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Plenary Article

Bidirectional regulation of P body formation mediated by eIF4F complex formation in sensory neurons

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ABSTRACT

Processing (P) bodies are RNA granules that comprise key cellular sites for the metabolism of mRNAs. In certain cells, including neurons, these RNA granules may also play an important role in storage of mRNAs in a translationally dormant state. Utilizing nerve growth factor (NGF) and interleukin 6 (IL6), which stimulate cap-dependent translation in sensory neurons, and adenosine monophosphate activated protein kinase (AMPK) activators, which inhibit cap-dependent translation, we have tested the hypothesis that cap-dependent translation is linked to P body formation in mammalian sensory neurons. Treatment with NGF and IL6 decreases, whereas metformin increases biochemical association of the P body marker and translational repressor/decapping activator Rck/p54/dhh1 with the 5′-mRNA-cap suggesting an ordered assembly of P bodies. Likewise, diverse AMPK activators enhance P body formation while NGF and IL6 decrease P bodies in sensory neurons. This bidirectional P body plasticity readily occurs in the axonal compartment of these neurons. These studies indicate that P body formation is intricately linked to cap-dependent translation in mammalian sensory neurons suggesting an important role for these organelles in the regulation of mRNA metabolism in the adult PNS.

1. Introduction

RNA granules are cytoplasmic foci composed of RNA and proteins involved in the regulation of RNA movement and metabolism and intricately linked to translation control [9]. Two prominent types of RNA granules are P bodies, and stress granules. P bodies are thought to be important sites of mRNA metabolism in cells because they contain deadenylation and decapping enzymes. The shortening of the 3′ poly-A tail and/or 5′-m7GTP cap removal leads to the rapid decay of mRNAs effectively terminating their cellular life cycle [9]. P bodies are defined at the cytological level as aggregates of mRNA and protein composed of one of several accepted P body markers such as the translational repressor/decapping activator Rck/p54/dhh1 (hereafter referred to as Rck) or the decapping enzyme Dcp2. Interestingly, P bodies are devoid of eukaryotic initiation factors (eIFs) with the exception of the 5′-m7GTP mRNA cap-binding protein eIF4E [20]. In contrast, stress granules are cellular RNA granules composed of proteins including eIFs and mRNAs yet largely devoid of enzymes involved in mRNA decapping. These structures are induced by cellular stress (e.g. starvation or arsenite exposure) in a wide variety of organisms and cell types and appear to play an important role in storing mRNAs stalled at translation initiation when translation integrity may be compromised [3].

Decapping activators such as Rck, Scd6/Rap55 and Pat1 can repress translation. This suggests that mRNA decapping/repression is preceded by two steps: (1) inhibition of signaling to translation factors followed by (2) the exchange of the translation initiation factors for components of decapping/repression machinery. Translational repression and possible storage or ultimate degradation of mRNA culminates this process. Cumulatively, this indicates that the 5′-mRNA-cap is a site of direct competition between translation initiation factors and decapping/repression machinery [6,17,18,23]. However, one unresolved issue is the mechanism that leads to the assembly of decapping/repression machinery. We asked whether the manipulation of signaling pathways that regulate the formation of eIF4F complex in mammalian primary sensory neurons would inversely control the assembly of decapping/repression enzymes, such as Rck on the 5′-mRNA-cap.

While P bodies have been extensively studied in yeast and some mammalian cell types, very little is known about their role and regulation in neurons. Interestingly, several previous studies have suggested that neuronal P bodies may serve a dual function in RNA

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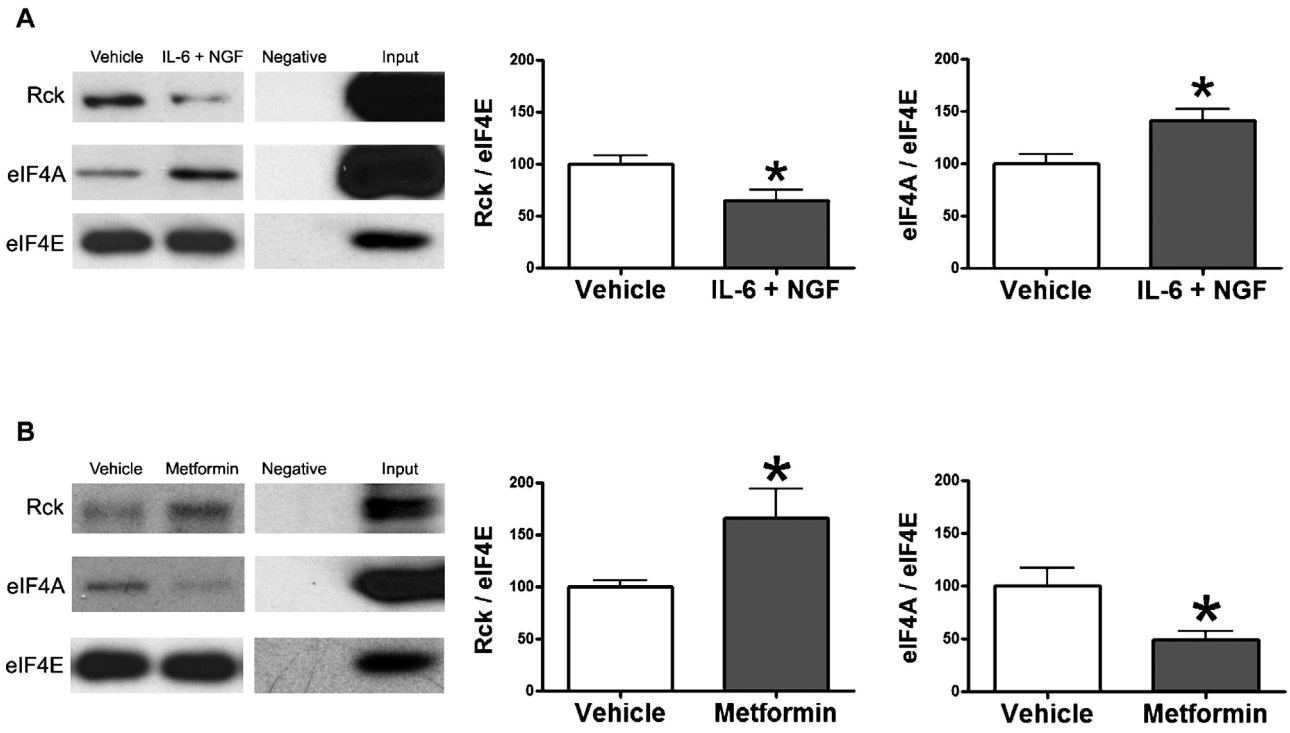

Fig. 1. eIF4F complex formation is inversely related to m7GTP/eIF4E Rck binding. (A) Representative western blots of TG neurons co-treated with IL-6 (50 ng/ml) and NGF (20 ng/ml) resulting in enhanced association of eIF4A with m7GTP beads while decreasing the association of Rck. (B) In contrast, treatment of TG neurons with metformin (20 mM) reduced association of eIF4A while enhancing the association of Rck with m7GTP beads. Levels were standardized to eIF4E association with m7GTP beads in all conditions. $^{*}p < 0.05$. $n = 8$ per condition.

transport and storage in addition to their role in RNA metabolism [4,8,19]. In support of this, stimulation of hippocampal neurons with NMDA induces a translocation of neuronal P bodies away from the soma to distal dendritic sites [8]. Moreover, brain derived neurotrophic factor (BDNF) and glutamate, two factors known to stimulate local, dendritic protein synthesis, reduce P bodies in central neurons [25]. Interestingly, while these studies support a role for P bodies in regulation of translation in central dendrites, they also suggest that P bodies are excluded from axons [8]. Using primary cultures of trigeminal ganglion (TG) neurons we demonstrate that P bodies are reciprocally controlled by factors that either stimulate (NGF and IL6 [14]) or inhibit (AMPK activators [15,16,24]) the formation of eIF4F complex on the mRNA cap in sensory neurons. We also show robust regulation of P bodies in the axonal compartment of these PNS neurons in apparent contrast to the CNS. Our findings reveal a novel mechanism of translation control and mRNA regulation in the PNS.

2. Materials and methods

2.1. Primary neuronal cultures

Male ICR mice (Harlan, 20–25 g) were used. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Arizona and were in accordance with International Association for the Study of Pain guidelines. Trigeminal ganglia (TG) were excised aseptically and cultured as we have described previously [14]. Experiments were done on day 5 in vitro.

2.2. 5′ mRNA cap complex analysis

Protein was extracted from the cells in lysis buffer (50 mM Tris HCl, 1% Triton X-100, 150 mM NaCl, and 1 mM EDTA at pH 7.4) containing protease and phosphatase inhibitor mixtures (Sigma) with an ultrasonicator on ice, and cleared of cellular debris and nuclei by centrifugation at 14,000 RCF for 15 min at 4 °C. After the protein extraction, 50 μg protein was incubated with 7-methyl GTP (m7GTP) Sepharose 4B beads (GE Healthcare) in the presence of 100μM GTP for 2 h at 4 °C. Unconjugated sepharose 4B beads were used for the negative controls. The beads were then pelleted and washed twice with lysis buffer. After a final centrifugation the pellet was suspended in 1X Laemmli sample buffer containing 5% (v/v) β-mercaptoethanol and eIF4E, Rck and eIF4A bound to the precipitated beads was analyzed by western blotting as described previously [14].

2.3. Immunocytochemistry (ICC)

Following the appropriate treatments the cells were washed with phosphate buffer saline (PBS) and fixed with ice-cold acetone/methanol. The coverslips were then blocked with 5% normal goat serum (NGS) for 3 h. Rck (1:1000, MLB International) and Dcp2 (1:2000, Sigma) antibodies were incubated in 5% NGS overnight at 4 °C. Neurons were immunodetected with peripherin (1:200, Sigma) and neurofilament heavy chain (NFH, 1:300, Sigma) antibodies in 5% NGS and incubated overnight in 4 °C. Alexa 488 goat anti-mouse and Alexa 555 goat anti-rabbit secondary antibodies (Invitrogen) were used to label proteins of interest. The coverslips were mounted onto slides.

2.4. Image correlation analysis (ICA)

Immunofluorescent micrographs were acquired on Ziess LSM 710 inverted microscope using a 40×, 1.3 numerical aperture oil immersion objective. ICA was performed using a plug-in for ImageJ provided by Li et al. [13] at the Wright Cell Imaging Facility, University Health Network Research, Canada (http://www.uhnresearch.