression of *Pou4f1* with either *Neurog1* or *Neurog2*, or expression of *Neurog1* with *Isl2* and other transcription factors, can convert human or mouse fibroblasts into some types of somatosensory neurons *in vitro*.^{16,17} These results support the critical roles of the *Neurog1*, *Neurog2*, *Pou4f1*, and *Isl1* in the specification of mammalian somatosensory neurons.

DEVELOPMENT OF A β -LTMRs

The A β -LTMRs constitute ~10% of all DRG neurons.^{18,19} They are derived from the early NTRK2⁺/NTRK3⁺/RET⁺ large-diameter neurons generated by the *Neurog2* and *Neurog1* waves.³ While there is a high degree of overlap between RET and NTRK2 and between NTRK2 and NTRK3 at E11.5, they become increasingly segregated by E14.5⁵ (see Figure 1), and these neurons then begin to express the neurofilament heavy chain protein NEFH.²⁰ Since A β -LTMRs can be further subdivided based on their adaptation properties (RA or SA), we will discuss their developmental mechanisms separately.

A β RA-LTMRs

A β RA-LTMRs include Meissner's corpuscles, Pacinian corpuscles, and Lanceolate Endings (Figure 2). *Meissner's corpuscles*, or RAI-LTMRs, are located within the dermal papillae of glabrous skin. They feature disc-like lamellar stacks of Schwann-like cells innervated by A β fibers (Figure 2). Multiple A β fibers can innervate a single corpuscle, and individual A β fibers can innervate 1 or 2 corpuscles.^{1,21,22} Meissner's corpuscles have small receptive fields, show RA-type response properties, and respond best to low frequency (~30–40 Hz) vibration.^{1,23} They likely act as velocity detectors of skin deformation,¹ and the activation of Meissner's corpuscle fibers in human subjects causes a 'fluttering' sensation.²³ *Pacinian corpuscles*, or RAI-LTMRs, are oval-shaped end organs composed of an outer core of lamellar perineural cells and an inner core of Schwann cells (Figure 2). Their location varies between species; while both rodents and primates have Pacinian corpuscles in their interosseous membranes, joints, tendons, muscles, and other deep tissues, primates additionally have Pacinian corpuscles in the subcutaneous fat pads of their fingers, palms, and soles.²⁴ Each corpuscle is innervated by a single A β axon.²⁵ Pacinian corpuscles have large, poorly defined receptive fields and respond best to high-frequency vibration (250–300 Hz) of the skin.^{1,23} *Lanceolate endings* surround whisker hair follicles along with guard and awl (but not zigzag) follicles in mouse back hairy skin. They form a palisade of endings surrounding the follicle, with each ending composed of a flattened stack of Schwann cells innervated by an A β axonal branch¹⁸ (Figure 2). The palisades surrounding one hair follicle can be innervated by multiple individual axonal fibers and individual axons can innervate multiple hair follicles.²⁶ In addition, A β lanceolate endings can coinnervate hair follicles

along with lanceolate endings from A δ - and/or C-LTMRs.¹⁸ A β lanceolate endings most likely function as detectors of hair movement velocity.¹

Centrally, the A β RA-LTMR central axon enters the spinal cord and bifurcates to send branches extending in both the rostral and caudal direction in the spinal cord dorsal column. Almost all ascending axons of A β RA-LTMR reach the dorsal column nuclei (DCN).^{27,28} Several interstitial collaterals from each rostral/caudal axon enter the dorsal horn gray matter and synapse with spinal cord interneurons (Figure 2). Each A β RA-LTMR subtype arborizes differently in the dorsal horn. Meissner's corpuscle afferents arborize at the medial aspect of layers III-V.^{27,29,30} The dorsal horn collateral arborization of Pacinian corpuscle neurons features a dorsal zone of innervation focused in layer III and a small ventral zone of innervation focused in layer V.^{27,31,32} The dorsal horn terminals of A β hair afferents (lanceolate endings) form distinctive 'flame-shaped' arbors.^{27,33}

A β RA-LTMR Specification

Most A β RA-LTMRs can be identified by their expression of *Ret* early in development (E11.5-E13.5, the 'early RET⁺' DRG neurons), a subset of which also coexpress *Ntrk2*.^{5,20,34} (Figure 1). Postnatally, large diameter RET⁺ neurons will express *Ret* only, *Ret* and *Ntrk2*, or *Ret* and *Ntrk3*. Since *Ntrk2*^{GFP} knock-in mice preferentially label A δ - rather than A β -LTMRs in adults,¹⁸ *Ntrk2* expression in A β RA-LTMRs must be low in adulthood. Consistent with this, a recent study used single-cell RNA sequencing to perform unbiased clustering of adult mouse DRG neurons and identified three clusters of putative large diameter LTMRs: one *Ntrk2*^{low}/calbindin 1⁺ population (potential A β RA-LTMRs³⁵), one *Ntrk2*^{high} population (potential A δ -LTMRs¹⁸), and one *Ntrk3* positive population (potential A β SA-LTMRs, see below).³⁶

A β RA-LTMRs begin to express the transcription factor *Maf* (formerly known as *c-Maf*) around the same time that they express *Ret*, followed by the expression of the transcription factor *Mafk* and the RET coreceptor *Gfra2*.^{20,34,37} *Maf* is required for the maintenance of *Ret*, *Gfra2*, and *Mafk* expression in A β RA-LTMRs (although *Ntrk2* expression is independent of *Maf*).^{36,37} In addition, sensory neuron conditional deletion of *Maf* using *Isl1*^{Cre} results in RA-LTMRs that are hyperexcitable and display a slower adaptation property. These changes can be partially explained by the downregulation of the potassium channel KCNQ4 in these mutants.³⁵ *Mafk* null mice also show a partial reduction in *Ret* and *Gfra2* expression, indicating a role for *Mafk* in maintaining expression of these genes. *Mafk* and *Maf* function redundantly to some extent in A β RA-LTMRs.³⁴ Moreover, RET signaling plays a critical role in A β RA-LTMR specification in addition to its role in promoting survival.^{20,38} Neural crest conditional *Ret* mutants (generated using *Wnt1*^{Cre}) have a deficit in the maintenance of *Gfra2* and *Mafk* expression, and mice null for the RET-GFRA2 ligand *Nrtn* also show a deficit in the maintenance of *Gfra2* expression.^{20,34} The expression of *Maf* is unchanged in *Ret* mutants.³⁵

The development of NTRK3⁺ and NTRK2⁺ mechanoreceptors is coordinated by the transcription factors *Runx3* and *Shox2*. *Runx3* is highly expressed at E11.5 by NTRK3⁺ proprioceptor precursors, acting to promote the expression of *Ntrk3* and to repress the expression of *Ntrk2* and *Shox2*.^{5,39} A low level of *Runx3* is seen at E11.5 in NTRK2⁺

neurons, which subsequently maintain *Ntrk2* and down-regulate *Ntrk3*⁵ (see Figure 1). While almost all DRG neurons express *Shox2* at E11.5-E12.5, its expression is only maintained in subsets of *Ntrk2* and *Ret* expressing neurons. Since neural crest specific deletion of *Shox2* (using *Wnt1^{Cre}*) causes a reduction in *Ntrk2* expression while ectopic expression of *Shox2* increases *Ntrk2* expression, *Shox2* must promote the expression of *Ntrk2*.^{39,40} In addition, *Shox2* appears to play a role in repressing *Ntrk3* in the RET⁺/NTRK2⁺ cells, although ectopic *Shox2* does not downregulate the level of *Ntrk3* in RET negative cells.^{39,40}

Aβ RA-LTMR Central Terminal Development

The development of Aβ RA-LTMR interstitial collaterals highly depends on RET signaling (Figure 2). Neural crest conditional deletion of *Ret* greatly reduces the dorsal horn collateral projections of Aβ RA-LTMRs.^{20,41} RET is unusual in comparison to other receptor tyrosine kinases in that it does not bind directly to its ligands. Instead, the ligand must first bind to a GPI-anchored GDNF family receptor α (GFRA) coreceptor which then leads to the recruitment, dimerization, and activation of RET.⁴² *In vitro* studies have demonstrated that RET can be activated by a coreceptor expressed in the same cell as RET (*cis*-signaling) or by a soluble coreceptor (*trans*-signaling).^{43,44} For the developing Aβ RA-LTMRs, *Gfra2* is coexpressed with *Ret* in the early RET⁺ DRG neurons while *Gfra1* is expressed in the dorsal spinal cord, dorsal root entry zone, and neighboring DRG neurons. This expression pattern suggests that both *cis*- and *trans*-RET activation could occur to promote the growth of Aβ RA-LTMR central projections.^{20,45,46} Indeed, recent work from our lab demonstrated that activation of RET either in *cis* by GFRA2 or in *trans* by GFRA1 can support the growth of Aβ RA-LTMR central terminals, which provides the first *in vivo* example of *trans*-RET signaling.⁴⁷

In contrast to the requirement of RET signaling in Aβ RA-LTMR central projection development, neural crest specific deletion of *Ntrk2* has no obvious effect on the spinal cord terminals of Aβ RA-LTMRs.²⁰ Since the loss of *Ntrk2*, but not *Ret*, severely affects Meissner's corpuscle development in the periphery (see below),^{20,48} NTRK2 and RET signaling likely play distinct roles in peripheral versus central terminal development of Aβ RA-LTMRs.

Both *Maf* sensory neuron conditional knockout and *Shox2* neural crest conditional knockout mice were reported to have reduced Aβ-LTMR central innervation in spinal cord dorsal horn layers III-V.^{35,40} Since *Maf* sensory neuron conditional knockout DRGs show a downregulation of *Ret* and *Gfra2*,^{35,37} the *Maf* phenotype could be caused either by downregulation of RET signaling or by RET-independent mechanisms. In addition, given that neural crest conditional deletion of *Ntrk2* does not affect Aβ-LTMR central terminals,²⁰ the reduction of Aβ-LTMR central innervation in *Shox2* neural crest conditional mutants is likely through an *Ntrk2*-independent mechanism.

Aβ RA-LTMR Peripheral Terminal Development

In mice and rats, Aβ-LTMR axons destined to innervate Meissner's corpuscles arrive at the dermal papillae shortly after birth and initiate the formation of the corpuscle. Local Schwann

cells differentiate to form corpuscle lamellar cells around 1 week after birth, and corpuscle maturation continues until about postnatal Day 20 (P20).^{25,49} Early postnatal nerve crush experiments demonstrate that corpuscle formation is dependent on axon innervation.⁴⁹ BDNF–NTRK2 signaling is essential for this process (Figure 2); Meissner's corpuscles do not form in mutant mice that have a nonfunctional *Ntrk2* kinase domain or in *Bdnf* null mice, although they do form in *Ntf5* null mice.^{48,50} Since *Ntrk2* and *Bdnf* knockout mice both have a ~30% reduction in DRG cell number, the lack of Meissner's corpuscles in these mice could simply be caused by cell death of Meissner corpuscle neurons.^{51,52} However, other lines of evidence have also suggested that the role of NTRK2 signaling in Meissner corpuscle formation could be independent of DRG neuron survival. Similar to observations in *Ntrk2* null mice, neural crest conditional knockout of *Ntrk2* prevents corpuscle formation. However, myelinated axons are still present in the dermal papillae in these mice around P14.²⁰ In addition, immunostaining of Meissner's corpuscles localizes NTRK2 to the lamellar cells but not the axon.⁵³ Furthermore, overexpression of either *Bdnf* or *Ntf5* in the skin increases the size of Meissner's corpuscles but does not increase DRG cell number.^{54,55} These results suggest that Meissner's corpuscle formation might require local NTRK2 signaling in the lamellar cells.

Since *Shox2* promotes *Ntrk2* in DRG neurons, *Shox2* mutant mice have a severe reduction in Meissner's corpuscles as well.³⁹ Sensory neuron conditional deletion of *Maf* also causes a severe reduction in the number of Meissner's corpuscles.³⁵ Since *Ntrk2* levels are unchanged in these mice, this effect must be independent of NTRK2 signaling. While the Meissner's corpuscle-innervating neurons express *Ret*, it does not appear to play a major role in Meissner's corpuscle formation. These corpuscles are still present after deletion of *Ret* from the neural crest lineage, although they may appear underdeveloped and disorganized.²⁰

Similar to Meissner's corpuscles, Pacinian corpuscles fail to form upon neonatal denervation.⁴⁹ Like all A β RA-LTMRs, the neurons that innervate Pacinian corpuscles arise from the early RET⁺ DRG population.²⁰ In contrast to other classes of A β RA-LTMRs, the development of Pacinian corpuscles is completely dependent on RET signaling (Figure 2). No Pacinian corpuscles are formed in neural crest conditional *Ret* mutant, in *Gfra2* null, or in *Nrtm* null mice.²⁰ It remains unclear why different classes of RA-LTMRs display differential requirements for RET signaling. Other neurotrophic factor signaling pathways may also contribute to Pacinian corpuscle formation, though it remains to be determined whether they function in primary afferents or the accessory cells.⁵⁶

Maf sensory neuron conditional knockout mice have a reduced number of Pacinian corpuscles, and the remaining corpuscles are smaller and morphologically abnormal.^{35,37} As in Meissner's corpuscles, this phenotype could be caused by a downregulation of RET signaling in these mutants, although RET-independent mechanisms are also possible. Humans with *Maf* missense mutations have difficulty sensing vibration at frequencies detected by Pacinian corpuscles.³⁵ Interestingly, these patients show no deficit in sensing vibrations at lower frequencies that are normally detected by Meissner's corpuscles. Another transcription factor, the ETS transcription factor *Etv1*, is expressed in both DRG neurons and the Schwann cells that make up the inner core of the Pacinian corpuscle. *Etv1* mutant

mice lack Pacinian corpuscles.⁵⁷ At present, it is unclear in which cell types *Etv1* functions to control Pacinian corpuscle formation.

The molecular mechanisms directing the formation of A β -LTMR lanceolate endings are much less clear. This is because the analysis of mutant phenotypes for lanceolate endings is more difficult due to the presence of three types of lanceolate endings (A β , A δ , and C) intermingled around individual hair follicles.¹⁸ Additionally, a high degree of variability exists in both the number of endings surrounding one follicle and the number of follicles innervated by one A β -, A δ -, or C-LTMR axon,²⁶ further complicating analysis of mutant phenotypes. For example, although neural crest conditional deletion of either *Ret* or *Ntrk2* severely affects Meissner's and Pacinian corpuscle development, A β lanceolate endings still appear to be present in the hairy skin of these mice.^{20,51} The lack of clear phenotypes could reflect technical difficulty in quantifying this specific phenotype and/or suggest potential functional redundancy between RET and NTRK2 signaling. *Shox2* neural crest conditional mutant mice show poorly developed lanceolate endings at P6, but it is difficult to determine which neuronal type is affected.³⁹ *Maf* sensory neuron conditional knockout mice show a severe deficit in NEFH⁺/NTRK2^{low} (most likely A β RA-LTMR) lanceolate endings, suggesting that *Maf* plays an important role in the development of all types of A β RA-LTMRs.³⁵

A β SA-LTMRs

The SA A β -LTMRs are Merkel cell-neurite complexes and Ruffini endings (Figure 3). *Merkel cell-neurite complexes*, or SAI-LTMRs, are SA-type mechanoreceptors with very small, restricted receptive fields that respond to very light indentation of the skin.⁵⁸ Based on their response properties and small receptive fields, they are well adapted for high-resolution discrimination of shape and texture.⁵⁹

The Merkel cell-neurite complexes are found in both glabrous and hairy skin. Each complex, known as a 'touch spot' in glabrous skin and a 'touch dome' in hairy skin, features an A β neuronal fiber associated with a cluster of ~5–150 Merkel cells in the basal epidermis^{1,25} (Figure 3). Touch domes in the hairy skin are associated with hair follicles in rodents but are present in the interfollicular skin in other mammalian species.²⁵ Interestingly, the primary afferents that innervate Merkel cells may be different between hairy and glabrous skin. In the glabrous skin, Merkel cells are innervated exclusively by large diameter NTRK3⁺/NEFH⁺ A β fibers. In contrast, neonatal touch dome Merkel cells in the mouse hairy skin are innervated by both NTRK3⁺/NEFH⁺ A β and RET⁺/NTRK1⁺ fibers.⁶⁰ Most of these RET⁺ neurites are missing in *Ntrk1* null mice, indicating that they are developmentally distinct from the large diameter early RET⁺ A β RA-LTMRs discussed above. The physiological significance for the dual innervation of neonatal touch dome Merkel cells by both large-diameter and small-diameter fibers is unclear. Notably, some postnatal large-diameter RET⁺ DRG neurons are also NTRK3⁺,³⁴ which could give rise to the Merkel cell-innervating RET⁺ neurites that remain in *Ntrk1* null mice. The exact developmental history of this RET⁺/NTRK3⁺ population has yet to be determined (see Figure 1). Centrally, the dorsal horn collaterals of Merkel cell-innervating A β SA-LTMRs dive into layers IV-V and then make a medial C-shaped turn before innervating layers III-V (Figure 3).²⁷

The nature of the functional relationship between Merkel cells and their innervating axons has long been an area of contention. Recent work suggests a two-receptor-site model in which both the Merkel cell and the innervating neurite are mechanosensitive.^{61–64} This idea is supported by the following evidence: (1) Merkel cells and neurites make connections that look very similar to synapses in ultrastructural studies,^{65,66} (2) Merkel cells have the machinery for neurotransmitter release,⁶⁷ (3) blocking glutamatergic transmission from Merkel cells reduces SAI responses,⁶⁸ (4) genetically ablating Merkel cells eliminates SAI responses,⁶⁹ (5) Merkel cells express the mechanosensory channel *Piezo2* and they display both *Piezo2*-dependent mechanically activated currents and calcium action potentials (APs),^{62,63} (6) both Merkel cell specific ablation of *Piezo2* and inhibition of Merkel cell APs greatly diminishes the sustained phase of the SAI response,^{62,63} while the dynamic phase is still recorded when Merkel cells are silenced, when *Piezo2* is knocked down or ablated from Merkel cells, or when Merkel cell APs are inhibited,^{61–63} and (7) neurites innervating Merkel cells express *Piezo2* and conditional ablation of *Piezo2* from both the neurite and the Merkel cells greatly reduces both dynamic phase and static phase firing of A β SAI fibers.⁶⁴ Therefore, Merkel cells and their innervating afferents work together to encode different aspects of the SAI response.

Ruffini endings, or SAI-LTMRs, have SA response properties, large receptive fields, and primarily respond to stretching of the skin.^{1,70,71} Early work identified the end organs of SAI-LTMRs as elongated corpuscles of perineural cells and Schwann cells innervated by single A β axons⁷² (the Ruffini ending, Figure 3). Later work found that, although mouse, raccoon, monkey, and human skin feature numerous SAI units based on physiological recordings, Ruffini endings are difficult to identify histologically in these skin samples.^{22,71,73–76} Therefore, the anatomical structure of SAI-LTMR fibers in the skin is currently obscure. The central terminals of SAI-LTMRs in the dorsal horn travel into layer III, where they split into at least two branches that then project ventrally to innervate layers III–VI²⁷ (Figure 3).

A β SA-LTMR Specification and Central Terminal Development

In contrast to the A β RA-LTMRs, which can be exclusively labeled based on their expression of *Ret* early in development,²⁰ A β SA-LTMRs currently have no exclusive molecular markers. Merkel cell-innervating A β SA-LTMR fibers are NTRK3⁺, SLC17A7⁺ (formerly known as VGLUT1), and NEFH⁺.^{60,77} However, proprioceptors are also NTRK3⁺,⁷⁸ whereas SLC17A7 and NEFH mark all proprioceptors and A β LTMRs. *Ntrk2*, *Ntrk3*, and *Runx3* mutants all have deficits in A β SA-LTMR peripheral development (see below).^{51,77,79} However, whether NTRK3⁺ A β SA-LTMRs are derived from the NTRK3⁺/RUNX3⁺ lineage, the NTRK3⁺/NTRK2⁺ lineage, or both, is currently unclear (Figure 1). As mentioned above, recent single-cell RNA-seq clustering of adult DRG neurons identified one cluster of putative LTMRs defined by high expression of *Ntrk3* and no expression of the proprioceptor marker parvalbumin (potential A β SA-LTMRs).³⁶ In addition, a small population of putative mechanosensory DRG neurons coexpresses *Runx1* and *Runx3* into adulthood, and subsets of these neurons express *Ntrk2* and *Ntrk3*.⁸⁰ Given that *Runx3* is important for A β SA-LTMR development, some of these neurons may be A β SA-LTMRs as well.

Shox2 may function in A β SA-LTMRs to promote *Ntrk2* expression, as *Shox2* neural crest conditional mutant mice have deficient Merkel cell touch dome innervation.⁴⁰ Furthermore, physiological recordings from *Maf* sensory neuron conditional knockout mice found that SAI units have higher mechanical thresholds, indicating that *Maf* directs some aspects of SAI-LTMR specification. Although central innervation deficits of A β sensory neurons are seen in *Shox2* neural crest conditional and *Maf* sensory neuron conditional knockouts,^{35,40} it remains to be determined whether these effects are specific for the central projections of A β RA-LTMRs, A β SA-LTMRs, or both populations.

A β SA-LTMR Peripheral Terminal Development

Unlike the end organs of Meissner's and Pacinian corpuscles, Merkel cell clusters are found in the skin prior to sensory innervation.²⁵ The embryological origin of Merkel cells is debatable since they share characteristics with both epidermal and neural cells. Chick-quail chimera experiments and genetic labeling of neural crest cells using *Wnt1*^{Cre} mice suggest that Merkel cells arise from the neural crest.^{81,82} However, some recent findings have also supported an epidermal origin for Merkel cells. For example, the transcription factor *Atoh1* is required for Merkel cell development, and conditional deletion of *Atoh1* from epidermal cells, but not the neural crest, prevented Merkel cell development.⁸³

Several classes of neurotrophins function in Merkel cell-neurite complex development⁸⁴ (Figure 3). NGF/NTRK1 signaling is important for a subpopulation of Merkel cell-neurite complexes. *Ntrk1* kinase domain knockout mice have reduced numbers of Merkel cells and innervating neurites around the whisker pad hair follicle.⁷⁷ The remaining Merkel cell complexes are maintained into adulthood, suggesting that this subset of complexes is NTRK1-independent. *Ngf* knockout mice have a similar, but less severe, phenotype.⁸⁵ Similarly, NTRK1 is required for some innervation of touch dome Merkel cells in the mouse body. In *Ntrk1* nulls, the proportion of Merkel cells innervated by NTRK1⁺ fibers is greatly reduced while the number of Merkel cells innervated by NTRK3⁺ fibers is unchanged.⁶⁰ These results are consistent with the fact that some hairy skin Merkel cell-innervating fibers are NTRK1⁺/RET⁺, though they do not express NEFH and may not be SAI-LTMRs. RET signaling plays a relatively minor role in Merkel cell-neurite complex development.^{20,34,60}

NTRK2 signaling also plays a role in Merkel cell-neurite complex development. In *Ntrk2* knockout mice, there is a large reduction in the number of Merkel cells in glabrous and hairy skin.⁵¹ Overexpression of *Bdnf* in the skin increases the number of Merkel cells present in glabrous, but not hairy, skin.⁵⁴ BDNF/NTRK2 signaling also modulates the physiological response of Merkel cell-neurite complexes. *Bdnf* heterozygous and null mice have SAI units with increased mechanical thresholds, although the number and morphology of Merkel cells in touch domes appear unaffected. Injection of BDNF into the skin rescues this deficit in *Bdnf* heterozygotes.⁸⁶ *Shox2* neural crest conditional knockout mice have a reduction in Merkel cell innervation, possibly because of the reduced *Ntrk2* expression in these mice.³⁹

Among all neurotrophins, NTF3/NTRK3 signaling plays perhaps the most critical role in the development of Merkel cell-neurite complexes. Both Merkel cells and a majority of the innervating neurites express *Ntrk3*.^{60,77,79} In *Ntrk3* kinase domain knockout mice, fewer Merkel cells are present at birth and the number of Merkel cells continues to decrease during

the first two postnatal weeks, indicating a continuous dependency of Merkel cells on NTRK3 signaling during postnatal development.⁷⁷ *Ntf3* knockout mice show an even more severe deficit in Merkel cell complex formation at birth.⁸⁷ In *Ntrk3* null mice, no Merkel cells or innervating fibers are present at birth, suggesting that NTRK3 may have kinase-independent roles in the development and survival of Merkel cells.^{77,85} *Runx3* mutants have defective Merkel cell innervation of whisker follicles, possibly due to the reduced *Ntrk3* expression in trigeminal neurons.⁷⁹

Finally, NGFR signaling is also important for Merkel cell development. In *Ngfr* nulls, the number and appearance of Merkel cells are normal at birth but begin to decrease in number starting around 2 weeks of age, with very few remaining in adulthood.^{85,88}

Knowledge of the development of Ruffini endings is very limited compared to that for the other classes of A β -LTMRs, mostly due to difficulty in reliably identifying these endings. Ruffini-like endings appear to be dependent on NTRK2 signaling (Figure 3). Periodontal and whisker hair Ruffini endings are absent in *Ntrk2* knockout mice and are either greatly reduced or are morphologically deficient in *Bdnf* knockouts.^{89,90} Loss of *Ntf5* leads to morphological deficits in periodontal Ruffini endings but has no effect on whisker hair Ruffini endings.⁹¹ Interestingly, the number of whisker hair associated Ruffini endings increases in *Ntf3* knockout mice.⁸⁵

DEVELOPMENT OF A δ -LTMRs

A δ -LTMRs, also known as D hair cells, have RA responses and are highly sensitive to mechanical force.^{1,18} They represent ~7% of adult DRG neurons.¹⁸ A δ -LTMRs form palisades of lanceolate endings around zigzag and awl/auchenne (but not guard) hair follicles in the back hairy skin and have large receptive fields¹⁸ (Figure 4). Centrally, A δ -LTMRs enter the spinal cord and send a single projection that runs rostrally for a few segments before turning ventrally to innervate the dorsal gray matter. They arborize in layer III of the dorsal horn, dorsal to the A β -LTMRs^{18,92} (Figure 4). Owing to their high sensitivity, these cells are tuned to respond to very gentle touch of hairy skin or to gentle displacement of hair follicles.¹

A δ -LTMR Specification

A δ -LTMRs can be identified based on their high expression of *Ntrk2* in adulthood.¹⁸ Thus, they are likely to be derived from the NTRK2⁺ lineage (Figure 1). While *Wnt1*^{Cre} (neural crest conditional); *Shox2*^{fllox/fllox} mice do not survive past P6, adult analysis of *Wnt1*^{Cre}; *Shox2*^{fllox/+} mice shows a severe reduction in the number of NTRK2⁺ DRG neurons, suggesting that *Shox2* is involved in A δ -LTMR specification.³⁹ In addition, while *Pou4f2* is coexpressed with *Pou4f1* by most DRG neurons around E15.5,⁹³ genetic tracing of DRG neurons that express *Pou4f2* around P0 exclusively labels neurons that match the peripheral terminal morphology and central innervation patterns of A δ -LTMRs.⁹⁴ However, analysis of *Pou4f2* null mice and of DRG neurons after single-cell *Pou4f2* deletion shows no obvious deficits in the specification or peripheral and central terminal development of A δ -LTMRs,^{93,94} suggesting either that *Pou4f2* plays a relatively minor role in the development of A δ -LTMRs or its function is compensated by other transcriptional factors such as *Pou4f1*.

While *Maf* is expressed by NTRK2⁺ cells at P15 (most likely A δ -LTMRs), no obvious deficit is found upon physiological recordings from *Maf* mutant A δ -LTMR units.³⁵

A δ -LTMR Central and Peripheral Development

Both BDNF and NTF5 signal through NTRK2.⁶ The terminal development of multiple A β -LTMR subtypes depends on BDNF–NTRK2 signaling and appears to be independent of NTF5 (see above).^{48,50} In contrast, A δ -LTMR survival and development is highly dependent on NTF5–NTRK2 signaling (Figure 4). *Ntf5* knockout mice show a depletion of A δ -LTMR axons and a lack of A δ -LTMR units in physiological recordings.⁹⁵ Although A δ -LTMRs are not dependent on BDNF for survival or for the development of mechanosensitive terminals,⁸⁶ BDNF plays a role in localizing the lanceolate endings of these neurons. A δ -LTMRs are localized to the caudal side of hair follicles and, consequently, these neurons preferentially respond to hair deflection in the caudal-to-rostral direction. Interestingly, BDNF is localized to the caudal side of the follicle, and conditional deletion of BDNF from the skin disrupts both the caudal localization of the neuronal terminals and the direction selectivity of these neurons.⁹⁶ These results reveal differential roles for BDNF and NTF5 in A δ -LTMR development.

A δ -LTMRs are additionally dependent on NTF3 signaling. *Ntf3* heterozygous mice have a ~50% reduction in the prevalence of A δ -LTMR units recorded in skin-nerve preparations along with a reduction in A δ caliber axons in the saphenous nerve.⁸⁷ This result suggests either that A δ -LTMR survival is dependent on NTRK3 signaling during early development or that NTF3 signaling may influence the expression of functional channels/ receptors that change physiological properties of A δ -LTMRs.

As stated above, *Shox2* neural crest conditional mutant mice show poorly developed lanceolate endings at P6, but it is difficult to determine if this phenotype includes A δ -LTMRs or not.³⁹ Consistent with physiological recordings, *Maf* sensory neuron conditional deletion does not affect NTRK2⁺/NEFH[−] (likely A δ -LTMR) lanceolate endings.³⁵

The molecular mechanisms controlling the central terminal development of A δ -LTMRs are currently unclear. *Shox2* neural crest conditional mutant mice have a reduction in sensory innervation of layers III/IV of the dorsal horn, but this reduction could be caused by deficits in the development of A β -LTMRs, A δ -LTMRs, or both populations.⁴⁰

DEVELOPMENT OF C-LTMRs AND C TACTILE FIBERS

C fiber low-threshold mechanoreceptors, the slowest conducting mechanoreceptor subtypes, make up a surprisingly large fraction of DRG neurons (~15–30%)¹⁸ and may mediate the pleasurable component of affectionate social touch.⁹⁷ In mice, one population of C fiber mechanoreceptors, which has been termed *C-LTMRs*, can be identified based on its expression of tyrosine hydroxylase (*Th*), *Slc17a8* (formerly known as *Vglut3*), and *Fam19a4*.^{18,98–101} These neurons have intermediately adapting responses to sustained mechanical stimuli and respond to gentle mechanical force. They form lanceolate endings surrounding zigzag and awl/auchenne, but not guard, hair follicles in back hairy skin¹⁸ (Figure 5). A second potential type of mouse C fiber low-threshold mechanosensory fiber

expresses the Mas-related G-protein coupled receptor member B4 (*Mrgprb4*).^{102,103} These fibers do not show any mechanical sensitivity *in vitro*. Nevertheless, *in vivo* calcium imaging suggests that they respond to pleasant stroking of the skin and could mediate light-touch.¹⁰³ These neurons have therefore been termed *C tactile fibers*. MRGPRB4⁺ fibers terminate as free nerve endings in hairy skin, with each neuron innervating a large patch of skin¹⁰² (Figure 5).

Like A δ -LTMRs, C-LTMRs and C tactile fibers enter the spinal cord and send a single projection that runs rostrally for one or two segments. These projections send collaterals into the dorsal horn gray matter and form flame-shaped terminal arbors that terminate in layer II^{18,102} (Figure 5).

C-LTMR and C Tactile Fiber Specification

Both TH⁺ and MRGPRB4⁺ neurons are part of the NTRK1⁺ lineage, which are small diameter, unmyelinated neurons deriving from the *Neurog1* wave of neurogenesis⁷⁸ (see Figure 1). Similar to the role that *Runx3* plays in the specification of the NTRK2⁺/NTRK3⁺ lineage, *Runx1* plays critical roles in the development of C fibers. One main function of *Runx1* is to segregate NTRK1⁺ DRG neurons during prenatal and early postnatal development.⁵ *Runx1* is initially expressed in almost all NTRK1⁺ neurons, but by E17.5, ~50% of NTRK1⁺ neurons have downregulated *Runx1*. Neurons that maintain *Runx1* expression will express *Ret* and its coreceptors (the 'late RET⁺' population, including nonpeptidergic nociceptors, pruriceptors, and both populations of C fiber mechanoreceptors), whereas those that decrease *Runx1* expression will eventually express the neuropeptide *Calca* and become the peptidergic nociceptors.¹⁰⁴ TH⁺ C-LTMRs maintain *Runx1* expression into adulthood.⁹⁹ In contrast, although *Runx1* is required for the expression of *Mrgprb4*, MRGPRB4⁺ neurons downregulate *Runx1* shortly after birth.^{104,105} *Runx1* and the downstream zinc finger transcription factor *Zfp521* form a transcriptional circuit that segregates TH⁺ C-LTMRs from MRGPRB4⁺ C tactile fibers and two other related C fiber populations (MRGPRD⁺ nonpeptidergic nociceptors and MRGPRA3⁺ pruriceptors). In this interaction, *Runx1* promotes expression of *Zfp521* and represses *Mrgprb4* and *Mrp3* in future TH⁺ C-LTMRs. *Zfp521* then acts to segregate TH⁺ C-LTMRs and MRGPRD⁺ nociceptors by promoting the expression of *Th*, *Slc17a8*, and *Fam19a4* and repressing genes specific for nonpeptidergic nociceptors in TH⁺ C-LTMRs.¹⁰⁰ Last, *Runx1* regulates the mechanosensitivity of TH⁺ C-LTMRs by promoting the expression of the mechanosensor *Piezo2*.⁹⁹ Recent transcriptional profiling of purified C-LTMRs revealed that their molecular profile is distinct from MRGPRD⁺ nociceptors but has a high degree of overlap with A β - and A δ -LTMRs.¹⁰⁶ This is interesting considering that C-fiber and A-fiber LTMRs have distinct developmental lineages (see above), suggesting that separate developmental mechanisms may converge to promote a common transcriptional profile for low-threshold mechanosensation.

NGF/NRTK1 signaling is necessary for the survival, specification, and central and peripheral terminal development (see below) of the NTRK1⁺ lineage. Both C-LTMRs and C tactile fibers do not survive in *Ngf* null mice.¹⁰⁷ In *Ngf*^{-/-}, *Bax*^{-/-} double null mice (in which cell death is bypassed), these neurons fail to express *Runx1*, *Ret*, *Gfra2*, or the *Mrg*

family of receptors, suggesting that NGF signalling is the master regulator for expression of functional genes in C fibers in addition to its role in survival.³⁸ Last, while *Ret* is not required for survival of these neurons, neural crest conditional deletion of *Ret* results in a severe reduction of *Mrgprb4* expression,³⁸ indicating that RET signalling is necessary for MRGPB4⁺ neuron specification and/or functional gene expression.

C-LTMR and C Tactile Fiber Central and Peripheral Terminal Development

In addition to its role in survival and specification, NGF–NTRK1 signaling is required for both the peripheral and central innervation of all small-diameter NTRK1⁺ lineage neurons,¹⁰⁸ including both TH⁺ and MRGPRB4⁺ C fiber mechanoreceptors (Figure 5). *Runx1* also controls the development of their peripheral and central terminals. While TH⁺ C-LTMRs normally enwrap hair follicles and grow a palisade of longitudinal lanceolate endings, *Runx1* mutant C-LTMRs still grow circumferential terminals around hair follicles but fail to form lanceolate endings.⁹⁹ MRGPRB4⁺ neurons are part of a class of nonpeptidergic late RET⁺ neurons that bind the isolectin IB4.¹⁰² *Runx1* has been shown to control the laminar position of IB4⁺ spinal cord projections, with *Runx1* mutant projections terminating more dorsally in the dorsal horn than wildtype terminals.¹⁰⁴ Since TH⁺ C-LTMRs do not bind IB4,¹⁸ it is unknown whether *Runx1* plays a similar role in these neurons. Finally, neural crest conditional *Ret* deletion does not affect the survival of RUNX1⁺ neurons up to P14,³⁸ and *Gfra2* knockout does not affect the survival or peripheral terminal development of TH⁺ C-LTMRs.

CONCLUSION

This review has summarized the complex intrinsic and extrinsic molecular mechanisms that specify A β RA-, A β SA-, A δ -, and C-LTMR neurons as well as guide their peripheral end organ formation and central projection wiring. All subtypes of mammalian LTMRs are derived from a common population of neural crest progenitors that delaminate from the developing neural tube. Early developmental events segregate this common progenitor pool into the early RET⁺ lineage (future A β RA-LTMRs), the NTRK3⁺ lineage (which includes A β SA-LTMRs), the NTRK2⁺ lineage (which includes A δ -LTMRs), and the NTRK1⁺ lineage (which includes the late RET⁺ C-LTMRs and C tactile fibers). A specific combination of transcription factor and trophic factor activity within each lineage further orchestrates the development of their distinct end organs as well as their subtype-specific connectivity in the spinal cord.

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FIGURE 1.

Illustration showing specification of different subtypes of mammalian cutaneous low-threshold mechanoreceptors (LTMRs). The major known genes that specify each subtype at different stages are shown here. The final column shows main molecular markers to identify each subtype of LTMR in adult mice. Instances where the developmental lineage or gene expression for a given population is unclear are indicated with dotted lines or question marks. Reference citations are shown at the upper left-hand corner of each panel.

FIGURE 2.

Illustration showing molecular mechanisms that control peripheral and central terminal development of Aβ rapidly adapting-low-threshold mechanoreceptors (RA-LTMRs). Genes that are important for Aβ RA-LTMR development are indicated in the dorsal root ganglion (DRG) cell body. Signalling molecules that direct terminal development are indicated in the spinal cord and skin. In the epidermis, SB/SS = stratum basalis/stratum spinosum, SG = stratum granulosum, SC = stratum corneum.

FIGURE 3.

Illustration showing molecular mechanisms that control peripheral terminal development of A β slowly adapting-low-threshold mechanoreceptors (SA-LTMRs). Genes that are important for A β SA-LTMR development are indicated in the dorsal root ganglion (DRG) cell body. Signalling molecules that direct terminal development are indicated in the skin.

FIGURE 4.

Illustration showing molecular mechanisms that control peripheral and central terminal development of Aδ-low-threshold mechanoreceptors (LTMRs). Genes that are either important for Aδ-LTMR development are indicated in the dorsal root ganglion (DRG) cell body. Signalling molecules that direct terminal development are indicated in the skin.

FIGURE 5.

Illustration showing molecular mechanisms that control peripheral and central terminal development of C-low-threshold mechanoreceptors (LTMRs) and C tactile fibers. Genes that either specifically mark the C-LTMRs or C tactile fibers or are important for their development are indicated in the dorsal root ganglion (DRG) cell body. Signalling molecules that direct terminal development are indicated in the spinal cord and skin.