Molecular Neurobiology
https://doi.org/10.1007/s12035-024-04478-w

REVIEW

SUMOylation and DeSUMOylation: Tug of War of Pain Signaling

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Received: 31 May 2024 / Accepted: 2 September 2024

Abstract
SUMOylation is a post-translational modification that attaches a small ubiquitin-like modifier (SUMO) group to a target protein via SUMO ligases, while deSUMOylation refers to the removal of this SUMO group by sentrin-specific proteases (SENPs). Although the functions of these processes have been well described in the nucleus, the role of SUMOylation and deSUMOylation in regulating ion channels is emerging as a novel area of study. Despite this, their contributions to pain signaling remain less clear. Therefore, this review consolidates the current evidence on the link(s) between SUMOylation, deSUMOylation, and pain, with a specific focus on ion channels expressed in the sensory system. Additionally, we explore the role of SUMOylation in the expression and function of kinases, vesicle proteins, and transcription factors, which result in the modulation of certain ion channels contributing to pain. Altogether, this review aims to highlight the relationship between SUMOylation and deSUMOylation in the modulation of ion channels, ultimately exploring the potential therapeutic role of these processes in chronic pain.

Keywords SUMOylation · DeSUMOylation · Ion channels · Pain

Background

Neuropathic pain is a pathological condition resulting from a lesion or disease in the somatosensory nervous system. This condition affects 7–10% of the general population [1, 2] and is characterized by abnormal pain responses, numbness, burning, stabbing, or electric shock-like pain and tingling, among other symptoms. Notably, people suffering from neuropathic pain also experience anxiety, depression, and sleep disturbances, significantly reducing their quality of life [2]. Despite efforts to understand the cellular and molecular mechanisms underlying neuropathic pain, current pharmacological treatments are effective in less than 50% of patients and induce adverse effects that limit their clinical utility [2]. Thus, there is an urgent need to explore the mechanisms implicated in the pathophysiology of neuropathic pain to identify and validate new treatment targets.

Primary sensory neurons play a key role in processing and transmitting nociceptive information from the periphery to the central nervous system through the activation of ion channels and receptors [3]. Specifically, the electrical activity in sensory neurons is mainly controlled by different ion channels such as voltage-gated sodium, calcium, and potassium channels. Moreover, these ion channels can be modulated by post-translational modifications (PTMs), which influence their turnover rates and localization in the plasma membrane [4, 5], thereby affecting neuronal excitability. In this context, SUMOylation is a PTM that enables rapid cellular responses to dynamic changes in various cell types, including neurons through the addition of small ubiquitin-like modifier (SUMO) proteins. Although some targets of SUMO proteins (i.e., nuclear proteins and transcription factors) have been well established, the SUMOylation of ion channels is an emerging field in pain research. This review focuses on understanding the patterns of SUMOylation and deSUMOylation. It discusses the impact of increased SUMOylation on ion channels in chronic pain conditions and highlights the role of sentrin-specific proteases (SENPs) in regulating ion channel SUMOylation.

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Published online: 14 September 2024

SUMO Isoforms and Function

SUMOylation is a reversible PTM that involves the covalent binding of an ~11-kilodalton SUMO protein to lysine residues in target proteins [6]. This modification modulates the activity and stability of the target protein by promoting or preventing protein-protein interactions [7, 8]. In mammals, four SUMO members have been identified and designated as SUMO-1 to SUMO-4 [6]. SUMO-1 to -3 are ubiquitously expressed, whereas SUMO-4 expression is restricted to specific tissues such as the spleen, kidney, and lymphatic nodes [9]. SUMO-2 (Smt3A) is 92 amino acids, and SUMO-3 (Smt3B) is 93 amino acids long. Due to their close homology, SUMO-2 and SUMO-3 are frequently referred to as SUMO-2/3 since they share ~97% sequence identity in humans [6]. Notably, SUMO-2 and SUMO-3 can form poly-SUMOylation chains that are involved in various cellular processes including replication, protein recycling, mitosis, and meiosis. SUMO-2 is more abundant than SUMO-3 and plays an important role in synaptic plasticity [10–12]. Moreover, SUMO-2 null mice present developmental impairments and are not viable beyond embryonic day 10.5, as result of poor proliferation and increased cell death [10]. In contrast, SUMO-1 null mice are viable since this deficiency can be compensated by SUMO-2/3 [9, 13]. SUMO-1 shares only ~48% and 46% sequence identity with SUMO-2 and SUMO-3, respectively [14, 15]. On the other hand, SUMO-4 contains a unique proline residue (P90) that prevents the efficient maturation required for E2 conjugation [16]. Furthermore, SUMO-4 has been associated with type 1 and type 2 diabetes [17]. Table 1 lists the proteins involved in each step of the SUMOylation cycle.

SUMO-Activating, Conjugating, and Ligating Enzymes

SUMOylation involves a series of enzymatic reactions, which start with the cleavage of the inactive precursor form of SUMO by the SENP enzyme, exposing a conserved di-Glycine motif at the C-terminus. Then, the di-Glycine motif of SUMO is activated by the heterodimer SUMO-activating enzyme subunit 1 and 2 (SAE1/2) in an ATP-dependent process [18]. This activation results in the formation of a SUMO adenylate intermediate, followed by the SUMO-thioester bond with E1, which precedes the SUMO-thioester bond with SAE2. After that, SUMO is transferred to a cysteine residue on ubiquitin-conjugating 9 (Ubc9) [18]. Finally, either E3 ligase or Ubc9 covalently forms an isopeptide bond between SUMO and the lysine residues on the target protein, following a Ψ-K-X-E pattern, where Ψ is a large amino acid in the target protein [19] (Figure 1). It is well established that the SUMOylation state in a protein can either inhibit or promote interactions with other proteins, triggering the assembly of complexes. Therefore, the SUMOylation is reversible and effectively controlled by distinct mechanisms [20].

Table 1 Proteins involved in each step of the SUMOylation cycle, including SUMO isoforms, SUMO-conjugating enzymes, and de-SUMOylating enzymes

Category	Name	Function(s)
SUMO isoforms	SUMO-1 SUMO-2 SUMO-3 SUMO-4	Small ubiquitin-like modifiers; covalently attached to target proteins, influencing their function, localization, and stability.
SUMO-activating enzyme	SAE1/2 (SUMO E1)	SUMO-activating enzyme 1/2; activates SUMO proteins by forming a high-energy thioester bond with SUMO, which is essential for SUMOylation. This enzyme is the first step in the SUMOylation cycle.
SUMO-conjugating enzyme	Ubc9 (SUMO E2)	Ubiquitin-conjugating enzyme 9; transfers the activated SUMO from SUMO E1 to target proteins. Ubc9 is the primary E2 enzyme responsible for the conjugation of SUMO to substrates.
SUMO ligase	SUMO E3	SUMO E3 ligase; enhances the transfer of SUMO from Ubc9 to target proteins.
De-SUMOylating enzymes	SENP1 SENP2 SENP3 SENP5 SENP6 SENP7	SUMO-specific proteases; remove SUMO from substrates. Involved in maintaining the balance of SUMOylation in the cell.

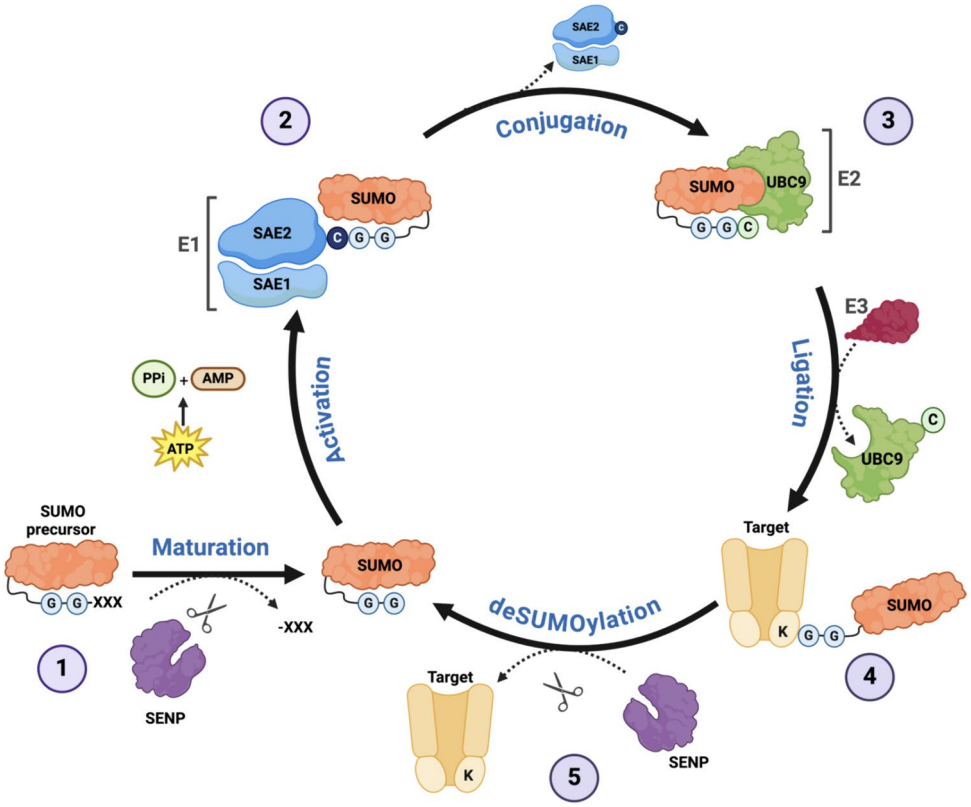


Fig. 1 SUMOylation and deSUMOylation process. SUMOylation and deSUMOylation are dynamically balanced processes under physiological conditions. (1) SUMOylation starts with the maturation of SUMO proteins through proteolytic cleaved by SENP enzyme. (2) The next step involves the activation of mature SUMO by the SUMO E1 enzyme (SAE1/SAE2) in an ATP-dependent process. (3) Once activated, SUMO proteins are conjugated to the cysteine residue of the E2 enzyme Ubc9, (4) while SUMO E3 ligase catalyzes the ligation of SUMO to a specific residue in the target protein. (5) Finally, SUMO proteins are cleaved off from their target proteins by SENPs in a process known as deSUMOylation, resulting in free SUMO proteins available for another catalytic cycle. ATP, adenosine triphosphate; SUMO, small ubiquitin-like modifier; SAE1, SUMO-activating enzyme subunit 1; SAE2, SUMO-activating enzyme subunit 2; Ubc9, ubiquitin-conjugating enzyme 9. Figure was created using Biorender.com

SENP Family

SUMO-specific proteases (SENPs) are cysteine proteases that cleave inactive SUMO forms. Furthermore, SENPs also catalyze the deconjugation of SUMO proteins from their substrates, which is an important activity for maintaining the balance between SUMOylated and deSUMOylated proteins [21]. In mammals, there are six SENP members (SENP1–3 and SENP5–7) that exhibit differences in their cellular distribution, specificity for different SUMO members, and regulation of SUMO maturation [22–25]. SENPs have both endopeptidase and isopeptidase activities [26]. Endopeptidase activity involves cleaving the SUMO precursor or pro-SUMO at the C-terminal, while isopeptidase activity deconjugates SUMO proteins from the target protein (Figure 1) [26]. It has been demonstrated that SENP1 prefers pro-SUMO-1 > pro-SUMO-2 > pro-SUMO-3, whereas SENP2/5 prefers pro-SUMO-2 > pro-SUMO-1 > pro-SUMO-3. This preference is linked to amino acid sequences at the C-terminal [27]. Notably, SENP6 and SENP7 exhibit poor endopeptidase activity, cleaving pro-SUMO-1, -2, or -3 at a low detectable rate [28]. On the other hand, the isopeptidase activity of SENP1 shows a preference for SUMO-2 > SUMO-3 > SUMO-1, while SENP2/5 has similar activity towards SUMO-2 and SUMO-3 [29]. Finally, SENP6 and SENP7 show notable isopeptidase activity for di-SUMO-2, di-SUMO-3, and poly SUMO chains [28, 30, 31]. In addition to the protective role of SUMOylation in a plethora of cellular processes in eukaryotes, including chromosome cohesion, mitosis, and transcription [32, 33]. In the central nervous system, SUMO-2, -3, and E2 SUMO-conjugating enzyme Ubc9 colocalize with synaptophysin and PSD95, suggesting their expression in pre- and post-synaptic neurons [34–37]. The expression of SUMO-1 in presynaptic sites has been controversial due to the lack of effective antibodies [34]. However, the development of a nanochannel device highly sensitive to the last 12 amino acids in the C-terminal of mature SUMO-1 allows its detection by

immunofluorescence imaging [38]. Furthermore, SENP1, SENP6, and SENP7 colocalize with synaptophysin and drebrin, a postsynaptic marker in hippocampal neurons of mice [22]. These findings suggest a potential role of deSUMOylation machinery in presynaptic sites. On the other hand, activation of the metabotropic glutamate receptor (mGluR5) leads to the accumulation of SENP1 and Ubc9 in dendritic spines, resulting in the modulation of synaptic transmission and plasticity [39]. Additionally, mGluR1 activation prevents SENP1 accumulation in the synapses [40]. The accumulation of SENP1 mediated by mGluR5 and mGluR1 depends on protein kinase C (PKC) and Ca^{2+}/calmodulin protein kinase II (CaMKII) activity [40]. Indeed, PKC phosphorylates mGluR7, facilitating SUMOylation by Ubc9 and deSUMOylation by SENP1, which results in its internalization in hippocampal neurons [41]. On the other hand, it has been reported that the SUMOylation is linked to cellular stress as heat shock [33], DNA damage [42], cancer [43, 44], and oxidative stress [45]. In this sense, oxidative stress influences SUMOylation through various mechanisms. For instance, high concentrations of H_2O_2 increase protein SUMOylation, while low concentrations induce protein deSUMOylation by inducing a disulfide bond between the catalytic cysteines of the SUMO E1 and E2 enzymes [46]. Moreover, H_2O_2 can also activate deSUMOylation machinery, by reversibly oxidizing a cysteine motif in the SENP3 regulates its stability and localization protecting this protease from proteasomal degradation [47, 48]. On the other hand, ROS inactivate SENPs, either reversibly through disulfide bond-induced dimerization or irreversibly by overoxidation of their catalytic cysteine [49]. These findings show the dynamic nature of the SUMOylation/deSUMOylation processes under oxidative stress conditions.

Expression of SUMOylation and DeSUMOylation Machinery in the Nervous System

The expression of the SUMOylation and deSUMOylation machinery has been reported in the sensory system. For instance, Ubc9, SUMO-1, and SENP1 are expressed in the spinal cord and in small-, medium-, and large-sized DRG neurons [50, 51]. Additionally, databases of deep RNA-sequencing show that Ubc9, SUMO-1, and SENP1 genes are expressed across different subpopulations of DRG neurons, including peptidergic, C-low threshold mechanoreceptors (LTM), non-peptidergic, Aβ- rapidly adapting (RA) + Aδ-LTMRs, and others [52]. Under pain conditions, alterations in the SUMOylation and deSUMOylation machinery become evident. Carrageenan-induced inflammatory pain increases the levels of SUMO-1-conjugated proteins in DRGs [50, 53]. Similarly, it has been reported that nerve injury increases the expression of Ubc9 in DRG neurons in mice [52], suggesting that the changes in the expression of SUMOylation and deSUMOylation machinery play an important role in pain signaling. While data for SENP expression in the DRG neurons has been limited due to its relatively understudied role in pain, available evidence suggests that SENP expression is directly proportional to the chronicity of the neuropathic pain [52], which could imply a compensatory mechanism to counteract prolonged SUMOylation effects. These findings, thus far, suggest that SUMOylation and deSUMOylation processes may play an essential role in modulating cellular processes in pain pathways. However, their specific contribution to the regulation of ion channels during chronic pain remains to be fully elucidated. Therefore, the following sections will focus on the current evidence on SUMOylation/deSUMOylation processes in ion channels and their potential implications in pain signaling.

Pain Modulation Through SUMOylation and DeSUMOylation of Voltage-Gated Sodium Channels

$Na_V1.7$ Channels

The VGSC family comprises nine members ($Na_V1.1$–1.9), with at least 3 subtypes ($Na_V1.7$, 1.8, and 1.9) being highly expressed in nociceptors, where they control action potential (AP) generation and frequency of firing [3, 54]. Specifically, $Na_V1.7$ is a tetrodotoxin (TTX)-sensitive channel encoded by the gene *SCN9A* [55]. $Na_V1.7$ channels are expressed in relevant sites for nociceptive transmission, including primary sensory neurons of dorsal root ganglia (DRG) and trigeminal ganglion (TG) [56–58] and the superficial lamina of the spinal dorsal horn [59]. We have reported that $Na_V1.7$ trafficking, localization, and conductance are strongly regulated by a variety of post-translational modifications, including SUMOylation. In the context of pain, $Na_V1.7$ turnover is tightly regulated by the SUMOylation state of its interacting protein, the collapsin response mediator protein 2 (CRMP2). The addition of SUMO-1 to CRMP2 increases NaV1.7 membrane localization and channel activity [60, 61]. In contrast, increasing the expression and activity of SENP1 promotes CRMP2 deSUMOylation resulting in reduced NaV1.7 channel expression [60]. CRMP2 is phosphorylated at Ser522 by cyclin-dependent kinase 5 (Cdk5) and then SUMOylated at lysine 374 (K374) by Ubc9 [61–64]. Disruption of the Ubc9-CRMP2 interaction with a decoy peptide reversed the nociceptive behavior in a neuropathic pain model [64, 65]. Additionally, CRMP2 lacking SUMOylation recruits the endocytic complex (Numb, Eps15, and Nedd4-2) to promote clathrin-mediated endocytosis of $Na_V1.7$ in female

mice [62, 66, 67]. These findings suggest that preventing the SUMO conjugation of CRMP2 by Ubc9 decreases $Na_V1.7$ expression and conductance, resulting in an antinociceptive effect in rodents. Interestingly, compound 194 disrupts the CRMP2-Ubc9 interaction by targeting the SUMOylation site in CRMP2 (K374) and reduces $Na_V1.7$ currents and neuronal excitability in several pain models [64–66, 68–70]. Furthermore, CRMP2 SUMO deficiency in sensory neurons of TG results in decreased $Na_V1.7$ currents and excitability [69]. In this context, intranasal delivery of compound 194 alleviated pain caused by chronic constriction injury of the infraorbital nerve in rats [69], supporting the notion that inhibiting SUMO conjugation of CRMP2 by Ubc9 with compound 194 exerts a beneficial effect in chronic pain conditions. Accordingly, we reported that reducing CRMP2 SUMOylation by replacing K374 with alanine reversed and prevented neuropathic pain-induced mechanical allodynia, suggesting that the SUMOylation of CRMP2 represents a promising strategy for pain treatment [70, 71]. Additionally, we found that increasing the isopeptidase activity of SENP1 through CRISPR/Cas9 mediated SENP1 overexpression which disrupts the interaction between CRMP2 and $Na_V1.7$ by inducing CRMP2 deSUMOylation and NaV1.7 clathrin-mediated endocytosis [60]. Collectively, these findings show that the SUMOylation/deSUMOylation process impacts $Na_V1.7$ plasma membrane expression, current density, and neuronal excitability, which converge to regulate nociceptive behaviors during chronic pain conditions.

Pain Modulation Through SUMOylation and DeSUMOylation of Voltage-Gated Calcium Channels

$Ca_V1.2$ Channels

VGCCs are grouped into two subfamilies according to their activation thresholds: high voltage-activated (HVA) and low voltage-activated (LVA). The HVA subfamily encompasses $Ca_V1.1$-1.4 (L-type), $Ca_V2.1$, $Ca_V2.2$, and CaV2.3 (P/Q-, N-, and R-types, respectively) channels, while the LVA subfamily comprises $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ channels (T-type) [72]. The SUMOylation of collapsin response mediator protein 4 (CRMP4) increases calcium influx through $Ca_V1.2$ channels, resulting in heightened thermal pain sensitivity [73]. These findings show that CRMP4 interacts with $Ca_V1.2$ to regulate its function and signal transmission during pain. The SUMOylation site in CRMP4 has been identified as K374 residues [63]: a GST-pulldown assay reported that CRMP4 interacts with SUMO-1 to -3, while mutation of K374 residues abolished this interaction, which confirmed that K374 mediates the SUMOylation of CRMP4. In contrast, a study reported that overexpression of deSUMOylated CRMP4 induces thermal allodynia compared to wild-type CRMP4 [73]. Considering that deSUMOylation of CRMP4 is linked with neurite outgrowth [73], it is possible that aberrant neurite outgrowth explains why deSUMOylated CRMP4 increases pain in naïve animals. However, further research is needed to determine the precise role of deSUMOylation of CRMP4 in the pathophysiology of neuropathic pain, where the neurite outgrowth not only contributes to nerve injury but also recovery.

$Ca_V2.2$ Channels

The neuronal channels (N-type) $Ca_V2.2$ are mainly located in the synapse and form complex interactions with other proteins such as soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, syntaxin-1 and synaptosomal-associated protein 25 kDa (SNAP-25), calcium/calmodulin-dependent serine protein kinase (CASK), CRMP-2, CaMKII, and Rab3-interacting molecule 1α (RIM1) [74]. $Ca_V2.2$ channels play an important role in regulating intracellular calcium concentrations and neurotransmitter release into the synaptic cleft. We have reported that phosphorylation of CRMP2 at serine 522 (S522) leads to the interaction between $Ca_V2.2$ channels and CRMP2 [75]. In this regard, the administration of the small molecule *S*-lacosamide inhibits cyclin-dependent kinase 5 (Cdk5)-dependent phosphorylation of CRMP2, resulting in a reduction of the membrane localization of the $Ca_V2.2$ channel [76]. We also reported that CRMP2-$Ca_V2.2$ interaction is disrupted by a 15-amino acid peptide derived from CRMP2 and linked to the cell-penetrating peptide TAT. Our findings show the 15 amino acid peptides decreased $Ca_V2.2$ plasma membrane expression, Ca^{2+} current density, and excitatory synaptic transmission in vitro and in vivo [77]. Additionally, the application of this peptide on spinal cord slices significantly decreased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) with no changes in the amplitude, suggesting that the 15-amino acid domain of CRMP2 does not exert a postsynaptic effect in the spinal dorsal horn [77]. Remarkably, the interaction of CRMP2-$Ca_V2.2$ is disrupted by the systemic administration of CBD3063, a peptidomimetic small molecule. CBD3063 selectively ameliorates N-type Ca^{2+} currents in DRG neurons, resulting in pain relief in inflammatory and neuropathic pain models [78]. While SUMOylation of CRMP2 regulates the trafficking and activity of $Na_V1.7$ channels in DRG neurons, it remains elusive whether $Ca_V2.2$ channels are directly SUMOylated under physiological or pathological conditions. In a heterologous system, the co-expression of SUMO-1, but not the SUMO conjugation-deficient mutant lacking the double-glycine motif (SUMO-1ΔGG), increased $Ca_V2.2$ Ca^{2+} current density in the presence of

Ubc9 [79]. However, a single mutation of K394, located in the I–II loop, resulted in the loss of function of $Ca_V2.2$ channels [80]. Furthermore, SUMO-1 and Ubc9 produced a hyperpolarizing shift in the midpoint ($V_{1/2}$) of activation. However, SUMO-1 or SENP1 alone did not affect N-type currents, suggesting that levels of endogenous SUMO-1 are not enough to be conjugated with $Ca_V2.2$ by Ubc9 or removed by SENP-1 [79]. On the other hand, we demonstrated that DRG neurons transfected with CRMP2 show a lower intracellular calcium concentration ($[Ca^{2+}]_i$), while transfection with CRMP2AAA, a SUMO resistant mutant, did not induce any change in ($[Ca^{2+}]_i$ [80]. These results suggest that CRMP2 SUMOylation is inversely correlated with Ca^{2+} influx [80]. Interestingly, we found that SENP1 overexpression did not change $[Ca^{2+}]_i$ or the surface expression of $Ca_V2.2$ channels in DRG neurons [60]. In line with these findings, a study reported that superior cervical ganglion neurons transfected with SUMO-1 show an increase in the paired excitatory postsynaptic potential (EPSP) ratio and reduced the inter-stimuli intervals, implicating presynaptic transmitter release likely involving $Ca_V2.2$ channels [79]. Collectively, our findings and those of others support the notion that other SENPs could effectively modulate CRMP2 SUMOylation and consequently the function and expression of $Ca_V2.2$ channels. However, further research is required to explore whether CRMP2 deSUMOylation by SENPs could represent a strategy to reduce neuronal excitability. We have demonstrated that CRMP2 phosphorylation at S522 is required for its interaction with $Ca_V2.2$ channels under neuropathic pain conditions, where the $Ca_V2.2$ channels are upregulated [81]. Furthermore, phosphorylation of S522 is required for the addition of a SUMO group to CRMP2 in K374, which upregulates $Na_V1.7$ activity and expression in DRG neurons [62, 64]. However, whether $Ca_V2.2$ channels expressed in sensory neurons are directly SUMOylated remains unknown. We have identified that the first loop in both $Na_V1.7$ and $Ca_V2.2$ channels is a site of interaction with CRMP2. CRMP2 interacts with the first intracellular loop (706–720 a. a.) of the $hNa_V1.7$ channel with a K_D ~1 µM [82]. On the other hand, CRMP2 binds to the distal C-terminal region of the $Ca_V2.2$ channel with a K_D of 0.3 µM and with lower affinity to the first loop with a K_D of ~24 µM [83]. The region in CRMP2 interacting with CaV2.2 is limited to 15 a. a. (484_ARSRLAELRGVPRGL_498), located towards its C-terminus [83]. In chronic pain, NaV1.7 and CaV2.2 channels are highly active due to their overexpression along the sensory neurons. NaV1.7 mainly contributes to the neuronal excitability and CaV2.2 to the transmitter release. It is possible that CRMP2 localization, patterns of PTM may facilitate a preferent interaction for a specific channel depending on the insult type. Moreover, in chronic pain states, a large variety of ion channels (see below) are dysregulated, contributing to neuronal excitability.

$Ca_V3.2$ Channels

Low-voltage–activated T-type calcium channels regulate neuronal excitability and are involved in exocytosis [84, 85]. In pain conditions, T-type channels contribute to neurotransmitter release in the spinal dorsal horn [86]. Specifically, the $Ca_V3.2$ isoform has been reported to play a significant role in pain signaling as silencing or inhibiting $Ca_V3.2$ channels produce a pain-free phenotype characterized by an increase in mechanical and thermal thresholds [87, 88]. $Ca_V3.2$ channels are ubiquitinated by the WW domain-containing E3 ubiquitin protein ligase 1 (E3 ubiquitin ligase WWP1) in the III–IV intracellular linker [89]. Knockdown of ubiquitin-specific peptidase 5 (USP5) increases $Ca_V3.2$ ubiquitination and decreases $Ca_V3.2$ channel activity, producing analgesia in inflammatory and peripheral injury models [89]. Another study reported that SUMOylation regulates the interaction between $Ca_V3.2$ and USP5, with residue K113 being the target site for USP5 SUMO-1/2 conjugation [90]. Notably, the interaction between $Ca_V3.2$ and the SUMO-resistant USP5-K113R mutant was strong, suggesting that SUMO modification of USP5 reduces its affinity for $Ca_V3.2$ channels [90]. The study revealed that peripheral nerve damage reduces the SUMOylation of USP5, which in turn increases its interaction with $Ca_V3.2$, causing the channel to remain active and present in the plasma membrane for an extended period [90]. Therefore, it should be noted that inhibiting deSUMOylation of USP5 could be a potential strategy to enhance USP5 interactions with the $Ca_V3.2$ channel and consequently promote antinociceptive effects. It is still unclear as to whether the SUMOylation of USP5 post-nerve injury is due to a decrease in SUMO ligase activity or an increase in SENP activity. Regardless, it is essential to emphasize that the processes of SUMOylation and de-SUMOylation greatly influence the functionality of Ca_V channels.

Pain Modulation Through SUMOylation and DeSUMOylation of Potassium Channels

Kir7.1 Channels

The potassium (K^+) channel family comprises approximately 80 members, divided into voltage-gated (K_V), two-pore (K_{2P}), calcium-activated (K_{Ca}), and inward-rectifying (K_{ir}) channels [91]. It has been demonstrated that dysfunction in K^+ channels is associated with the development and maintenance of pathological conditions like neuropathic pain [92].

Specifically, Kir7.1 channels are expressed in the neurons of the spinal cord [93]. In naïve animals, genetic silencing or pharmacological blockade of Kir7.1 contributes to the development of mechanical allodynia, suggesting that Kir7.1 downregulation promotes pain behaviors [94]. SUMO-1 and Kir7.1 colocalize in the spinal cord of neuropathic animals, implying that SUMO-1 may regulate the function or expression of Kir7.1 in pain conditions [94]. Administration of GA, ubiquitin-activating enzyme (E1) inhibitor, or 2-D08, a Ubc9 inhibitor, prevented Kir7.1-SUMOylation and increased the expression of Kir7.1 in the plasma membrane of the spinal cord in neuropathic animals [94]. The research suggests that inhibiting Kir7.1 SUMOylation could reduce sensory neuron excitability by amplifying K^+ currents and affecting action potential duration or resting membrane potential. However, it is important to conduct additional studies to identify the target amino acids for SUMOylation in Kir7.1 and investigate if deSUMOylation via specific SENPs contributes to pain.

Kv2.1 Channels

Voltage-gated potassium channel subunit 2.1 ($K_V2.1$) plays a crucial role in the nervous system by controlling activity-dependent excitability [95]. SUMO proteins modulate $K_V2.1$ function through reversible, enzyme-mediated attachment to specific lysine residues [96]. SUMOylation of $K_V2.1$ in hippocampal neurons influences neuronal firing by altering the half-maximal activation voltage of the channels. Moreover, SUMO proteins and $K_V2.1$ channel interact both within and outside channel clusters on the neuronal surface. Heterologous expression of $K_V2.1$ channels revealed that residue K470 undergoes SUMOylation [96]. Although $K_V2.1$ channels are composed of four subunits, no more than two non-adjacent subunits are simultaneously modified by SUMO [96, 97]. Indeed, SUMOylation at one site shifts half-maximal activation voltage by 15 mV, while modification at two sites results in a full response [96]. Collectively, these findings suggest that SUMOylation exerts control over neuronal excitability by modulating $K_V2.1$ channels at the cell surface. The widespread presence of the SUMO pathway, combined with the extensive tissue expression and physiological significance of $K_V2.1$ channels, suggests that the regulation of excitability observed in hippocampal neurons might also occur in other structures, such as DRG neurons. Indeed, it has been reported that $K_V2.1$ channels are highly expressed in medium- and large-diameter DRG neurons [98]. However, it is important to highlight that the effects of SUMOylation on the function of $K_V2.1$ channels in sensory neurons are still largely unknown. Abnormal regulation of $K_V2.1$ leads to potassium ions outflow, triggering apoptotic cascades, mitochondrial dysfunction, and inflammation responses, contributing to the pathological process of diabetic periphery neuropathy [99, 100]. Therefore, it is likely that the SUMOylation of $K_V2.1$ has a broad impact on pain signaling at the DRG level. However, further research is still required to test this.

$K_V4.2$ Channels

The regulation of the function and expression of voltage-gated potassium channels subunit 4.2 ($K_v4.2$) by SUMOylation has been studied in heterologous systems [101–104]. In HEK cells, $K_V4.2$ is SUMOylated at residues K437 and K579, which are conserved mouse, rat, and human [101]. Notably, SUMOylation at K579 decreases the current amplitude (I_A) of $K_v4.2$, while SUMOylation at K437 increases the surface expression of $K_V4.2$ by reducing its internalization [102]. These findings suggest that SUMOylation can independently regulate $K_v4.2$ surface expression and I_A to promote opposing effects. Moreover, $K_V4.2$ channels are modified by SUMO-2/3, but not by SUMO-1, in hippocampal neurons [102]. These results are particularly relevant considering that K_V4 channels are expressed in excitatory interneurons in spinal lamina II and $K_V4.2$ knockout increases the excitability of dorsal spinal neurons, resulting in mechanical and thermal hypersensitivity in mice [101, 105]. However, it is important to note that the effects of SUMOylation or deSUMOylation on the function and expression of K_V4 channels in sensory neurons remain largely unknow.

$K_{2P}1$ Channels

The potassium two-pore domain (K_{2P}) channels are constitutively open, allowing K^+ ions to leak outward. These channels help sustain the hyperpolarized resting membrane potential and impact the cell's electrical characteristics. SUMOylation of $K_{2P}1$ has been shown to reduce K^+ currents in oocytes [104]. In contrast, a mutant $K_{2P}1$ channel in which the K274 was replaced with glutamate produced large K^+ currents. Additionally, the removal of SUMO from K274 by SENP1, but not by the inactive C603S-SENP1 enzyme, results in intact K^+ currents through $K_{2P}1$. These findings suggest that SUMOylation in K274 is responsible for the silencing of $K_{2P}1$ channels [104]. The expression and function of $K_{2P}1$ channels are altered during inflammatory and neuropathic pain conditions [106–108]. However, whether SUMOylation plays a role in the mechanisms underlying the altered function of $K_{2P}1$ in sensory neurons during chronic pain conditions remains unknown.

Pain Modulation Through SUMOylation and DeSUMOylation of Other Ion Channels

TRPV1 Channels

Transient receptor potential vanilloid 1 (TRPV1) is a calcium-permeable nonselective ion channel activated by capsaicin, noxious heat (> 43 °C), pH (<5.9), and noxious stimuli [109, 110]. TRPV1 channels are abundantly expressed in small sensory C-fibers in DRG [111] and TG neurons [112]. In the spinal cord, TRPV1 is localized in both pre- and post-synaptic neurons in lamina I and II, as well as in astrocytes [113]. Beyond the spinal level, the TRPV1 receptor is also found at supraspinal levels such as the periaqueductal grey and medial prefrontal cortex, contributing to the descending modulation of nociception [114]. In sensory neurons, TRPV1 channels undergo SUMOylation by Ubc9 [51] and deSUMOylation by SENP1 [50]. Acute and chronic peripheral inflammation increases TRPV1 SUMOylation in DRGs [50]. Conditional deletion of Ubc9 in DRG neurons decreases capsaicin-evoked Ca^{2+} transients, Ca^{2+} currents, and deactivation time of TRPV1 channels, while the expression of deSUMOylated TRPV1 reduces nocifensive responses induced by intraplantar capsaicin injection [51]. Additionally, knockout of SENP1 or SUMOylation of TRPV1 has been linked to increased thermal hyperalgesia in an inflammatory pain model [50]. In contrast, another study reported that TRPV1 SUMOylation specifically reduces TRPV1 activation induced by heat, but not by capsaicin, protons, and voltage [112]. It has been reported that replacing residue K822 with an arginine reduces the response to heat but does not modify the membrane expression of TRPV1 channels or affect their voltage dependence, capsaicin sensitivity, or proton-induced currents [50]. Conversely, the mutation of residue K324 to an alanine decreases the sensitivity to capsaicin [51]. Collectively, these findings highlight that the SUMOylation of specific residues of TRPV channels regulates different functions of the channel. Indeed, in vivo studies have reported that nocifensive responses induced by intraplantar capsaicin are decreased in sensory neurons from (SNS)-$TRPV1^{K325A}$ knock-in mice compared to controls [51]. Similarly, $TRPV1^{K822R}$ expression in wild-type mice significantly reduces the thermal hypersensitivity induced by carrageenan injection [50]. These data emphasize that preventing TRPV1 SUMOylation in inflammatory pain models results in an antinociceptive effect. In contrast, it has been reported that increased deSUMOylation processes are associated with the loss of epidermal nerve fibers in chronic stages of diabetic peripheral neuropathy (DPN), which is linked with the development of mechanical and thermal hypoalgesia [115]. Furthermore, SNS-$TRPV1^{K325A}$ mice fail to develop hypoalgesia in the late phases of DPN, without changes in the peripheral nerve fiber density [51]. These findings suggest that during DPN, SUMOylation increases the function of TRPV1, while deSUMOylation of metabolic enzymes induces the loss of sensory neurons by increasing oxidative stress, thereby hastening DPN [51].

TRPA1 Channels

Transient receptor potential ankyrin 1 (TRPA1) is a nonselective cation channel activated by chemical irritants such as mustard oil and allicin or noxious cold temperatures (10–20 °C) [116]. In the sensory system, TRPA1 channels are expressed in DRG neurons, nerve roots, intestinal neurons, spinal cord neurons, and skin basal keratinocytes [117, 118]. High expression of TRPA1 has been linked with cold hypersensitivity in inflammatory, neuropathy, and cancer-induced pain [119]. Heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) is a member of the RNA binding protein family involved in RNA stability regulation [120, 121]. Indeed, it has been reported that hnRNPA1 can regulate the expression of TRPA1 [118], through various PTMs including phosphorylation, SUMOylation, and glycosylation [121, 122]. Specifically, SUMOylation of hnRNPA1 at K113 and K183 regulates extracellular vesicle sorting and mRNA translation, respectively [123, 124]. SUMOylation of hnRNPA1 prolongs the half-life of TRPA1 mRNA by reducing its degradation, thereby increasing the TRPA1 mRNA stability via the coding sequence (CDS) rather than through its interaction with 5′ or 3′ untranslated regions (UTRs), suggesting that SUMO-1 stabilizes TRPA1 mRNA [118]. Moreover, overexpression of SUMO-1 increased the interaction between hnRNPA1 and TRPA1 mRNA, while hnRNPA1 knockdown prevented this effect [118]. In this context, CFA-induced inflammatory pain increases hnRNPA1 SUMOylation in DRGs, correlating with the development of cold hypersensitivity [118]. Moreover, overexpression of hnRNPA1 in DRGs facilitated CFA-induced increased TRPA1 protein expression at the plasma membrane, an effect absent when expressing the hnRNAPA1 SUMO-null mutant K3R, the SUMOylation site that promotes hnRNPA1 cytoplasmic localization [118]. These findings were consistent with a higher response of TRPA1 to allyl isothiocyanate in HEK cells overexpressing hnRNAP1 in the presence of SUMO-1 compared to the K3R mutants [118]. Taken together, these findings suggest that hnRNAP1 SUMOylation enhances TRPA1 channel activity in inflammatory pain, contributing to the maintenance of CFA-induced cold hypersensitivity.

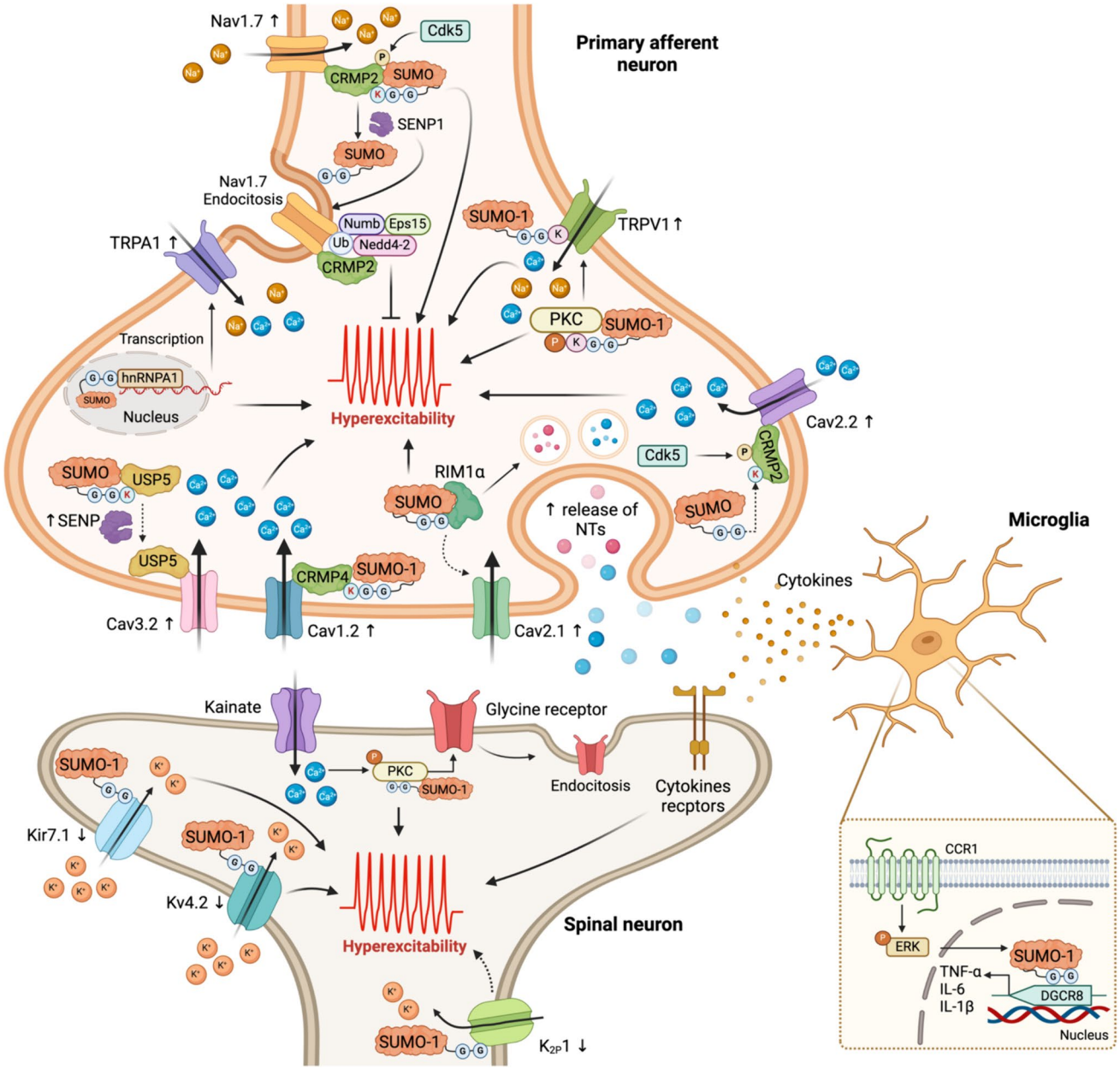
HCN Channels

The hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are homo- or heterotetramers assembled from four isoforms (HCN1–4), with each subunit being composed of six transmembrane segments [125]. HCN channels mediate an inward cation current (I_h), allowing the influx of Na^+ and K^+ ions after membrane hyperpolarization, which is regulated by cyclic nucleotides, particularly cyclic adenosine monophosphate (cAMP) [125, 126]. It has been reported that HCN2 channels are SUMOylated by both SUMO-1 and SUMO-2/3 in rodent brains [127]. Furthermore, SUMOylation of HCN2 at K669 increases its surface expression and the hyperpolarization-activated current (I_h) in heterologous expression systems [127]. Inflammatory pain increases SUMO-2/3-induced SUMOylation of the HCN2 channel in small neurons from the L5 nerve, while SUMO-1-induced SUMOylation of HCN2 is reduced in medium and large neurons from the L5 nerve 1 day post CFA-injection. These findings suggest that the SUMOylation of these channels could be contributing to the hyperalgesia observed after CFA injection [128]. Conversely, another study reported that CFA injection increases HCN2 SUMOylation in medium and large cells at day 1 in the lumbar nerve L6, but not in the L4 nerve [129]. Therefore, these findings suggest that inflammation may induce differential expression of SUMO forms, which could have varying impacts on the function of ion channels during inflammation-induced pain. The discrepancies between these studies could be attributed to the fact that sensory fibers from the L5 and L6 nerves mostly project to the hind paw and visceral organs, respectively [129], while sensory fibers from the L4 nerve project to the hindlimb, knee, and hip joint [129]. This implies that DRG neurons may exhibit a unique and dynamic pattern of HCN2 SUMOylation. Another explanation could be the specificity of SENPs and E3 ligases for different SUMO isoforms [29, 130]. Considering these factors, it is likely that multiple mechanisms regulate the function and expression of HCN2 during CFA-induced inflammation.

Pain Modulation Through the SUMOylation of Kinases and RIM1

Protein kinase C (PKC) is a family of serine/threonine kinases divided into classic (α, βI, βII, and γ), novel (δ, ε, θ, and Ƞ) and typical (ζ and ι/λ) based on their calcium and diacylglycerol dependence [131]. Genetic [132] and pharmacological [133] studies have demonstrated that these different PKC isoenzymes play important roles in several cellular functions such as proliferation, apoptosis, neuronal excitability, and neurotransmitter release [134, 135]. It has been reported that the activation of kainate, but not AMPA or NMDA receptors, induces glycine receptor endocytosis in a calcium- and PKC-dependent manner, resulting in reduced glycine receptor activity in the spinal cord [136]. Interestingly, kainate treatment decreased the expression of glycine receptors in neurons overexpressing SENP1, but not in SENP1 knockout neurons. Furthermore, the substitution of K465 with arginine in PKC prevents its SUMOylation and abolishes kainate-induced glycine receptor endocytosis, suggesting that K465 is the key site of PKC SUMOylation in this kinase [136]. Collectively, these findings suggest that kainate-induced glycine receptor endocytosis requires PKC activation in response to intracellular Ca^{2+} elevations and the subsequent SENP1-dependent deSUMOylation of PKC. Several studies have demonstrated the role of PKCε in inflammation-induced hyperalgesia [136]. Notably, PKCε SUMOylation increases in response to peripheral inflammation [53, 135]. It has been reported that phosphorylation of PKCε at serine 729 (S729) promotes its SUMOylation at K534, resulting in the interaction between PKCε and TRPV1 [53]. In this context, expression of SUMO-resistant PKCε failed to induce inflammatory-induced thermal hyperalgesia [53]. Furthermore, TRPV1 knockout mice injected with SUMO-resistant PKCε exhibited blunted thermal hyperalgesia compared to wild-type mice, suggesting that PKC SUMOylation modulates thermal hyperalgesia through TRPV1 [53].

Extracellular signal–regulated kinases (ERK), a member of the family of mitogen-activated protein kinases (MAPK), is another important kinase in pain signaling [137]. ERK is expressed in the spinal cord, astrocytes, microglia, and DRG neurons after the peripheral noxious stimulation [138]. Phosphorylation of ERK has been related to the activation and inflammatory response of microglia, as well as increased nociceptive behaviors in neuropathic pain conditions [138]. The activation of spinal C-C chemokine receptor type 1 (CCR1) increases the levels of phosphorylated ERK, leading to the SUMOylation of DiGeorge syndrome critical region 8 (DGCR8) in microglia after exposure to LPS [139]. DGCR8 SUMOylation induced by SUMO-1 stabilizes its expression and reduces its ubiquitin-dependent degradation, resulting in the release of proinflammatory cytokines such as TNF-α, IL-6, and IL-1B [139]. Interestingly, SUMOylation of DGCR8 was prevented by CCR1 knockdown or an ERK-inhibitor, suggesting that CCR1/p-ERK/DGCR8 axis plays an important role in neuropathic pain–induced microglial activation [139]. While this study underscores the significance of SUMOylation in glial cells, it also opens up new research directions. These include determining the SUMOylation site in DGCR8 and assessing the impact of overexpressed SENPs on microglial activation and inflammation triggered by neuropathic pain.



RIM1

It is well known that neurotransmitter release is mediated by synaptic vesicles, which are strictly dependent on Ca^{2+} domains in the vicinity of the presynaptic terminal [140]. The role of Rab3-interacting molecule 1α (RIM1α) in the active zone is essential for presynaptic function. In this context, the increase in $[Ca^{2+}]_i$ levels induces vesicle priming, where RIM1α interacts with Munc13-1. Then, RIM1α engages Rab3α in vesicle docking and synaptotagmin during exocytosis [141]. In cortical neurons, RIM1α is SUMOylated at K502, facilitating its interaction with $Ca_V2.1$ through its PDZ domain. This interaction promotes Ca^{2+} influx that precedes the fast phase of vesicle exocytosis of the readily releasable pool. In contrast, deSUMOylation of RIM1α by SENP1 reduces its binding to the $Ca_V2.1$ channel [141]. In the absence of SUMOylation, RIM1α still facilitates the docking/priming of synaptic vesicles and maintains the active zone structure, suggesting that SUMOylation acts as a molecular switch in the active zone, controlling interactions and defining the function of different protein pools [141]. Although this evidence demonstrates the potential role of SUMOylated RIM1α in the function or expression of $Ca_V2.1$ channels located in the synaptic terminals of DRG neurons, further studies are needed to explore the precise role of RIM1α SUMOylation in the context of pain.

◂**Fig. 2** SUMOylation and deSUMOylation process new targets to modulate the excitability in sensory neurons. SUMOylation and deSUMOylation processes play a relevant role in the modulation of different ion channels, kinases, and other proteins implicated in noxious transmission in sensory neurons. In primary afferent neurons, SUMOylation of CRMP2 increases the trafficking, expression, and function of Nav1.7 channels, increasing neuronal excitability. In contrast, deSUMOylation of CRMP2 recruits the endocytic complex composed of Ub, Numb, Eps15, and Nedd4-2, leading to endocytosis of Nav1.7 channels. Another ion channel modulated by SUMOylation is the TRP family. Specifically, SUMOylation of hnRNAP1 prolongs the half-life of TRPA1 mRNA, resulting in increased expression of TRPA1 and increased pain sensitivity. Direct SUMOylation of TRPV1 increases its activity. Additionally, SUMOylation of PKC enhances the expression and currents of TRPV1, contributing to an increase in the excitability in sensory neurons. The SUMOylation process also indirectly regulates the function of some calcium channels. SUMOylation of CRMP4 by SUMO-1 leads to increased Ca^{2+} currents through Cav1.2 channels. Similarly, SUMOylation of CRMP2 results increases Cav2.2 currents in a heterologous system, though its effect on Cav2.2 in sensory neurons is unknown. Conversely, deSUMOylation of USP5 leads to strong interaction with Cav3.2, resulting in an increase of intracellular calcium in sensory neurons. At the spinal level, SUMOylation modulates ion channels implicated in the control of neuronal excitability. In this context, SUMOylation of potassium channels such as Kir7.1, Kv4.2, and $K_{2P}1$ reduces K+ currents, increasing the excitability in spinal neurons. Furthermore, the activation of kainate receptors induces the phosphorylation of PKC, promoting its SUMOylation, which results in the endocytosis of glycine receptors in the spinal cord. Finally, the role of SUMOylation has been reported in glial cells. In microglia, the activation of ERK induces SUMOylation of DGCR8, leading to the transcription of genes related to pro-inflammatory cytokines such as TNF- α, IL-6, and IL-1 β. These pro-inflammatory cytokines can sensitize spinal neurons, contributing to increased neuronal excitability and pain. Dashed arrows represent mechanisms that remain unknown in the sensory neurons and require further research to determine their potential contribution to pain signaling. Cav2.2, voltage-gated calcium channel subunit alpha1B; Cav3.2, voltage-gated calcium channel subunit alpha1H; CRMP2, collapsin response mediator protein 2; CRMP4, collapsin response mediator protein 4; DGCR8, DiGeorge syndrome critical region gene 8; Eps15, endocytic adaptor epidermal growth factor receptor substrate 15; ERK, extracellular signal-regulated kinase; hnRNAP1, heterogeneous nuclear ribonucleoprotein A1; IL-1β, interleukin-1 Beta; IL-6, interleukin-6; K^+, potassium ion; $K_{2P}1$, two-pore domain potassium channel TREK-1; Kir7.1, inwardly rectifying potassium channel 7.1; Kv4.2, potassium voltage-gated channel subfamily D member 2; Nav1.7, voltage-gated sodium channel alpha subunit 7; Nedd4-2, neural precursor cell expressed developmentally down-regulated protein 4-2; Numb, Numb protein; PKC, protein kinase C; TNF-α, tumor necrosis factor Alpha; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1; Ub, ubiquitin; USP5, ubiquitin-specific protease 5. Figure was created using Biorender.com

Conclusions and Future Perspectives

SUMOylation and deSUMOylation are dynamic post-translational modifications that significantly impact the function, expression, and localization of several ion channels. (Figure 2). These processes influence downstream signaling pathways by modifying the activity of kinases and other signaling molecules that interact with ion channels, thereby affecting pain signaling. The dynamic balance between SUMOylation and deSUMOylation provides a mechanism for fine-tuning pain sensitivity. Therefore, small changes in the SUMOylation status of ion channels can have significant effects on pain perception, ranging from hypersensitivity to reduced pain sensitivity. While current investigations have shed light on these processes, it is necessary to acknowledge the existing gaps in our understanding, particularly in the discovery and development of novel therapeutic strategies specifically targeting the SUMOylation and deSUMOylation modifications to modulate neuronal excitability and pain behaviors in different models. To advance our understanding of SUMOylation and deSUMOylation in pain mechanisms, it is essential to deepen our comprehension of these processes and their implications for pain management. Investigating the precise sites of SUMOylation on ion channels, receptors, kinases, and others, elucidating the role of specific SENPs in modulating SUMOylation dynamics and exploring the crosstalk between SUMOylation and other post-translational modifications will be crucial steps in defining the potential therapeutic applications of SUMOylation/deSUMOylation processes. Furthermore, studies examining the effects of pharmacological interventions targeting SUMOylation pathways in preclinical pain models will provide valuable insights into the therapeutic potential of these strategies and guide the development of tailored therapeutic interventions. Some clinical studies have reported that SUMO-1 and Ubc9 are upregulated in the human skin of patients with diabetic peripheral neuropathy [142]. Furthermore, patients with carpal tunnel syndrome (entrapment neuropathy) present high levels of Ubc9 that decrease after carpal tunnel surgery [143]. These observations suggest that SUMOylation and associated machinery may play distinct and condition-specific roles in neuropathic pain conditions. This variability highlights the need for targeted therapeutic strategies tailored to the specific SUMOylation profiles associated with different pain types. Future research should focus on developing specific inhibitors or activators of SUMOylation pathways. Additionally, creating personalized pain management approaches based on individual SUMOylation patterns could enhance treatment efficacy and reduce adverse effects.

Inhibitors of the SUMOylation machinery including SUMO-E1, -E2, -E3, and SENP1 inhibitors have been reported [144]. A small molecule inhibitor of Ubc9, named 2-DO8, has been found to block SUMOylation, but also cause neuronal damage [145]. This adverse effect could be attributed to Ubc9's ability to conjugate SUMO to certain substrates in the absence of an E3 ligase [146]. Excessive or inappropriate SUMOylation of neuronal proteins could impair their function and lead to neuronal damage. This suggests that targeting Ubc9 requires a more refined approach to avoid unwanted side effects on neuronal cells. In this regard, selectively inhibiting the SUMOylation of specific proteins

by targeting particular lysine residues, as demonstrated with compound 194, has shown greater efficacy and minimized unwanted side effects [147].

Overall, these findings clearly suggest that SUMOylation and deSUMOylation are promising targets to treat pain that require further translational validation.

Author Contributions ACR, KG, and RK conceived the idea for the article. ACR performed the literature search and wrote the first draft with help from KG. ACR and EJR-P created the figures using BioRender.com. EJR-P edited the article. All authors critically revised the work.

Funding The work was funded by an award from the National Institutes of Health: National Institute of Neurological Disorders and Stroke, K99NS134965 to KG; National Institute of Neurological Disorders and Stroke, NS098772, NS120663 to RK; and National Institute on Drug Abuse, DA042852 to RK.

Data Availability No data was generated for this study.

Declarations

Ethics Approval This is a review paper. The University of Florida Research Ethics Committee has confirmed that no ethical approval is required.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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