Translational Controls in Pain

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Abstract

Pain is an unpleasant but essential sensation. On a cellular level, pain typically originates in sensory neurons called nociceptors. They undergo rapid increases in cap-dependent translation in response to noxious stimuli. The specificity of translational controls in nociceptors is governed by regulatory factors and mRNAs that collaborate to ensure precise temporal and spatial regulation of protein synthesis. Multiple signaling pathways bridge extracellular cues to nascent translation including: the mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), and the integrated stress response (ISR). The torrent of information on both mechanisms and targets of translational controls in nociceptive circuits supports an enticing corollary. Targeted inhibition of aberrant translation in the cells responsible for the genesis of pain signals in the periphery affords a new strategy to prevent or reverse chronic pain states. We describe the implications of emerging insights into translational controls predominantly in the peripheral nervous system on the search for safer and more specific pain therapeutics.

Keywords: Pain, ISR, mTOR, AMPK, nociception, translation, anti-nociceptive mechanisms, hyperalgesia, allodynia

Introduction

The nervous system facilitates a crucial role in detection of harmful cues through a conserved process termed nociception(Tracey, 2017). It serves a critical function in the prevention of tissue damage and increases organismal fitness. Humans with congenital insensitivity to pain (CIP) often perish in childhood due to injuries or infections that fail to be recognized(Indo et al., 1996). Nociceptors are sensory neurons tasked with detection of noxious stimuli (e.g. heat, inflammatory cytokines, neurotrophic factors, capsaicin). They play a key role in both the detection and propagation of pain signals to the spinal cord that are ultimately communicated to the somatosensory cortex of the brain (**Figure 1 A**). After an injury, nociceptors undergo remarkable changes in their activity (termed plasticity) that often outlive the healing process(Pace et al., 2018). Nociceptor sensitization refers to a failure of nociceptors to return to their resting state and may play a major role in the transition from acute to chronic pain(Ferrari et al., 2010). Translational control have emerged as a dominant theme in nociceptor plasticity(Khoutorsky and Price, 2018, Melemedjian and Khoutorsky, 2015). Here we provide an overview of the tremendous body of evidence in support of translation as an integral component of pain signaling. We emphasize the critical role of nociceptors given their key function in the detection and relay of pain signals.

A primer on pain physiology

Nociceptors are pseudounipolar neurons tasked with detection of harmful stimuli and propagation of these signals to the spinal cord (**Figure 1 B**). The nucleus and many of the organelles in the nociceptor are housed in the cell body or soma. These are found in two different tissues, the dorsal root ganglia (DRG) adjacent to the spinal cord, or the trigeminal ganglia (TG) in the head. Approximately half of the neurons in the DRG and TG are nociceptors in most species. Protein and mRNA expression differ substantially between cell types giving rise to characteristic conduction velocity, diameter, and stimuli responsiveness. These differences manifest in the fibers (also known as axons) that extend from the soma (**Figure 1C**).

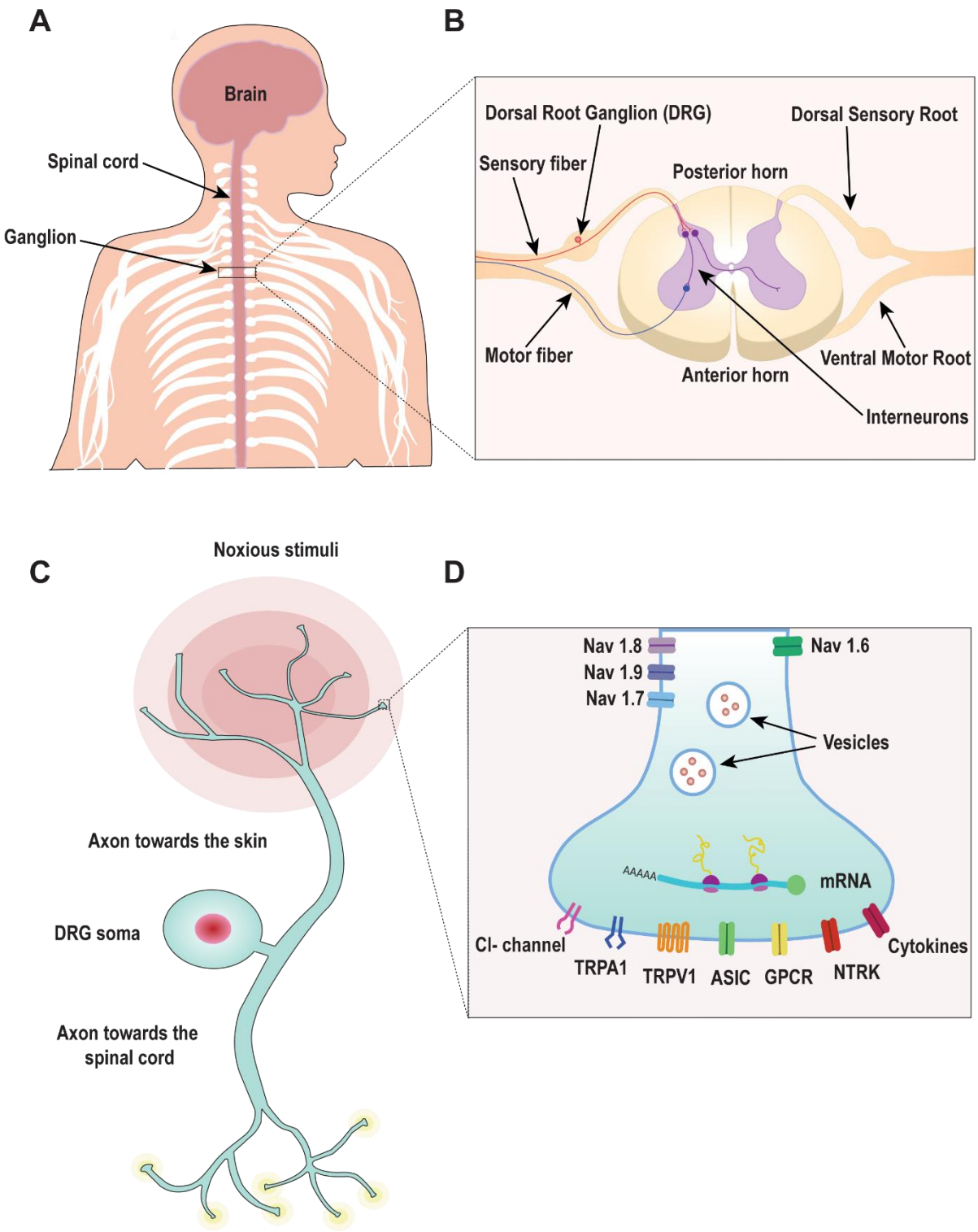

Figure 1 – (A) The anatomy of the pain. (B) Nociceptors are responsible for detecting harmful stimuli. Cell bodies of nociceptors are clustered in the DRG adjacent to the spinal cord. DRG neurons have one axon with two branches: one branch (sensory fiber)

innervates the skin, peripheral tissues, and internal organs. The opposing branch (sensory root) synapses with neurons in the spinal cord which then relay the signal to the somatosensory cortex of the brain via the thalamus. (D) Receptors expressed on the nerve endings of sensory fibers can detect various noxious stimuli such as heat (TRPV1), environmental irritants (TRPA1), pro-inflammatory mediators (e.g. NGF) (NTRK) and cytokines. Synaptic vesicles can store neurotransmitters at the synapse and are controlled by voltage gated calcium channels.

One end innervates the skin and other peripheral organs, including most of the viscera, and is responsible for the detection of noxious and/or damaging stimuli.

Injury can result in damage to the axon and its subsequent degeneration (Davies et al., 2019). All nociceptor axons are associated with Schwann cells through structures called remak bundles but most nociceptors are not myelinated. Following injury, Schwann cells have been demonstrated to play a critical role in the clearance of debris resulting from tissue degradation and secrete molecules, including nerve growth factor (NGF), that stimulate axonal growth. This process hinges on local production of proteins in axons (**Figure 1D**). Axons projecting into the skin shed any associated Schwann cells and encounter fibroblasts and keratocytes. After an injury, in addition to inflammatory mediators secreted by immune cells (*e.g.* IL-6), keratinocytes release ATP which contributes to changes in nociceptive activity and pain-associated behaviors (Moehring et al., 2018). Thus, the microenvironment surrounding the nerve fiber facilitates axonal regeneration and activity.

The most critical function accomplished by nociceptor axons is signal relay. Peripheral receptors are electrically silent in a resting state, but once threshold is reached they transmit action potentials back to the central nervous system (CNS) (Ferrari et al., 2013b, Ferrari et al., 2013c, Inceoglu et al., 2015, Khoutorsky et al., 2016, Melemedjian et al., 2010, Xu et al., 2014, Bogen et al., 2012, Moy et al., 2017, Barragan-Iglesias et al., 2018)

(Dubin and Patapoutian, 2010). These signals are received by interneurons in the dorsal horn of the spinal cord. The spinal cord transmits pain signals to the brain, where they are consciously perceived. Specific neurons act as checkpoints and determine whether a pain signal is relayed or not, thus not all signals are relayed (Tracey and Mantyh, 2007). Injury changes the electrophysiological and neurochemistry of neurons that detect and relay pain signals (Song et al., 2003). Hypersensitivity to noxious stimuli (hyperalgesia) or innocuous stimuli (allodynia) results from neuronal plasticity that causes a lowering of pain thresholds (**Figure 2**).

A growing body of evidence suggests that translational controls are integral to sustained changes in neuronal excitability that drive persistent pain states (Megat et al., 2019, Barragan-Iglesias et al., 2019, Moy et al., 2017, Ferrari et al., 2015a, Ferrari et al., 2015b, Melemedjian et al., 2014a, Ferrari et al., 2013a, Sonali Uttam, 2018, Khoutorsky et al., 2016, Khoutorsky et al., 2015, Melemedjian and Khoutorsky, 2015, Melemedjian et al., 2013a, Melemedjian et al., 2011, Obara et al., 2011, Geranton et al., 2009, Hunt et al., 2001). A major challenge moving forward is dissecting differential contributions of translational control to establishment as opposed to maintenance of pain states. Tremendous insights into nociceptor plasticity have resulted from studies of hyperalgesic priming. Priming refers to susceptibility to normally subthreshold noxious inputs following a noxious stimulus. The strength of this model is the ability to separate acute and prolonged pain states (Kandasamy and Price, 2015). The ever expansive pharmacopoeia for translational control applied to hyperalgesic priming will expand our understanding of acute and chronic pain. Increasingly precise genetic and optogenetic tools are also likely contribute key insights.

Local translation

Neurons must modulate their function in response to a range of physiologic stimuli. A key mechanism that facilitates rapid changes in sensory fibers is local translation from polarized populations of mRNA (Jung et al., 2014). RNA-localization is common in eukaryotes. An extreme example can be found in *Drosophila* embryos where ~70% of

genes show distinct patterns of subcellular localization (Tomancak et al., 2007, Lecuyer et al., 2007). Most of the corresponding proteins co-localize with their transcripts suggestive of a potential use of RNA localization to regulate sites of protein synthesis. Highly specialized cell types, including neurons, make extensive use of RNA localization. RNA-seq on neuronal processes suggests highly specific mechanisms of mRNA trafficking (Andreassi et al., 2010, Cajigas et al., 2012, Gumy et al., 2011, Minis et al., 2014, Zivraj et al., 2010, Taylor et al., 2009). During transit, translation of the mRNA is repressed often via protein factors recruited to the 3' untranslated region (UTR) (**Figure 3A**). The repertoire of RNA-binding proteins bound to mRNAs destined for local

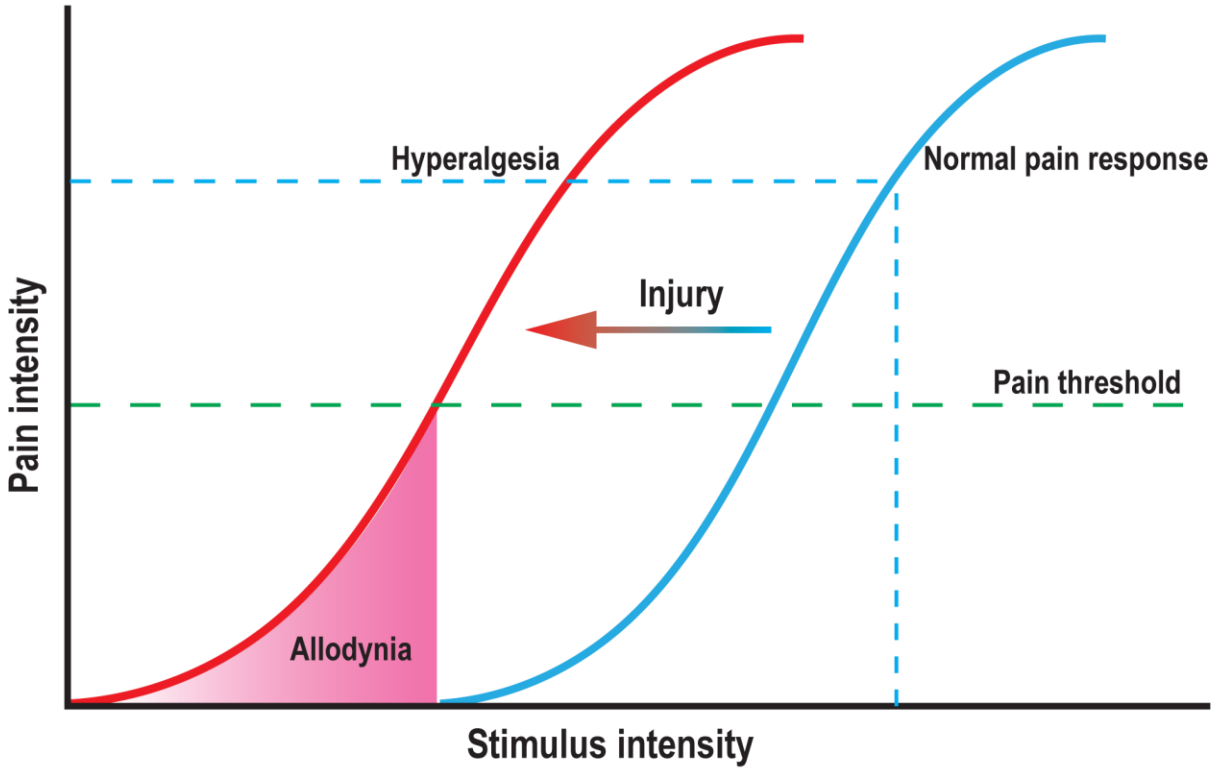

Figure 2 – Injury changes pain responses. Allodynia (pain due to a stimulus that does not usually provoke pain) and hyperalgesia (increased pain from a stimulus that usually elicit pain) are commonly observed in patients after an injury. Maladaptive central changes and nociceptor sensitization contribute to the generation and maintenance of allodynia and hyperalgesia which can ultimately lead to chronic pain (Cervero and Laird, 1996).

translation is controlled by a variety of signaling mechanisms (Gumy et al., 2011, Taylor et al., 2009, Zivraj et al., 2010, Willis et al., 2011, Merianda et al., 2009, Yudin et al., 2008, Willis et al., 2007). These multi-protein complexes serve critical roles in both trafficking of the mRNA and ensuring that translation is repressed until the transcript has arrived at the appropriate location within the cell. Thus, tremendous precision is achieved through cis-acting elements present in mRNA that provide all subsequent regulatory potential by trans-acting factors.

Local translation serves a key biological function. Neuronal protein synthesis can occur in the soma, synapse, or in axons. In nociceptors, axons can span vast distances (in some cases a meter or longer). Local translation provides a means to accomplish protein biosynthesis at the site where polypeptides are required. This provides a rapid solution to the problem of generating new proteins on demand that can guide critical processes to the function of afferent fibers (such as axonal growth) (Kar et al., 2018, Brittis et al., 2002). Local translation requires instructions provided by mRNA, and executed through the combined actions of ribosomes, tRNAs, and regulatory factors. Regulatory factors play a critical role in triggering translation of the correct target at the appropriate moment when it is required. Among the best characterized examples of activity-dependent protein synthesis is local translation of the immediate early gene Arc. Arc is translated in dendrites as an integral component of learning and memory processes in the hippocampus and amygdala (Tzingounis and Nicoll, 2006, McIntyre et al., 2005, Guzowski et al., 2000, Guzowski et al., 1999). However, the role of Arc in peripheral neurons is unclear. While Arc is translated in the spinal cord, it appears to be dispensable for inflammatory pain (Hossaini et al., 2010). What are the regulatory features present in mRNA that dictate the specificity of local translation in nociceptors? While the answer is likely transcript specific, emerging evidence suggests that analogous mechanisms to neurons in the CNS are employed, making use of untranslated regions (UTRs) to impart changes in mRNA localization (Willis et al., 2011, Baj et al., 2016).

Multiple lines of evidence suggest a key role for local translation in pain. First, axons are key sites of protein synthesis particularly in nociceptors (Kar et al., 2018, Terenzio et al., 2018, Merianda and Twiss, 2013, Barragan-Iglesias et al., 2018). Injection of protein synthesis inhibitors into the paw blocks behavioral responses to inflammatory mediators that increase nociceptor excitability (Melemedjian et al., 2010, Black et al., 2018). Second, disruption of mRNA polyadenylation specifically in the DRG blocks hyperalgesic priming through local translation of CamKIIα (Ferrari et al., 2013b, Bogen et al., 2012). Third, NGF increases axonal localization of a subset of mRNAs (Willis et al., 2005). Fourth, injection of NGF into humans promotes mechanical hypersensitivity without inflammation through a mechanism that is locally regulated (Rukwied et al., 2010). Additionally, injection of NGF into an axonal branch of a single nociceptor sensitizes only that branch (Obreja et al., 2018). Fifth, proteomics of neuromas suggests that and pulse chase labeling experiments suggest that local translation of cytoskeletal factors drives hyper-excitability after nerve damage (Huang et al., 2008). Finally, several groups have identified Nav1.8 mRNA as axonally localized after peripheral nerve injury (Thakor et al., 2009, Hirai et al., 2017). Knockdown of Nav1.8 in the sciatic nerve fiber but not the DRG blocks neuropathic pain caused by sciatic nerve entrapment (Ruangsri et al., 2011).

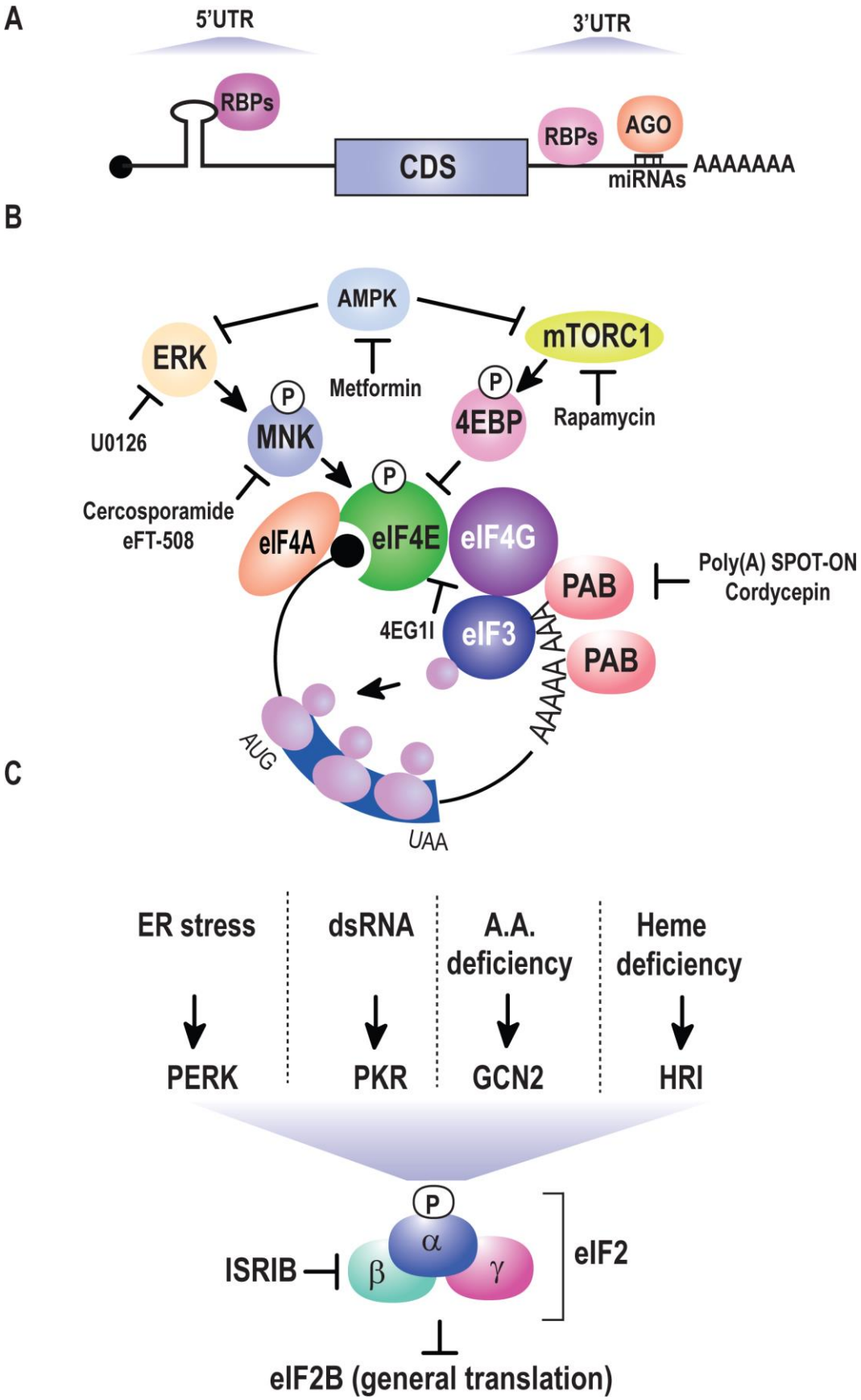
A
5'UTR
3'UTR
RBPs
CDS
RBPs
AGO
miRNAs
AAAAAAA
B
AMPK
ERK
mTORC1
Metformin
U0126
MNK
4EBP
Rapamycin
Cercosporamide
eFT-508
eIF4A
eIF4E
eIF4G
PAB
Poly(A) SPOT-ON
Cordycepin
4EG1I
eIF3
PAB
AAAAAAA
AUG
UAA
C
ER stress
dsRNA
A.A. deficiency
Heme deficiency
PERK
PKR
GCN2
HRI
α
β
γ
eIF2
ISRIB
eIF2B (general translation)

Figure 3 – (A) mRNA is comprised of a 5' UTR, the coding sequence (CDS), the 3'UTR, and the poly(A) tail. The M7G cap structure (black ball) is found on the 5' end of the transcript. Structures in the 5' UTR can influence translation efficiency and can also recruit RNA-binding proteins (RBPs). Similarly, the 3' UTR contains regulatory elements that can be bound by trans-acting RNAs (e.g. miRNAs) as well as proteins. The transcript is appended with a poly(A) tail. (B) Translation initiation and key inhibitors. AMPK negatively regulates both mTOR and ERK. Erk controls MNK which ultimately phosphorylates eIF4E. mTOR controls eIF4E availability through phosphorylation of 4EBPs. eIF4G interacts with PABP to promote initiation. eIF4G facilitates recruitment of the 40S ribosomal subunit through interactions with eIF3 (not shown). (C) Stimuli that engage the ISR are indicated as well as downstream kinases that act on eIF2α. eIF2α-dependent translation is blocked by the small molecule ISRIB.

mRNA structure

Eukaryotic mRNAs are resplendent with regulatory features. These include the ubiquitous 5' 7-methylguanosine (^{m7}G) cap on the 5' end of the transcript (**Figure 3A**). The cap is bound by the cap binding protein eIF4E (Sonenberg et al., 1979). Loss of the ^{m7}G renders the mRNA susceptible to rapid 5'->3' degradation by exonucleases (*e.g.* Xrn1). mRNAs possess two UTRs that either precede the coding segment on the 5' side or follow the stop codon on the 3' end. The UTRs harbor regulatory information in the form of *cis*-acting structures and sequences which are bound by *trans*-acting regulatory factors. These include RNA-binding proteins and regulatory RNAs that act in consort with RNA-binding proteins. The 5'UTR has distinct classes of regulatory elements that includes internal ribosomal entry sequences (IRES) and upstream open reading frames (uORFs). IRES elements can overcome the need for eIF4E mediated translation initiation through recruitment of translation factors. The function of uORFs is generally to reduce protein output of the main reading frame but they can also change the reading frame, add additional protein sequence, or encode functional peptides (Barbosa et al., 2013). A key

property of 5'UTRs is structural content. Secondary structure in the 5'UTR can increase dependency on the helicase eIF4A and further refine translational output. Similar to the 5' UTR, the 3'UTR can encode binding sites for regulatory factors and serves as a major repository of information that can enhance or reduce translational efficiency. The 3'UTR also provides a critical function in neurons as a source of information for specification of local translation (Menon et al., 2004, Huang et al., 2003, Aronov et al., 2001). Part of the challenge in elucidating their targets are the dynamic changes in 3'UTR length caused by alternative polyadenylation (APA). The final step on mRNA maturation is addition of the Poly(A) tail to the 3' end of the mRNA (AC and M, 2008). Poly(A) tail length is intimately linked to translational efficacy and the Poly(A) binding protein appears to be integral to pain signaling(Barragan-Iglesias et al., 2018). APA provides a mechanism to modulate poly(A) site selection and appears to be critical for localization of ion channels (e.g. $Na_v1.8$) required for nociception (Hirai et al., 2017). Finally, targeted disruption of polyadenylation by the small molecule cordycepin reverses pain hypersensitivity (Ferrari et al., 2013a).

Initiation

Protein synthesis is the culmination of a complex process initiated with the birth of RNA during transcription and the emergence of nascent peptides on the ribosome. Translation can be described in a series of four subsequent steps – translation initiation, elongation, termination, and ribosome recycling. Translation initiation is the rate-limiting step and has garnered tremendous attention as the bulk of translational control is thought to occur at this step (Hinnebusch et al., 2016). Inhibition of translation initiation in nociceptors abolishes sensitization and exemplifies the central role that translation initiation plays in pain plasticity(Melemedjian et al., 2010, Melemedjian et al., 2014b, Moy et al., 2017). In mammals, the main initiation pathway is termed cap-dependent translation and is responsible for the initiation of most translational events under non-stress conditions (Aitken and Lorsch, 2012). However, alternative pathways exist and are essential for survival under stress conditions and viral infections (Holcik and Sonenberg, 2005). Among the best-studied examples of alternative initiation pathways are IRES. They reside

in the 5'UTR and can directly recruit the ribosome to the mRNA. While their function in pain is unclear, cellular IRES initiate translation of mRNA subsets when cap-dependent translation is compromised and could mediate translation of nociceptive factors (Komar and Hatzoglou, 2011).

Cap-dependent translation

Cap-dependent translation hinges on multiple complexes that recruit the ribosome to the mRNA (Aitken and Lorsch, 2012) (**Figure 3B**). The eukaryotic initiation factor 4E (eIF4E) associates with the 5' 7-methylguanosine (^{m7}G) cap of the mRNA (Sonenberg et al., 1979). eIF4E is controlled both at the level of phosphorylation at a single site and through sequestration by a protein partner (eIF4E binding protein (4E-BP)) (Pause et al., 1994, Waskiewicz et al., 1997). eIF4E interacts with the scaffold protein eIF4G which in turn binds the helicase eIF4A. Collectively, this tripartite complex (referred to as eIF4F) stably associates with the ^{m7}G cap. eIF4F phosphorylation globally affects mRNA translation, and in some cases alters the translation of specific subsets of mRNAs – frequently proteins that are important for cell survival (Hsieh et al., 2012). Once assembled, eIF4F recruits the 43S pre-initiation complex (PIC) to the ^{m7}G cap. The PIC consists of the small ribosomal subunit (40S) bound to the initiation factor eIF2, initiator tRNA Met-tRNA$_i^{Met}$, and GTP. Though intrinsically active, eIF4A helicase activity is further stimulated by complex formation and unwinds the 5' UTR of the mRNA to facilitate ribosomal scanning of the 5' UTR. Thus, the translation of many mRNAs with highly structured 5' UTRs is eIF4A-dependent. Upon encountering the AUG start codon, the large ribosomal subunit (60S) joins the complex to form the 80S ribosome, and eIF2 is released. The joining of the large ribosomal subunit concludes successful translation initiation and transitions the ribosome into the elongation phase. eIF4G further interacts with PABP and circularizes the mRNA, possibly facilitating re-initiation after a successful round of translation (Wells et al., 1998).

eIF4F

Several signaling cascades converge on eIF4F. Multiple lines of evidence suggest that eIF4E is central in the development of pain pathologies. While the interaction of eIF4G with eIF4E is crucial for pain amplification, as evidenced by pharmacological studies (e.g. 4EGI1) (Moerke et al., 2007), a specific role of eIF4A in pain remains poorly understood. A possible reason might be that the high expression level of eIF4A and its eIF4F-independent helicase properties complicate tight regulation (Duncan and Hershey, 1983) (Galicia-Vazquez et al., 2012). In contrast, eIF4E has a low expression level, thus minor changes in availability by sequestration or modification can have extensive consequences on translation initiation. Two major pathways directly affect and modulate eIF4E activity; the mechanistic target of rapamycin (mTOR), and the mitogen-activated protein kinase (MAPK) pathways (**Figure 3B**) (Melemedjian et al., 2010, Moy et al., 2017).

The mechanistic target of rapamycin (mTOR) signaling cascade is a dominant regulatory feature of translational control (Yanagiya et al., 2012). The mTOR catalytic subunit exists in two multimeric protein complexes, one of which is sensitive to inhibition by rapamycin (mTORC1). In neurons, the mTORC1 pathway receives input from a large variety of upstream pathways that relay external input to mTORC1 which in turn creates cellular responses (Boutouja et al., 2019). mTORC1 upstream receptors include: NMDA, Trk, and IGF-1. The downstream targets of mTOR include regulators of translation like eIF4E binding proteins (4E-BPs), p70 S6 kinase (S6K), and eEF2 kinase. The three known 4E-BP isoforms (1, 2, and 3) show a tissue specific expression and the predominant isoform in the pain processing pathway is 4E-BP1 (Jimenez-Diaz et al., 2008, Melemedjian et al., 2011, Xu et al., 2010, Khoutorsky et al., 2015). Phosphorylation of 4E-BPs releases eIF4E from sequestration and allows it to engage in the eIF4F complex. Inflammatory pain models using injections of the upstream activators nerve growth factor (NGF) and interleukin 6 (IL-6), revealed a rapid induction of protein synthesis in nociceptors, which is concurrent with the activation of mTORC1 as monitored by phosphorylation (Melemedjian et al., 2010). Conversely, pharmacological inhibition of mTORC1 with rapamycin-related small molecules reduces pain hypersensitivity in a wide variety of pain models (Geranton et al., 2009, Jimenez-Diaz et al., 2008, Price et al., 2007). The endogenous endothelial growth factor receptor (EGFR) ligand, Epiregulin (EREG),

stimulates the mTOR pathway in DRG neurons and upregulates matrix metalloproteinase 9 (MMP-9) translation (Martin et al., 2017). MMP-9 is a regulator of inflammation and is transiently upregulated in DRG sensory neurons in models of neuropathic chronic pain (Manicone and McGuire, 2008, Kawasaki et al., 2008). Inhibitors of EGFR, used in cancer treatments, have been reported to also alleviate pain in patients with cancer-induced neuropathic pain (Kersten and Cameron, 2012, Moryl et al., 2006).

While mTORC1 is a global regulator of translation, it also appears to alter translation locally in the sciatic nerve and proprioceptive DRG neurons. During neuronal injury mTOR is transiently activated and translation of its own mRNA and other survival promoting molecules is up-regulated in a 3' UTR-dependent fashion (Terenzio et al., 2018). 3' UTRs frequently contain localization motifs suggesting that local mRNA pools can be deposited and activated upon a stimulus, in this case injury. Local pharmacological repression of mTOR leads to reduced neuron numbers. It is not known, however, if injury-induced local translation of mTOR affects nociception plasticity.

mTORC1 is also known to specifically regulate specific subsets of transcripts. For example, mTOR regulates expression of mRNAs that contain terminal oligopyrimidine tracts in their 5' UTRs (5' TOP mRNAs) via 4E-BPs (Thoreen et al., 2012). A critical issue in the field is systematic identification of mTOR targets that contribute to pain associated behaviors. While the molecular mechanisms by which these subsets are selected remains elusive an enticing hypothesis is that disabling cap-dependent translation favors alternative initiation pathways. While so far not investigated in nociceptors, this hypothesis is underpinned by increased IRES-dependent translation of Arc mRNA in dendrites when cap-dependent initiation is inhibited (Pinkstaff et al., 2001), which is consistent with the continued translation of IRES-containing mRNAs in the presence of mTOR inhibitors (Torin-1) (Thoreen et al., 2012).

S6K1 and 2 are downstream effectors of mTORC1. S6Ks that act on translation elongation by phosphorylation of initiation and elongation factors like eukaryotic elongation factor 2 (eEF2) (reviewed in (Zoncu et al., 2011)). Although an important

regulator of elongation, the role of S6K1/2 in pain is less clear than that of eIF4E. The investigation of S6K1 has been complicated by predominantly relying on genetic tools as small molecules targeting S6Ks lack in high specificity. In models of chronic inflammation pain, mTOR activation leads to S6K1 phosphorylation in DRG neurons but remains unaffected in neuropathic pain models (Liang et al., 2013). S6K1/2 double-knockout mice are more sensitive to mechanical stimuli with unaltered thermal sensitivity. The direct implications of S6K1/2 on elongation are masked by a negative feedback mechanism that in the long term activates the MAPK/ERK pathway. This leads to hyperexcitability of sensory neurons, allodynia, and spontaneous pain (Melemedjian et al., 2013a), which makes S6K1/2 a poor target for pharmacological intervention.

The MAPK pathway controls phosphorylation of a single residue, Ser209, in eIF4E via MAPK-interacting protein kinases (MNKs) 1 and 2 (Pyronnet et al., 1999, Waskiewicz et al., 1999). MNK1/2-mediated eIF4E phosphorylation contributes to the development of nociceptor sensitization and promotes chronic pain after injury (Moy et al., 2017). Both, phosphorylation-resistant eIF4E^{S209A} mutant mice, and reciprocally, MNK knockout mice show decreased pain hypersensitivity in response to most inflammatory mediators. Inhibition of eIF4E phosphorylation also inhibits hyperalgesic priming (Melemedjian et al., 2010, Moy et al., 2017). Similar to the mTOR pathway, MNK1/2-dependent phosphorylation of eIF4E Ser209 is suspected to promote tissue-specific alternative translation of mRNA subsets. Few eIF4E-phosphorylation dependent mRNA targets have been identified so far. In the pain processing pathway, known targets are matrix metalloproteases (MMP-2 and 9) and the key regulator of pain plasticity, *Bdnf*, in dorsal root ganglia (Moy et al., 2018). Translation of *Bdnf* mRNA is stimulated in response to inflammation and is important for pain plasticity and hyperalgesia (Obata and Noguchi, 2006) (Melemedjian et al., 2013b, Moy et al., 2018, Melemedjian et al., 2014a). In the DRG, eIF4E phosphorylation is required for hyperalgesic priming and promotes the translation of a specific *Bdnf* mRNA isoform (Bdnf-201), which has the longest and most structured 5' UTR of all *Bdnf* isoforms (Moy et al., 2018). The specific translation enhancement might reflect the stimulatory role of eIF4E on the RNA helicase eIF4A, though the specific effect of eIF4E-phosphorylation is unknown. These findings highlight

that local and tissue specific eIF4E-dependent translation is a feature of pain amplification.

eIF4E in chemotherapy induced peripheral neuropathy

While several relevant pathways for pain amplification have been identified, the translational alterations of their specific mRNA targets are mostly unknown. A ribosome profiling study identified regulators of the MAPK/ERK pathway as mRNA targets in the DRG and spinal cord dorsal horn in neuropathic pain (Sonali Uttam, 2018). Though ribosome profiling lends itself to the identification of translationally regulated mRNA expression, the cellular heterogeneity of the nervous system poses an obstacle and can confound the identification of cell type-specific changes in protein expression. A method that allows for cell-type specific analysis is translating ribosome affinity purification (TRAP) (Heiman et al., 2008), which uses a tagged ribosomal protein that is specifically expressed in the desired cell type. An initial study using TRAP has described translation in nociceptors in chemotherapy (paclitaxel)-induced pain in mice (Megat et al., 2019). Sequencing of mRNA bound to tagged ribosomes and further pharmacological and mutational validation suggests that MNK1 mediated eIF4E phosphorylation increases translation of the mTORC1-activator RagA complex. In mice, pain-associated behavioral effects of paclitaxel were reversed upon injection of a MNK inhibitor called eFT508. This suggests that pharmacological disruption of cap-dependent translation may provide a means to reverse neuropathic pain states. Consistent with this notion, elimination of the sole phosphorylation site on eIF4E results in profound deficits in pain behavioral responses to inflammatory mediators (Moy et al., 2018, Moy et al., 2017). This work suggests that cap-dependent translation is integral to the persistence chemotherapy-induced neuropathic pain.

eIF2

eIF2 is another key regulator of protein translation that promotes initiation (Holcik and Sonenberg, 2005), and is a known effector in neuropathic pain (Barragan-Iglesias et al.,

2019). Phosphorylation on Ser51 of the eIF2α subunit is the nexus of four pathways that collectively form the integrated stress response (ISR) (Khoutorsky et al., 2016, Sidrauski et al., 2015). These pathways (**Figure 3C**) are activated by viral infection (double-stranded RNA-dependent protein kinase, PKR), ER-stress (PKR-like ER kinase, PERK), amino acid deprivation (general control non-repressible 2, GCN2), oxidative stress and heme-deficiency (heme-regulated inhibitor, HRI) (Lu et al., 2001). Ser51 inhibits initiation by turning eIF2 into a competitive inhibitor of its GDP exchange factor (GEF) eIF2B, rendering it inactive (Yang and Hinnebusch, 1996, Pavitt et al., 1998, Krishnamoorthy et al., 2001, Jennings et al., 2013). eIF2α phosphorylation is increased in models of diabetes induced neuropathic pain and chronic inflammation (Barragan-Iglesias et al., 2019) (Khoutorsky et al., 2016). The targetability of individual pathways and subsequently the phosphorylation state of eIF2α make it an attractive pharmacological target. For example, activation of eIF2B by the small molecule ISRIB (Tsai et al., 2018) reverts eIF2α phosphorylation via PERK and relieves both translational inhibition and diabetic pain in mice (Barragan-Iglesias et al., 2019).

eIF2α phosphorylation generally inhibits translation but stimulates translation of upstream ORFs (uORFs) in the 5' UTRs of mRNAs (Barbosa et al., 2013, Hinnebusch et al., 2016). eIF2α phosphorylation can also impact read-through of uORFs through eIF2A-dependent mechanisms (Sendoel et al., 2017). Thus, it is tempting to speculate that this transient shift from main ORFs (mORF) to uORFs causes pain hypersensitivity, potentially by affecting the local biophysics of the cell and membrane. The molecular mechanism by which uORFs affect nociception remains to be investigated.

eIF2α in diabetic peripheral neuropathy

A reactive glycolytic metabolite associated with painful diabetic pain called methylglyoxal triggers neuropathic pain via the integrated stress response (Barragan-Iglesias et al., 2019). Intriguingly, MGO induced pain or diabetic pain caused by ablation of insulin producing cells (with streptozotocin) is reversed by the small molecule inhibited ISRIB

that targets eIF2B. While the relevant targets are unknown, the integrated stress response has been broadly implicated in neuronal function and is likely key in a variety of pain states linked to increases in eIF2a phosphorylation. Indeed, hemizygous loss of eIF2a phosphorylation decreases thermal but not mechanical hypersensitivity (Khoutorsky et al., 2016).

AMPK

AMP-activated protein kinase (AMPK) functions as a key energy sensor and has emerged as a therapeutic target for pain (Price et al., 2015, Taylor et al., 2013, Price and Dussor, 2013, Carling et al., 2012). Three subunits contribute to AMPK function (α, β, and γ). The γ subunit senses the AMP/ATP ratio and mediates allosteric effects on the α subunit. The catalytic domain is modulated by an upstream kinase (AMPKK). AMPK controls mTOR via two different pathways. AMPK directly inhibits mTOR activity through phosphorylation of raptor and indirectly inhibits mTOR via activation of the TSC complex. AMPK is a target for metabolic disease and cancer. AMPK agonists including metformin attenuate nascent translation and increase neuronal p-granules (Melemedjian et al., 2014a). AMPK activators appear to attenuate allodynia caused by peripheral nerve injury and reduce the excitability of nociceptors *in vitro (Melemedjian et al., 2011).*

Translational controls in the central nervous system

Re-consolidation mechanisms in pain

Reconsolidation has been coupled to protein synthesis inhibitors as a means of erasing memories and has clear implications for traumatic memories that can lead to pathological states (*e.g.* posttraumatic stress disorder). Analogous states may underlie certain nociceptive pain states. For example, mechanical hyperalgesia can be labile and susceptible to reversal by intrathecal delivery of protein synthesis inhibitors (Bonin and De Koninck, 2014). This work suggests that pain reconsolidation is likely spinally mediated and could be a useful strategy to reverse persistent pain.

Spinal modulation

Injury can increase the excitability of nociceptors and of the spinal cord circuitry. Central sensitization refers to increases in the excitability of the spinal circuit and play a major role in pain signaling. Central sensitization can amplify signals originating in the periphery (communicated by the nociceptors) destined for processing by the central nervous system. The implications are manifest in three ways: allodynia, hyperalgesia, and generalized pain to noninjured sites (secondary hyperalgesia). Central sensitization is driven in part by changes in synaptic strengthening at the dorsal horn.

A major structural model for understanding plasticity comes from understanding the key role of synaptic strength in learning and memory in the brain. Synaptic strength is modulated by opposing processes termed long-term potentiation (LTP) and long-term depression (LTD) in mammals. Learning and memory and LTP share several commonalities. Both LTP and long-term memory require protein synthesis and are blocked by mTOR inhibitors (Costa-Mattioli et al., 2009, Martin et al., 1999). Drugs that block LTP induction also attenuate hyperalgesia *in vivo (*Ruscheweyh *et al., 2011)*. Finally, electrical stimulation that induces LTP in rodents generates long-lasting increases in pain perception in humans (Biurrun Manresa et al., 2018). Conversely, electrical devices that induce LTD show some promise in reduction of pain perception and may be useful for treating chronic pain (Rottmann et al., 2010).

Opioid-induced hyperalgesia

Chronic administration of opioids can sensitize patients to acute pain through an effect called opioid induced hyperalgesia (OIH). Intriguingly, mTOR is activated in the dorsal horn of the spinal cord in a model of OIH (Xu et al., 2014). This drives an increase in nascent protein synthesis and eIF4E activity due to an increase in 4E-BP1 phosphorylation. OIH induced mechanical hyperalgesia can be reversed by intrathecal delivery of rapamycin. While the precise site of action is unclear as the delivery route is

not specific to the spinal cord, these data suggest that neuroplasticity in the nervous system caused by opioids is controlled, at least in part, by mTOR signaling.

Conclusions

Tremendous human suffering results from poorly managed pain. Chronic pain is estimated to impact the lives of a quarter of the population in the United States (Dahlhamer et al., 2018). Existing therapies for the treatment of chronic pain include numerous opioids that interact with reward circuity in the central nervous system contributing to their rampant misuse(Pathan and Williams, 2012). Advances in understanding the genesis of pain particularly in the peripheral nervous system have tremendous potential value in the identification of new therapeutic targets. Therapeutics with a peripheral site of action may provide safe and effective alternatives to opiates because they need not cross the blood brain barrier and target pain from where it originates. Translational control in peripheral sensory nociceptors has emerged as important regulator in pain sensitization and in the development and maintenance of various chronic pain conditions. Despite the identification of upstream signaling events that mediate translation in nociceptors, we still haven't elucidated the precise mechanism by which translation leads to nociceptor hyperexcitability and synaptic plasticity. Which mRNAs are efficiently translated or repressed during a particular pain state, and what are their functions? What are common among these mRNAs? Can these factors be targeted for inhibition? Hopefully, the answers to these key questions will provide the genesis for more effective pain management.

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