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Mas-Related G Protein–Coupled Receptors and the Biology of Itch Sensation

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Abstract

Chronic, persistent itch is a devastating symptom that causes much suffering. In recent years, there has been great progress made in understanding the molecules, cells, and circuits underlying itch sensation. Once thought to be carried by pain-sensing neurons, itch is now believed to be capable of being transmitted by dedicated sensory labeled lines. Members of the Mas-related G protein–coupled receptor (Mrgpr) family demarcate an itch-specific labeled line in the peripheral nervous system. In the spinal cord, the expression of other proteins identifies additional populations of itch-dedicated sensory neurons. However, as evidence for labeled-line coding has mounted, studies promoting alternative itch-coding strategies have emerged, complicating our understanding of the neural basis of itch. In this review, we cover the molecules, cells, and circuits related to understanding the neural basis of itch, with a focus on the role of Mrgprs in mediating itch sensation.

INTRODUCTION

DRG: dorsal root ganglia

TG: trigeminal ganglia

Mrgpr: Mas-related G protein–coupled receptor

TRPV1: transient receptor potential channel V1

TRPA1: transient receptor potential channel A1

Chronic itch is a challenge in the clinic that can result from a broad range of etiologies. Understanding the neural basis of itch at the molecular, cellular, and circuit levels can identify new therapeutic targets to treat this devastating symptom.

The sensation of itch is carried by primary afferent neurons, mainly small-diameter, unmyelinated C-fibers whose cell bodies are located within the dorsal root ganglia (DRG) or trigeminal ganglia (TG). These neurons transmit itch sensation from the skin to the central nervous system. In the spinal cord, these afferents synapse with secondary neurons in the dorsal horn that transmit itch signals to the brain.

Grossly, itch-sensing neurons are anatomically indistinguishable from pain-sensing neurons. As such, itch-sensing neurons were commonly believed to be a subpopulation of nociceptive neurons. One prevailing model, the intensity-coding theory, proposed that at low firing rates, neuronal activity would result in the sensation of itch, but at higher firing rates, activity would be interpreted as painful (24, 36). As molecular biology and neuroscience techniques have improved, this model has been challenged. Itch neurons are now recognized as being both molecularly and functionally distinct from pain neurons.

In this review, we compare the molecular characteristics of itch afferents with their pain counterparts. This discussion takes place in the context of reviewing two alternative theories for the neural basis of itch: labeled-line coding and intensity coding. Then, we cover the genetics of Mrgpr (Mas-related G protein–coupled receptor) proteins, as well as their expression and function in nonhistaminergic itch. Finally, we bridge the discussion of labeled-line and intensity coding from the periphery to the central nervous system by discussing spinal itch circuits.

AFFERENT ITCH NEURONS EXPRESS DISTINCT G PROTEIN–COUPLED RECEPTORS

Pain and itch can result from stimuli as varied as chemicals, temperature, and mechanical force. To detect stimuli, nociceptive afferents utilize a host of channels and receptors. For example, many small-diameter nociceptive neurons express the transient receptor potential (TRP) channel V1 (10, 11). At 42°C, the threshold for noxious heat, TRP channel V1 (TRPV1) fluxes cations and depolarizes its host neuron (11). Similarly, pungent chemicals, such as cinnamaldehyde and mustard oil, cause irritation by activating another channel, TRP channel A1 (TRPA1) (29, 43).

In addition to expressing channels, receptors also play a part in sensing noxious substances. Among detection-capable proteins, G protein–coupled receptors (GPCRs) occupy a critical functional niche. GPCRs are a large, seven-transmembrane domain superfamily of proteins that share an ability to interact with a G protein messenger for intracellular signaling (21, 33). GPCRs have critical roles in detecting stimuli as varied as lipids, ions, and proteins, and they are expressed in almost every cell of the body (35). There are more than 800 GPCRs in humans, and they are among the proteins most targeted for drug development (21).

The GPCR profile of a neuron dictates its molecular sensitivity. Transitively, GPCR expression can distinguish functional populations of neurons. In an unbiased study of mouse DRG sensory neurons using single-cell transcriptomics, three GPCRs were identified as the prevailing markers for three separate classes of itch-sensitive afferent neurons: 5-hydroxytryptamine (serotonin) receptor 1F (5-ht1f), Mrgpra3, and Mrgprd (**Figure 1*a*,*b***) (71). On a macrotranscriptome level, the three classes were found to be distinct from one another and from other sensory

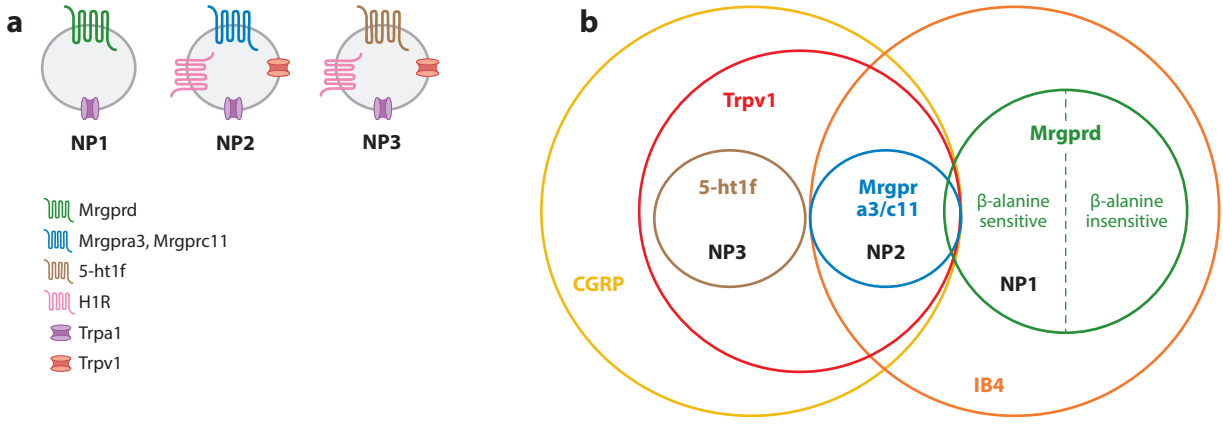

Figure 1

Itch-sensitive neurons can be classified into three distinct classes based on the expression of GPCRs. (*a*) The circles represent subtypes of itch nociceptors. These subtypes are named NP1–3 in accordance with Reference 71. Predominant itch-associated receptors are depicted for each class. NP1 neurons express Mrgprd (*green*). NP2 neurons express Mrgpra3 and Mrgprc11 (*blue*). NP3 neurons express 5-ht1f (*brown*). NP2 and NP3 neurons express H1R (*pink*). All three classes express TRP channels that are important for itch signaling. (*b*) A Venn diagram of the three classes of itch neurons and their relation to the expression of CGRP and Trpv1 and whether they bind to IB4. About half of Mrgprd-positive neurons are sensitive to β-alanine. These nonpeptidergic, IB4-positive neurons are believed to be NP1 itch neurons. NP2 neurons are both IB4-positive and CGRP-positive. NP3 neurons are IB4-negative, CGRP-positive. Abbreviations: 5-ht1f, 5-hydroxytryptamine (serotonin) 1F; CGRP, calcitonin gene-related peptide; GPCR, G protein–coupled receptor; H1R, histamine H1 receptor; Mrgpr, Mas-related G protein–coupled receptor; TRP, transient receptor potential; Trpa1, transient receptor potential channel A1; Trpv1, transient receptor potential channel V1.

neurons. However, the expression of each receptor was not 100% specific to each class. For example, there was some 5-ht1f expression (9%) detected in the Mrgpra3-positive class and vice versa (8%) (71).

Of the three markers, 5-ht1f was expressed outside the three designated classes of itch neurons. This receptor and other serotonergic receptors were expressed broadly in peripheral nervous tissue and have a known function in pain signaling (19, 63). However, Mrgpra3 and Mrgprd were specific to classified itch neurons, with only minimal expression in other neuronal cell type clusters. Importantly, itch neurons, although unique in their expression of transcripts such as Mrgpra3, also expressed transcripts with known pain signaling function, suggesting that itch neurons are a subpopulation of pain neurons (**Figure 1*a*,*b***) (71).

Not only do itch afferents express many receptors and channels involved in pain, but these proteins are also considered to be critical for itch transmission. In vivo electrophysiological recordings across a variety of model organisms, including mice, rats, and monkeys, have shown that many pruritogen-responsive neurons in the dorsal horn are sensitive to algogens such as capsaicin and mustard oil (15, 31, 60).

Genetic studies have buttressed conclusions drawn from these physiological studies. *Trpv1*$^{-/-}$ mice, animals with well-characterized deficits in pain sensation, scratch less when injected with histamine, and *Trpa1*$^{-/-}$ mice scratch less when injected with chloroquine, a well-known nonhistaminergic pruritogen (28, 75). Together, these data confirm that TRP channels are functional molecular intersections between pain and itch, providing a molecular basis for intensity-coding theory.

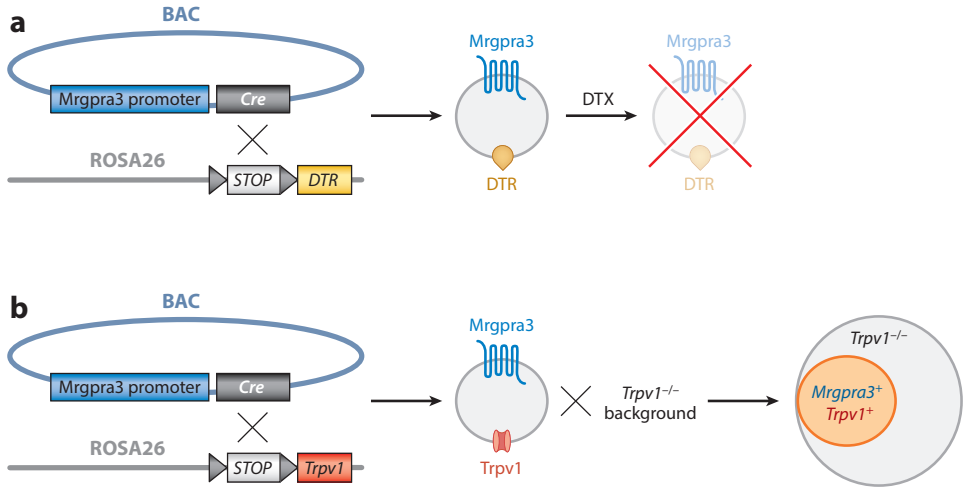

Figure 2

Genetic strategies for deletion and activation of specific populations of neurons. ROSA26 is a commonly used mouse locus for gene expression. Black triangles represent loxP sites that are recognized by *Cre*. Bars represent gene coding regions. (*a*) A depiction of the genetic mating strategy and experimental paradigm used to ablate specific neuronal populations in mice. By using various gene promoters, in this case Mrgpra3, to drive *Cre* expression, one can achieve specific expression of *DTR* (*yellow*) in select cell populations. Dosing the animal with DTX ablates *DTR*-positive cells, allowing precise removal of cells based on gene expression. (*b*) A diagram of the genetic mating strategy and experimental paradigm for achieving exclusive Trpv1 expression in a cell type of interest. Using promoter-driven *Cre* lines, one can express the channel *Trpv1* (*red*) in select populations. After crossing animals to a *Trpv1*-knockout mouse, Trpv1 expression is restricted to cells of interest. Subsequently, dosing with Trpv1 agonists, such as capsaicin, should act as a specific activator of these cells. Abbreviations: BAC, bacterial artificial chromosome; *DTR*, diphtheria toxin receptor; DTX, diphtheria toxin; Mrgpra3, Mas-related G protein–coupled receptor a3; Trpv1, transient receptor potential channel V1.

MRGPRA3 EXPRESSION CLASSIFIES AN ITCH-SPECIFIC LABELED LINE IN THE PERIPHERAL NERVOUS SYSTEM

In 2013, Han et al. (26) provided several lines of molecular evidence that directly contradicted the prevailing intensity theory. In their study, Mrgpra3 was found to label a population of nociceptors that coded exclusively for itch and not pain.

Mrgpra3-positive neuronal fibers exclusively innervated the epidermis (26). In vivo electrophysiological analysis of Mrgpra3-positive fibers revealed that they are responsive to mechanical stimuli, noxious heat, and various pruritogens, including histamine, BAM8–22 (bovine adrenal medulla 8–22), and chloroquine. The ablation of these neurons with diphtheria toxin (**Figure 2*a***) resulted in less pruritus in response to a wide range of pruritogens, including chloroquine, SLIGRL, BAM8–22, histamine, endothelin 1, and α-Me-HT (α-methylserotonin) (see the sidebar titled Itch Behavior in Rodents). Additionally, these animals exhibited less pruritus in two models of chronic itch: dry skin pruritus and allergic itch. Importantly, pain sensitivity in Mrgpra3-positive neuron–ablated animals was intact.

From these data, however, one cannot conclude that Mrgpra3-positive neurons are a labeled line for itch. If Mrgpra3-positive neurons are a subpopulation of nociceptors that code for both pain and itch, ablation would result in only partial loss of nociceptors, which could conceivably account for the observed behavioral data. To address this point, the authors developed a strategy to specifically activate Mrgpra3-positive neurons (**Figure 2*b***). They expressed Trpv1 in

ITCH BEHAVIOR IN RODENTS

In rodents, itch behavior is distinct from pain or grooming. Behavioral models of acute itch involve injecting substances into the nape of the neck or the cheek. Cheek injection has become favored as it is able to more accurately distinguish pain response from itch (56). In mice, a scratch is counted as itch behavior only when it is delivered by the hind limbs. Forelimb behavior, which accounts for the majority of grooming behavior, is not counted as itch. Because itch scoring can be subjective, it is important for experimenters and scorers to be blinded to the experimental condition whenever possible. Interestingly, both mouse strain and sex have an effect on the number of scratches delivered in response to pruritogens (23).

Mrgpra3-positive neurons and bred these animals to a global *Trpv1*$^{-/-}$ mouse line. This resulted in offspring that expressed Trpv1 only in Mrgpra3-positive neurons. Thus, upon injection of capsaicin, a ligand of TRPV1, only Mrgpra3-positive neurons were activated. Indeed, in these animals the injection of capsaicin resulted in high firing rates of Mrgpra3-positive neurons and no other neurons. After the introduction of capsaicin, only itch behavior and not pain was observed, providing strong evidence that these neurons signal exclusively for itch.

The intensity-coding theory predicts a rate-dependent shift in sensation that should be reflected by behavior. The activation of Mrgpra3-positive neurons with high-dose capsaicin resulted in itch and not pain. As such, the authors concluded that Mrgpra3-positive neurons, despite their expression of pain-related proteins, represented a long-sought labeled line for itch in the peripheral nervous system (PNS).

INITIAL CLONING OF THE MRGPR FAMILY

Mrgpra3 is a member of the Mrgpr family of receptors. These were first identified in a subtractive complementary DNA screen between wild-type and neurogenin1 (Ngn1) knockout mice, which lacked TrkA-positive, small-diameter nociceptive neurons. From this screen, a variety of nociceptor-specific sequences were cloned. Mrgpra1, the first Mrgpr, was pursued as a novel target for study because it was a GPCR with unknown function.

Mrgpra1 has 35% sequence homology with the known angiotensin receptor Mas1 (18). Based on this homology, it was named Mas-related G protein–coupled receptor a1. Using in situ hybridization, Mrgpra1 was found to be expressed in a subset of nociceptors in mouse DRG and TG, confirming the validity of the screening approach.

The receptor was identified at the turn of the millennium, before the human and mouse genomes were sequenced. However, bioinformatic analyses of databases still proved fruitful. Searches of public genomic sequences using the cloned Mrgpra1 sequence yielded 14 additional members of the MrgA subfamily, 27 members in two closely related subfamilies, MrgB and MrgC, and the single-gene subfamilies D, E, F, and G (**Figure 3**) (18). Many identified sequences were pseudogenes. Of the 50 murine Mrgpr sequences identified, only 27 had intact open reading frames (18). Searches of human genomic databases revealed eight intact human Mrgprs (**Figure 3**) (18). Four genes, *MRGPRX1–X4* appeared to be similar in sequence to the murine MrgprA subfamily. However, there was no clear homology between individual murine receptors and these four identified human receptors. Additional human Mrgprs—D, E, F, and G—all had clear, single-gene mouse orthologs.

Early functional analyses of Mrgprs focused on screening ligands related to pain signaling. Due to the specific expression of these receptors in nociceptive neurons, Mrgprs were believed to play

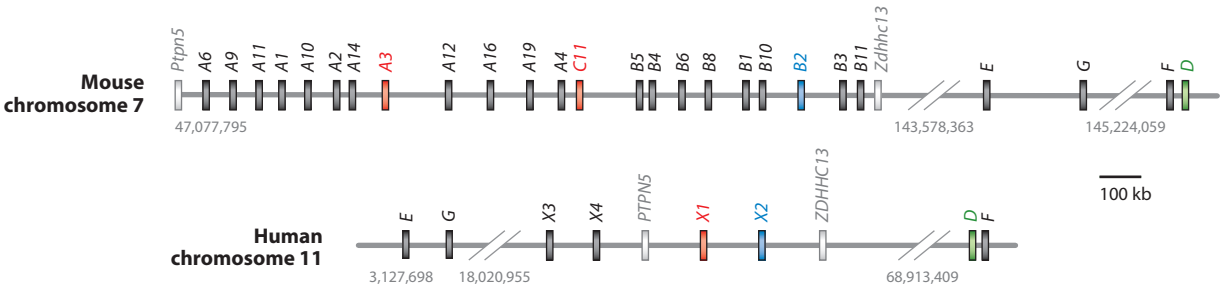

Figure 3

Diagram of mouse and human *Mrgpr* genetic loci. Mouse *Mrgprs* are clustered on chromosome 7. Human *Mrgprs* are present on chromosome 11. Each bar represents the coding sequence of a gene. Double forward slashes depict breaks. *Mrgprs* appearing in red, blue, and green have a known receptor function in itch. Colors are coordinated based on functional, not sequence-based, homology between mouse and human *Mrgprs*. Gray bars illustrate non-*Mrgpr* genes that are conserved within the region.

a part in pain. In a targeted screen, Dong et al. (18) identified RFamide neuropeptides as potent agonists of murine Mrgpra1. These neuropeptides had previously been linked to pain modulation. Notably, the supraspinal injection of these peptides resulted in hyperalgesia and reversed morphine tolerance (53). These data led to the hypothesis that RFamide neuropeptides function as part of an anti-opioidergic system (53). Both mice and humans express NPFF (neuropeptide FF), an RFamide neuropeptide that activates two different receptors, NPFF1 and NPFF2, at nanomolar affinity (32, 47). Due to this high affinity at an existing receptor, RFamide peptides are not believed to be physiological ligands of Mrgprs.

In 2002, two separate groups used degenerate oligonucleotide–primed PCR to clone a novel receptor isolated from rat DRG, brain, and spinal cord tissue (8, 37). Lembo et al. (37) termed the receptor rat sensory neuron–specific receptor (SNSR). Using the SNSR sequence as a probe, the authors cloned six receptors from a human genomic library that had 50–55% sequence identity to the original rat SNSR sequence and 80–98% sequence identity to each other. Phylogenetic analysis revealed that human SNSRs clustered with the mouse MrgA family, but the identified rat SNSR was most closely related to MrgC (37). The expression of both SNSRs and Mrgprs in nervous tissue was restricted to somatosensory afferents in the DRG and TG. Importantly, these identified human SNSRs were found to be identical or nearly identical in sequence when compared with previously identified Mrgprs. Due to sequence similarity, the International Union of Basic and Clinical Pharmacology (IUPHAR) has classified the SNSRs as Mrgprs, the official name for these receptors (6). Today, the union denotes the existence of several classes of Mrgprs: A, B, C, D, E, F, G, H, and a primate-specific X.

Lembo et al.'s (37) study uncovered additional ligands with activity against Mrgpr proteins. BAM8–22, a proteolytic cleavage product of proenkephalin A, was identified as a potent activator of MRGPRX1, a DRG-expressed human Mrgpr (37). Full-length BAM activated both the μ-opioid receptor and MRGPRX1. The peptide required its N terminus for interaction with opioid receptors, and BAM8–22, missing 7 N-terminal amino acids, did not interact with opioid receptors but retained activity against MRGPRX1.

SNSR: sensory neuron–specific receptor

MURINE-SPECIFIC MRGPR DIVERSITY

Studying the Mrgprs using mouse models has been complicated by a murine-specific abundance of these receptors. Mice have many more intact coding Mrgpr sequences than any other model organism or humans. Based on sequence phylogeny, the murine A, B, and C families are thought

Table 1 Mouse and human Mrgprs linked to known pruritogens

Mouse receptor	Human receptor	Pruritogen	Mouse EC_{50} (approximate μM)	Human EC_{50} (approximate μM)	Reference(s)
Mrgprc11	MRGPRX1	BAM8–22	0.03	0.03	27, 37
	MRGPRX2	SLIGRL	10	20	40
Mrgpra3	MRGPRX1	Chloroquine	28	300	39
Mrgprb2	MRGPRX2	PAMP-20	12.4	0.17	44
		Compound 48/80	3.7 μg/ml	0.47 μg/ml	44
Mrgprd	MRGPRD	β-alanine	44	15	57

Abbreviation: EC_{50}, half of the maximal effective concentration.

to most closely map onto the human X family, and the D, E, F, and G subfamilies are concordant from mouse to human (**Figure 3**) (18, 78). However, mice have dozens of intact Mrgpr coding sequences within their A, B, and C subfamilies, but humans have only a few MRGPRX family members, making a one-to-one comparison based on sequence difficult.

The murine-specific expansion of the *MrgprA* and *MrgprC* gene clusters is due to an L1 retrotransposon insert into the 3′ end of the *MrgprA* gene. Evolutionarily similar rodents, such as rat and gerbil, lack this genetic expansion. After the mouse diverged from its last common ancestor with these organisms, numerous rounds of unequal crossover are hypothesized to have taken place, resulting in the large number of similar open reading frames and pseudogenes in the region (78). In humans, the clustering of *MRGPRX* genes is also believed to have resulted from rounds of unequal crossover (78). With many similar, nonorthologous murine Mrgprs, identifying biologically relevant receptors for study is challenging. Furthermore, the lack of sequence homology makes it complicated to translate findings from mouse Mrgpr studies to specific human receptors. Over time, this problem has been whittled at by ligand activity studies, which have identified functional, not sequence-based, orthologs between mouse and human receptors. For example, Mrgpra3 is considered the mouse ortholog of MRGPRX1 based on shared ligands, such as chloroquine (**Figure 3**) (**Table 1**).

EXPRESSION OF MRGPRs

Mrgprs are a complex family of receptors with dozens of members. They are expressed in sensory neurons of the DRG and TG, numerous brain regions, the enteric nervous system, and mast cells. Early cloned Mrgprs, such as Mrgpra1 in mice and MRGPRX1 in primates, have specific expression in the DRG and TG (18, 37). However, since their discovery, the expression of Mrgpr family members in other tissues has become appreciated. Both murine and human MrgprE are expressed in numerous brain regions and in the enteric nervous system (5, 76). MrgprF has also been identified in the enteric nervous system (5). Ligands for both MrgprE and MrgprF, endogenous or exogenous, remain to be determined, and an *Mrgpre*$^{-/-}$ mouse displayed no obvious deficits in pain sensation (13). Mrgprb2, a murine receptor, and MRGPRX2, a human receptor, have been identified as being expressed in mast cells (30, 44, 64, 65). These are the only Mrgprs identified as being present in non-neuronal tissue.

MRGPRs ARE RECEPTORS FOR NONHISTAMINERGIC PRURITOGENS

Mrgprs, as GPCRs, engage neuronal signaling pathways that result in pruritus. In 2009, Liu et al. (39) published a landmark study showcasing Mrgprs as receptors whose activation resulted in itch

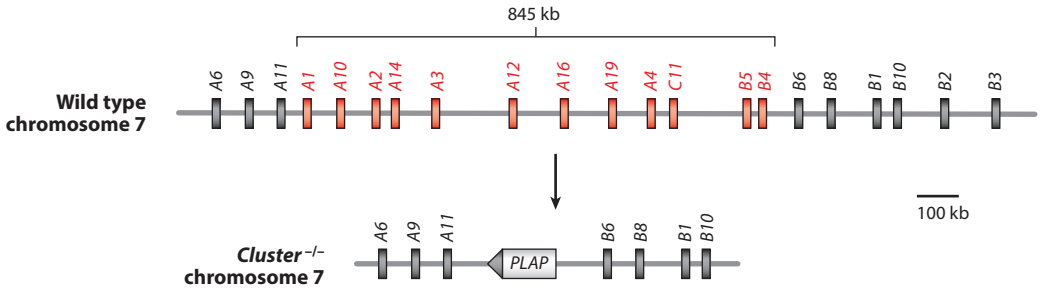

Figure 4

Illustration of Mas-related G protein–coupled receptor (*Mrgpr*) cluster knockout deletion. Each bar depicts the coding sequence of a gene. In an *Mrgpr* cluster knockout mouse, an 845-kb segment containing the coding sequences for 12 Mrgprs (*red*) is removed and replaced with a human placental alkaline phosphatase (PLAP) sequence. The nearby *Mrgprs* remaining in the resulting gene product are shown.

sensation. In their study, chloroquine, an antimalarial drug that causes pruritus in black Africans, was determined to be a ligand of Mrgpra3 in mice and MRGPRX1 in humans.

Chloroquine-mediated itch is nonhistaminergic. The injection of chloroquine does not result in wheal, flare, or other symptoms of mast cell degranulation. Additionally, antihistamine treatment fails to alleviate pruritus (1).

To test the role of Mrgprs in chloroquine-mediated pruritus, Liu et al. (39) generated a mouse that had a cluster of Mrgprs deleted. Due to the L1 retrotransposon element and unequal crossover events, the coding regions of the Mrgpr A–C subfamilies are present in a highly repetitive region of genomic DNA that is difficult to target for single-gene deletion. Additionally, many Mrgpr subfamily members have similar sequence identity, making compensatory action a possible experimental confounder in single-gene knockout studies (18). For these reasons, Liu et al. elected to pursue a cluster knockout strategy that resulted in an *Mrgpr* cluster knockout (*Cluster*$^{-/-}$) mouse in which 12 intact coding open reading frames were deleted (**Figure 4**).

The acute injection of chloroquine caused pruritus in wild-type mice, which was alleviated in *Cluster*$^{-/-}$ mice. DRG isolated from *Cluster*$^{-/-}$ animals failed to respond to chloroquine, as determined by either calcium imaging or electrophysiological recording. Heterologous expression of individual Mrgprs identified Mrgpra3 as the sole receptor within the cluster that had a chloroquine response. Importantly, *Cluster*$^{-/-}$ animals had intact acute pain responses across a variety of modalities and intact serotonin-elicited and mast cell–mediated itch (39). Compared with wild-type animals, these animals had similar numbers of nociceptive neurons and no appreciable changes in the proportions of sensory neuron types. Thus, the authors concluded that Mrgprs were not important for itch neuron survival or targeting neuronal fibers to the skin and that the differences assessed between wild-type and *Cluster*$^{-/-}$ mice in the presence of chloroquine were due to a lack of activity at Mrgpra3.

Since Liu et al.'s (39) study, several Mrgprs have been identified as receptors for different pruritogens, which, in turn, has informed models of peripheral itch sensation. Although the exact pruritogens and Mrgprs involved may differ, the general hypothesis is that the Mrgprs present on select peripheral pruriceptive neurons bind pruritogens. This interaction then results in neuronal changes that lead to firing and the sensation of itch.

ACTIVATION OF MRGPRC11 CAUSES PRURITUS

Lembo et al. (37) identified BAM8–22 as an activator of human MRGPRX1. In mice, BAM8–22 was determined to be a ligand of Mrgprc11 (39). The cutaneous injection of BAM8–22 in mice

led to robust scratching in wild-type mice and no phenotype in *Cluster*$^{-/-}$ mice (39). Injecting BAM8–22 into the volar forearm of healthy human volunteers produced itch, accompanied by pricking, stinging, and burning sensations (59). These sensations occurred with no evidence of wheal or neurogenic flare at the application site, suggesting a lack of mast cell involvement.

PAR: protease-activated receptor

In addition to BAM8–22, other nonhistaminergic pruritogens have been linked to MrgprC11. For example, pruritogens involved in protease-associated itch have been shown to have activity against this receptor. Applying serine and cysteine proteases to human skin results in inflammation and nonhistaminergic itch (2, 3, 50). Itch is hypothesized to result from proteases, most notably mucunain from cowhage, cleaving the N-terminal domain of protease-activated receptors (PARs) (40, 51). Subsequent autoactivation of the receptor by the cleavage product would then elicit itch.

SLIGRL is the protease-cleavage product derived from murine Par2. In a careful study, SLIGRL was shown to evoke itch by activating Mrgprc11 and not Par2 (40). *Cluster*$^{-/-}$ mice—mice without Mrgprc11—did not scratch upon injection of SLIGRL, but *Par2*$^{-/-}$ mice scratched robustly. Of note, activation of Par2 with SLIGRL, which does not activate Mrgprc11, produced thermal hyperalgesia and no itch (40). Thus, from these data, the authors concluded that the activation of Par2 resulted in hyperalgesia, and protease-associated itch was mediated by Mrgprc11 and not Par2.

ACTIVATION OF MRGPRD RESULTS IN PRURITUS

Mrgprd is another Mrgpr linked to nonhistaminergic pruritus. Unlike the murine Mrgpr A, B, and C subfamilies, Mrgprd has a clear sequence-based homolog, MRGPRD. Initial studies of Mrgprd showed that Mrgprd-positive neurons were nonpeptidergic, nociceptive neurons that selectively innervated the skin (79).

β-Alanine is the only identified agonist of Mrgprd (57). The intradermal injection of β-alanine in humans resulted in itch, with minimal reports of pain-related symptoms, such as pricking, stinging, and burning (38). In mice, both intradermal injection of and oral supplementation with β-alanine caused pruritus (38). Importantly, the introduction of β-alanine failed to elicit itch in *Mrgprd*$^{-/-}$ mice (38). β-alanine evoked robust firing in Mrgprd-expressing neurons but had no effect on neurons from knockout animals. In conjunction with psychophysical studies, these data suggest that the activation of Mrgprd by β-alanine results in the sensation of pruritus.

FUNCTIONAL HOMOLOGY OF MRGPRs: TRANSLATING MOUSE STUDIES TO HUMANS

Of note, both BAM8–22, an activator of murine Mrgprc11, and chloroquine, an activator of murine Mrgpra3, have been shown to activate human MRGPRX1 (39). In this case, two separate mouse receptors, Mrgpra3 and c11, are functional homologs of a single human receptor MRGPRX1, linked by shared ligands (**Table 1**). Interestingly, the homolog of a different activator of Mrgprc11, SLIGRL, was found to activate MRGPRX2 in humans. In this case, Mrgprc11, a single murine Mrgpr, is functionally linked to two separate human MRGPRs (**Table 1**).

MRGPRB2 AND MRGPRX2: MAST CELL–SPECIFIC RECEPTORS

Mrgprb2, a mouse receptor, and MRGPRX2, a human receptor, are the only two members of the Mrgpr family to have specific cell-type expression outside of nervous tissue. Both receptors are expressed in mast cells, which are resident sentinel cells of the skin, lung, and intestine (44, 64, 69).

Mast cells are capable of releasing a wide range of immunomodulatory factors, including histamine, prostaglandins, and cytokines (70). Canonical activation of mast cells occurs through the cross-linking of IgE (immunoglobulin E) receptors. However, numerous non-IgE peptides can activate mast cells, including various toxins, neuropeptides, and hormones (44, 61, 64, 65, 69). As a major reservoir for histamine in the skin, mast cells are key cellular mediators of both acute and chronic itch. Many clinically relevant pruritic conditions have a mast cell–dependent mechanism. These conditions are so numerous that it is convenient for itch disorders to be classified based on mast cell involvement, whether they are histamine dependent or independent.

MRGPRX2, a human receptor, was the first Mrgpr found to be expressed on mast cells. Early in vitro work identified many basic secretagogues, such as β-defensins, as being ligands of this receptor (64, 65). However, definitive conclusions were difficult to draw due to the lack of an animal model for in vivo validation.

Because of the unclear homology between murine A, B, and C Mrgpr subfamilies and the human MRGPRX receptors, an animal model was slow to be generated. Based on amino acid sequence alone, there was no identifiable ortholog between MRGPRX2 and any murine Mrgpr. However, a stringent RT-PCR screen detected the expression of Mrgprb2 in murine mast cells (44).

The analysis of heterologously expressed Mrgprb2 revealed that many basic secretagogue activators of MRGPRX2 also activated Mrgprb2, suggesting that Mrgprb2 was a functional ortholog of MRGPRX2 (44). This was confirmed in vivo, as *Mrgprb2*$^{-/-}$ mice had substantially decreased secretagogue-associated histamine release, inflammation, and airway contraction compared with wild-type controls. These secretagogues were shown to activate Mrgprb2 and X2 by a common THIQ motif, shared by many pharmaceuticals approved for clinical use. Interestingly, *Mrgprb2*$^{-/-}$ mice were protected from drug-induced anaphylaxis (44).

Basic secretagogues, such as compound 48/80 and proadrenomedullin peptide 9–20 (PAMP9–20), are widely used pruritogens that elicit itch in both mice and humans. Compound 48/80 is a promiscuous drug that interacts with numerous receptors. In itch studies, it is a reliable mast cell activator that produces histaminergic itch (36, 48). Given recent evidence showcasing compound 48/80 as an activator of Mrgprb2, the presumptive mechanism of this itch is mast cell degranulation through activation of Mrgprb2.

MRGPRs, PAIN, AND LABELED-LINE CODING

In the peripheral nervous system, Mrgpra3-positive neurons represent a labeled line for itch. However, other Mrgpr-expressing neurons have been linked to non-itch-related functions. For example, both Mrgprc11 and Mrgprd activation in mice can affect pain sensation.

The intrathecal application of BAM8–22, a ligand for Mrgprc11 in mice and MRGPRX1 in humans, has an analgesic effect in mice (25). Specifically, applying BAM8–22 attenuates inflammatory heat hyperalgesia, neuropathic mechanical allodynia, and central pain sensitization. The analgesic properties of BAM8–22 are absent in *Cluster*$^{-/-}$ mice, suggesting that Mrgprc11 activation is required for analgesia. This pain inhibition is thought to result from Mrgprc11 activity at central terminals inhibiting high voltage–activated calcium channels and interrupting neurotransmission. Thus, in theory, these analgesic properties of Mrgprc11 signaling do not deny the possibility that Mrgprc11-positive afferent neurons still code exclusively for itch.

Mrgprd-positive neurons are believed to represent a population of nonpeptidergic, mechanical pain-sensing afferents. The genetic ablation of Mrgprd-expressing neurons reduces sensitivity to noxious mechanical stimuli (12). This pain deficit was specific to mechanical injury, as noxious heat or cold responses were not affected (12, 49, 73). In vivo calcium imaging of Mrgprd-positive neurons revealed that they are directly activated by a painful pinch (72).

Mrgprd not only marks a specific population of nonpeptidergic nociceptors, but it also has intrinsic GPCR activity that affects neuronal firing. *Mrgprd*$^{-/-}$ mice exhibit decreased sensitivity to painful mechanical stimuli and noxious temperature ranges (12). This suggests that Mrgprd itself plays a part in pain transmission. Applying β-alanine, an Mrgprd ligand, reduced rheobase in Mrgprd-positive neurons and increased firing rates, indicating that Mrgprd, when activated, increases the excitability of expressing neurons by inhibiting potassium channel M-currents (14, 49).

STT: spinothalamic tract

Activating Mrgprd through agonists such as β-alanine causes itch and not pain. Interestingly, as assessed by calcium imaging and electrophysiological recording, β-alanine activates only about half of Mrgprd-positive neurons in the DRG (38). This indicates that only a subset of Mrgprd-positive neurons express the downstream channels necessary for neuronal activation. In this case, β-alanine-responsive Mrgprd-positive neurons could represent a specialized population of sensory neurons that code for itch, and nonresponsive Mrgprd-positive neurons could code for either itch and pain or pain alone. Additional studies that specifically activate all Mrgprd neurons and are combined with behavioral assessments are necessary to address these questions.

DOWNSTREAM SIGNALING EVENTS OF MRGPRs

In pathological contexts, Mrgprs on sensory neurons and mast cells can be activated by select ligands, which results in itch. Itch can occur either by direct elicitation of neuronal action potentials or indirectly through mast cell degranulation and histamine release. After an Mrgpr is bound by a ligand, canonical GPCR intracellular signaling is engaged. Many Mrgprs, including A1, C11, X1, and X4, are coupled to the G protein subunit α_{q11} for intracellular signaling (6, 27, 61).

In the DRG, Mrgpr ligand–mediated calcium increases were blocked by ruthenium red, a nonspecific inhibitor of numerous TRP channels (39). These data suggest that TRP channels lie downstream of Mrgpr signaling and are a major source of extracellular calcium influx.

Interestingly, only inhibitors of Gβγ signaling blocked chloroquine-mediated calcium flux, suggesting that MrgprA3 coupling to TRP channels is mediated primarily by the Gβγ subunit (28). In a divergent mechanism, BAM8–22-mediated calcium flux was blocked by inhibitors of phospholipase C signaling and not by gallein. Thus, MrgprC11 coupling to TRP channels was believed to depend on phospholipase C activity.

SPINAL PROCESSING: PAIN INHIBITS ITCH

TRPV1 and TRPA1 agonists produce pain and not itch. If TRP channels are important mediators of itch signaling, then why do agonists not cause itch? Among all TRPA1-expressing neurons, only a fraction express either Mrgpra3 or c11. Similarly, histamine receptor–expressing neurons are a subset of a much larger population of Trpv1-positive neurons. Therefore, the injection of TRP agonists will activate many pain neurons in addition to pruritogen-sensitive neurons.

Afferent pain neurons are hypothesized to activate spinal interneurons that inhibit itch sensation. This model is similar to Melzack & Wall's (45) gate control theory in which nonpainful mechanical inputs activate interneurons that inhibit pain (**Figure 5*a***). The model arose from observations surrounding the common experience of itch and scratching. Scratch, a pain-inducing activity, relieves itch. In primates, scratching decreased action potential discharges in the spinothalamic tract (STT) (16). This decrease in activity depended on scratching state. Upon cessation of scratching, STT neuron firing rebounded (16).

The pain–itch gate control theory predicts that inhibiting nociceptive afferent input decreases spinal interneuron activity and results in disinhibited, increased itch. Experimental data support

Figure 5

Type I incoherent feedforward loops. Excitatory connections are depicted with black arrows. Inhibitory connections are depicted with red inhibition lines. (*a*) Circuit depiction of gate control theory. Large-diameter, touch-sensitive Aβ neurons can activate interneurons (INs) which then inhibit pain-producing neurons. (*b*) Depiction of gastrin-related peptide (Grp)-positive interneuron circuit function. Grp-positive INs activate both pain- and itch-coding neurons. Grp-positive neuron activation of enkephalin-positive (Enk) INs inhibits pain sensation.

this prediction, as silencing the Trpv1-positive primary afferent nociceptors results in chronic, pathological itch in mice (34).

Until recently, the specific interneuron target of primary nociceptive afferents was a mystery. In 2010, a potential interneuron population, basic helix-loop-helix b5 (Bhlhb5)-dependent interneurons, was identified. Bhlhb5 is a transcription factor that is transiently expressed by a subset of postmitotic neurons that migrates to the superficial layers of the dorsal horn (52). The loss of Bhlhb5-dependent interneurons resulted in severe chronic itch, heightened acute behavioral itch responses, and no deficits in pain sensation (**Figure 6**) (52). These findings suggest that Bhlhb5-dependent neurons selectively inhibit itch and not pain.

In the earlier case of a peripheral injection of capsaicin resulting in the exclusive sensation of pain, Trpv1 channels on both pain- and itch-sensing afferents open, activating both populations.

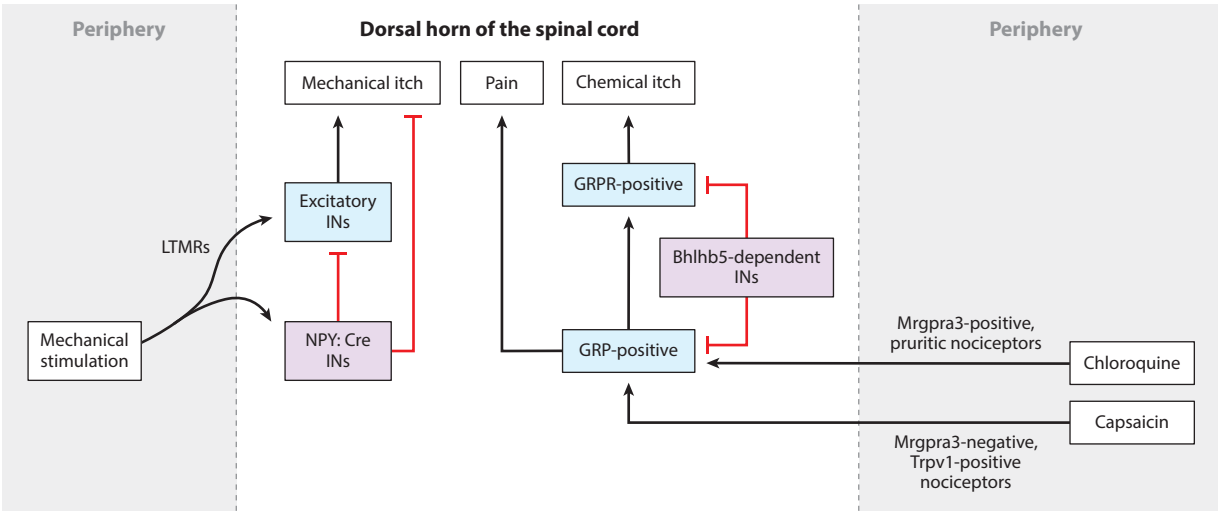

Figure 6

Mechanical and chemical itch pathways in the periphery and the spinal cord. Excitatory connections are depicted with black arrows. Inhibitory connections are depicted with red inhibition lines. Mechanical stimulation in the periphery activates low-threshold mechanoreceptors (LTMRs), which activate both excitatory interneurons (INs) and neuropeptide Y (NPY)-Cre–positive INs. Alternatively, Mrgpra3-positive fibers are activated by chloroquine, which activates Bhlhb5-dependent INs in the spinal cord along with gastrin-related peptide (GRP)-positive and gastrin-related peptide receptor (GRPR)-positive neurons. GRP-positive neurons code for both pain and itch.

However, in the spinal cord, pain afferents drive Bhlhb5 interneurons to silence itch activity. Thus, only pain is felt.

Bhlhb5-dependent interneurons are believed to gate chemical itch (**Figure 6**). Recently, a different class of interneurons, neuropeptide Y (NPY)-positive neurons, was found to regulate transmission of mechanical itch resulting from mechanical stimulation of hairy skin (**Figure 6**). Ablation of NPY-positive neurons causes the development of chronic itch-associated skin lesions similar to those in *Bhlhb5*$^{-/-}$ mice. In NPY-ablated animals, von Frey–graded mechanical stimulation of hairs resulted in significant increases in evoked itch compared with wild-type animals (9). This increase in itch occurred only at low-threshold mechanical stimulation (0.02–0.4 g) and not at high thresholds (>0.6 g) (9). Mechanical stimulation activates mechanoreceptors on large-diameter afferents, which can result in itch. However, large, myelinated afferents can also activate NPY-positive interneurons, which inhibit spinal cord neurons that transmit itch (9). In the absence of NPY-positive interneurons, mechanoreceptor-driven itch is uninhibited and chronic itch develops.

Bhlhb5$^{-/-}$ mice have normal NPY expression (52). Similarly, after NPY-positive interneuron ablation, numerous Bhlhb5-dependent interneurons are spared (9). Based on this and corroboratory behavior data, NPY-positive and Bhlhb5-dependent neurons are believed to have nonoverlapping functions of gating mechanical and chemical itch, and not pain.

GRPR: gastrin-related peptide receptor

Npra: natriuretic peptide receptor A

Grp: gastrin-related peptide

GASTRIN-RELEASING PEPTIDE RECEPTOR AND NATRIURETIC PEPTIDE RECEPTOR A: A LABELED LINE FOR ITCH IN THE SPINAL CORD?

There is evidence that the labeled-line specificity for itch seen in the PNS extends to the spinal cord. In 2001, histamine-sensitive, mechano-insensitive, and thermo-insensitive neurons in the cat spinothalamic tract were electrophysiologically isolated (4). These neurons were activated monosynaptically by slow-conducting peripheral C-fibers, with a latency and duration that paralleled psychophysical itch studies (4). In primates, a separate population of STT neurons sensitive to cowhage, a nonhistaminergic pruritogen, was identified (17). According to physiological analyses, these cowhage-sensitive neurons and histamine-sensitive neurons were nonoverlapping neuronal populations. Both were distinct from known classes of nociceptive neurons and were initially thought to be itch-dedicated spinal neurons. However, later study proved that neither population was purely responsive to itch stimuli. Both histamine-sensitive and cowhage-sensitive STT neurons were activated by painful stimuli, such as capsaicin and noxious heat. At the time of study, this result was viewed as strong evidence against the existence of an itch-specific labeled line (17, 54).

With the advent of mouse genetic tools, cellular heterogeneity in the spinal cord became accessible to study. As spinal neurons were dissected, evidence supporting the labeled-line theory emerged. Specifically, gastrin-related peptide receptor (GRPR) and natriuretic peptide receptor A (Npra), two receptors expressed in lamina I of the dorsal horn, have been put forth as itch-selective neurons in the spinal cord (**Figure 6**) (46, 68).

Grpr is necessary for itch and dispensable for pain. *Grpr*$^{-/-}$ mice scratched less in response to a variety of pruritogens, compound 48/80 (a mast cell activator), and SLIGRL and chloroquine (two nonhistaminergic pruritogens) (68). Importantly, these mice had normal responses to noxious mechanical and thermal stimuli, indicating that Grpr was not involved in pain signaling (67, 68). The ablation of Grpr-positive neurons in lamina I resulted in decreased itch and alterations in pain behavior (68). The activation of Grpr with gastrin-related peptide (Grp) homologs, such as bombesin and neuromedin B, elicited pruritus and not pain, suggesting labeled-line specificity (22).

Nppb: natriuretic polypeptide B

Npra is the receptor for natriuretic polypeptide B (Nppb), a neuropeptide expressed in Trpv1-positive peripheral itch afferents (46). *Nppb*$^{-/-}$ mice exhibited deficits in acute itch but had intact pain response to noxious thermal and mechanical stimuli (46). Activating Npra by injecting its cognate ligand, Nppb, elicits itch. The pharmacological antagonism of Grpr can block this itch, suggesting that Grpr signaling lies downstream of Npra. Concordantly, the ablation of Npra-positive dorsal horn neurons attenuated histamine-induced pruritus but not Grp-induced itch (46).

The pharmacological activation of either Grpr-positive neurons or Npra-positive neurons was sufficient for itch. However, more specific, genetic-based activation of these populations has yet to be carried out. Altogether, the behavioral, anatomical, and physiological data presented support the hypothesis that Npra-positive and Grpr-positive neurons represent a labeled line for itch in the spinal cord.

REVISITING LABELED-LINE AND SELECTIVITY THEORIES IN THE SPINAL CORD

Earlier data pointed toward Nppb and Grp as being critical neuropeptides that transmit itch information in the spinal cord. Grp protein has been detected in the DRG by immunocytochemistry, and the Grp transcript has been detected by in situ hybridization, quantitative PCR, and RNA sequencing (7, 42, 77). However, these findings have been questioned by other groups able to detect only low or absent levels of Grp transcript in the DRG by quantitative PCR or in situ hybridization (20, 46, 62). In a *Grp-eGFP* mouse, a mouse that has GFP (green fluorescent protein) linked to Grp protein, GRP was detected in the spinal cord and not in the DRG (20, 46, 62).

Although there is controversy over whether GRP is expressed in the DRG, Grp messenger RNA and protein have been detected in the spinal cord by multiple labs. A recent publication from our lab sought to determine the role of these Grp-positive neurons in the spinal cord (66). Grp was detected in glutamatergic neurons located in lamina II of the superficial dorsal horn. No Grp was detected in the DRG. Grp-positive neurons received direct monosynaptic input from Mrgpra3-positive neurons in the periphery. Surprisingly, Grp-positive neurons were also found to receive input from many Trpv1-positive, CGRP-positive, Mrgpra3-negative pain-sensing afferents (66).

In DRG-attached spinal slice recordings, both pain and itch stimuli were found to activate Grp-positive neurons (66). This dual input was reflected in behavior. Using a Cre–loxP strategy similar to that employed previously (**Figure 2*b***), Trpv1 was expressed specifically in Grp-containing neurons. Upon injection of capsaicin and activation of Grp-positive neurons, animals responded with both pain- and itch-associated behaviors (66). Strikingly, at high doses of capsaicin, pain behavior decreased while itch behavior remained elevated. This pattern of dose response resulted from a type I incoherent feedback circuit in which Grp-positive neurons evoke pain and itch while simultaneously activating enkephalin-producing interneurons that inhibit pain (**Figure 5*b***) (66).

Based on this striking phenotype from cell type–specific activation, Grp-positive neurons were hypothesized to code for both itch and pain. These data contradicted existing models in which Grp-positive neurons were hypothesized to be a component of the itch-specific labeled line in the spinal cord. Instead, Grp-positive neurons demonstrated intensity-dependent coding through which, at high levels of activity, itch information was transmitted faithfully while pain was inhibited by the parallel activation of enkephalin-containing interneurons (66). In this system, itch was coded monotonically but pain was most reliably signaled by inputs of medium intensity.

The presence of dual pain- and itch-coding neurons in the spinal cord holds explanatory value for human psychophysical studies of itch. Injecting pruritogens, such as histamine and cowhage, into humans produces itch along with weaker nociceptive sensations, such as burning, pricking,

and stinging (36, 59). These results can be accounted for by the neuronal coding exhibited by Grp-positive neurons.

Of note, itch stimuli and painful stimuli elicited different firing rates in Grp-positive neurons (66). Pruritogens, such as SLIGRL, histamine, and chloroquine, produced low firing rates in Grp-positive neurons, even at high concentrations, and pain-associated ligands, such as capsaicin, reliably produced high firing rates (66). Because the direct activation of Grp-positive neurons by capsaicin is an artificial paradigm, physiologically one could hypothesize that, even though Grp-positive neurons themselves can transmit itch and pain sensations, downstream neurons could represent a labeled line for itch. For example, at low firing rates, Grp-positive neurons could release different neurotransmitters than at high firing rates, thereby activating divergent neuronal populations that could code for separate sensations. To address this hypothesis requires specific genetic-based activation of candidate neuronal populations—Grpr-positive and Npra-positive—like that employed in Grp-positive neurons.

CONCLUSIONS

Viewed grossly, itch neurons are indistinguishable from nociceptive neurons in the periphery and spinal cord. At higher resolution, peripheral itch neurons express a host of exclusive molecular markers that differentiate them from pain neurons. However, itch-sensitive neurons also express proteins that have a long-standing role in pain. Despite this expression pattern, the activation of at least one population of itch-sensitive afferents, Mrgpra3-positive neurons, resulted in itch and not pain. Therefore, in the periphery, itch is capable of being transmitted by labeled-line coding.

Mrgpra3-positive neurons exist in the PNS. Thus, it is natural to wonder whether itch-related labeled-line specificity extends centrally into the spinal cord. Grp, Grpr, and Npra are all markers of spinal neuron populations that code for itch. Ablating Grp-, Grpr-, and Npra-positive neurons drastically reduces itch behavior. However, the activation of Grp-positive neurons exists in both pain and itch, reaffirming the selectivity theory, in which itch is transmitted by a population of pain-responsive nociceptors. Further experiments are required to determine whether labeled-line coding is preserved centrally.

In addition to issues concerning the neural basis of itch, many questions remain in the field. Roles for non-neuronal cell types in chronic itch conditions are becoming increasingly appreciated. In the periphery, keratinocytes are now understood to have an important role in numerous chronic itch conditions (55). Centrally, glia have proven to be important contributors to both acute and chronic itch (41, 58). How all these different cell types interact—with their varied signaling pathways, across time, and in different pathophysiological contexts—is a difficult question to address. Itch is an extensive medical problem throughout many communities (74). Much work remains to be done to perfect treatments and relieve a substantial source of suffering.

SUMMARY POINTS

1. Mrgprs are G protein–coupled receptors expressed within small-diameter nociceptors of the DRG (dorsal root ganglia) and TG (trigeminal ganglia).
2. Numerous Mrgprs function as novel itch receptors for nonhistaminergic pruritogens.
3. In the periphery, labeled-line coding for itch exists.
4. Mrgpra3-positive DRG afferents are itch-specific neurons that are distinct from pain-sensing neurons.

5. In the spinal cord, Grpr-positive, Npra-positive, and Grp-positive neurons transmit itch.
6. Inhibitory interneurons in the spinal cord mediate the inhibition of itch by pain.
7. Grp-positive neurons in the spinal cord code for both itch and pain.
8. Itch likely displays selectivity coding in the spinal cord, where itch is carried by a subpopulation of nociceptors that transmit both pain and itch.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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