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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.

Keywords

AMPK; eIF4F; P bodies; Translation initiation; mRNA degradation; Trigeminal ganglion

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

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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 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, 150mM 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 100 μ M 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 Zeiss LSM 710 inverted microscope using a 40 $\times$, 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.ca/facilities/wcif/fdownload.html>) as we have described previously using TG neurons [14]. To determine the extent of colocalization of Rck or Dcp2 with NFH/peripherin in neurons ICA was used [13] to calculate positive product of the difference of the mean (PDM) for each pair of images. $PDM = (\text{channel 1} - \text{mean channel 1 intensity}) \times (\text{channel 2} - \text{mean channel 2 intensity})$. The positive PDM values were used to generate an image that visualizes colocalization of Rck or Dcp2 with NFH/peripherin. Intensity correlation quotient (ICQ) values were calculated by dividing the number of positive PDM pixels by the total PDM pixels and then subtracted by 0.5. ICQ values were then expressed as a percent change between vehicle and treatment groups.

2.5. Drugs and primary antibodies

Recombinant mouse IL-6 was from R&D Systems and mouse 2.5S NGF was from Millipore. The following rabbit polyclonal antibodies were obtained from Cell Signaling: eIF4E (cat# 9742) and eIF4A (cat# 2425). Rck antibody was from MLB International (cat# PD009) and Dcp2 antibody was from Sigma (cat# D6319). Rapamycin was from LC Laboratories, A769662 was from Tocris and metformin was from Axxora.

2.6. Statistical analysis and data presentation

Data are shown as means and the standard error of the means ($\pm$ SEM) of eight independent cell culture wells. Graph plotting and statistical analysis used GraphPad Prism Version 5.03 (Graph Pad Software, Inc. San Diego, CA, USA). Statistical evaluation was performed by Student's t-test and the a priori level of significance at 95% confidence level was considered at $p < 0.05$.

3. Results

We have previously shown that NGF and IL6 increase cap-dependent translation via convergent signaling to the eIF4F complex [14] resulting in enhance eIF4F formation on the 5'-mRNA-cap. Moreover, AMPK activators such as metformin and A769662 decrease cap-dependent translation and eIF4F complex formation in sensory neurons [15]. To determine if

the regulation of eIF4F formation on the 5'-mRNA-cap is inversely correlated with the association of decapping/repression machinery (i.e. Rck) we utilized m7GTP-conjugated beads. We hypothesized that Rck, an integral component of P bodies [2], might bind to m7GTP-conjugated beads in an inversely related fashion to eIF4F complex formation. Treatment of sensory neurons with IL-6 and NGF enhances the association of eIF4A (indicative of eIF4F complex formation [21]) and reduces Rck binding to m7GTP-conjugated beads (Fig. 1A). We then conducted the inverse experiment. Metformin treatment, as expected, decreased eIF4A association with m7GTP beads while Rck association was increased (Fig. 1B). Therefore, Rck association with the 5'-mRNA-cap is inversely related to eIF4F complex formation in sensory neurons.

The translational repressor/decapping activator Rck is associated with P bodies. Thus, we hypothesized that NGF and IL6 or AMPK activators would regulate P body formation in sensory neurons. To test this we first used ICC for Dcp2, a distinct decapping enzyme that localizes exclusively to P bodies [1]. As predicted, IL-6 and NGF led to a decrease in Dcp2 puncta (shown as decreased ICQ%) in TG neurons suggesting a decreased number of P bodies (Fig. 2). Conversely, the AMPK activators metformin and A769662 increased Dcp2 ICQ values in TG neurons indicating increased P body formation. Likewise, the mammalian target of rapamycin complex 1 (mTORC1) inhibitor, rapamycin, also led to an increased number of P bodies in sensory neurons (Fig. 2). To confirm these findings with an independent ICC marker we utilized Rck. Consistent with the Dcp2 findings, NGF and IL6 decreased the formation of Rck immunoreactive puncta, whereas the AMPK activators metformin and A769662 increased Rck immunoreactive puncta (Fig. 3). Interestingly, treatment with rapamycin did not cause a significant change in the number of puncta immunoreactive to Rck (Fig. 3). This is likely a consequence of the selective inhibition of mTORC1 by rapamycin which, in contrast to AMPK activators, induces the phosphorylation of eIF4E through a negative feedback pathway [16]. These data suggest distinct mechanisms of P body assembly. Collectively, these findings indicate that cap-dependent translation in mammalian sensory neurons is inversely related to regulation of P bodies.

In the adult CNS P bodies localize almost exclusively to the somatodendritic domain even in primary cultures of CNS neurons [8]. This is clearly not the case in the adult PNS where we find robust P body regulation in TG neuron axons. In naïve TG neurons P bodies are found in axons consistent with a role of these structures in basal RNA metabolism and possibly transport (Fig. 4). Paralleling a role for IL6 and NGF in axonal translation regulation [14], these factors induced a profound loss of P bodies in the axonal compartment of TG neurons. Inversely, metformin treatment led to an enhancement of P bodies in the axons of TG neurons (Fig. 4). Hence, we observe a robust P body plasticity in the axons of adult PNS neurons.

4. Discussion

We reach several conclusions based on the work presented here. First, Rck associates with the 5'-mRNA-cap in a fashion that is inversely related to eIF4F complex formation. This suggests an ordered assembly of P bodies in sensory neurons. Second, using two independent P body markers, we show that inhibitors of cap dependent translation enhance, whereas promoters of cap dependent translation decrease P body formation in sensory neurons. Finally, we demonstrate robust P body bidirectional regulation in axons of sensory neurons. Hence, sensory neurons display dynamic regulation of P body assembly that may be related to a key function of these RNA granules in RNA metabolism, localization and/or storage in these cells.

These findings are the first evidence, to our knowledge, of the presence of P bodies in the adult mammalian PNS. Several previous studies have demonstrated the presence of P bodies in neurons and related their regulation to neuronal plasticity. These studies have shown that NMDA receptor stimulation drives P bodies into dendrites suggesting a role in RNA localization [8]. Moreover, BDNF or glutamate stimulation of hippocampal neurons in vitro leads to a decrease in local P body number suggesting a release of mRNAs from P bodies into the locally translatable pool [25]. Because local translation in dendrites is a key feature of BDNF-induced plasticity [7] these findings support a role for P bodies in synaptic plasticity. We have shown that NGF and IL6 lead to cap-dependent translation in the cell bodies and axons of sensory neurons and that this signaling event is required for pain hypersensitivity induced by these factors [14]. In contrast, we have shown that AMPK activators such as metformin and A769662 reduce pain hypersensitivity induced by NGF and IL6, nerve injury or incision [15,16,24]. AMPK activators also decrease cap-dependent translation in sensory neurons [15]. NGF and IL6 and AMPK activators regulate P body formation in a fashion inversely related to their effect on cap-dependent translation suggesting a new role for these RNA granules in PNS and possibly nociceptive plasticity.

Neuronal P bodies are enriched in proteins that mediate RNA decapping and decay (e.g. Rck and Dcp2) and also contain important RNA binding and transport proteins such as staufer and fragile X mental retardation protein (FMRP) [4]. The presence of these proteins in neuronal P bodies suggests a transport and storage function that maybe equivalent to maternal RNA granules [4,9,19]. Interestingly, in the adult CNS, P bodies are largely excluded from axons and are, instead, enriched in the soma and dendrites [8]. This localization is congruent with the distribution of staufer [12,22] and FMRP [11] in the adult CNS. In contrast, peripheral sensory neurons, which do not have dendrites, are enriched in FMRP and staufer in their peripherally and centrally projecting axonal fields [4]. Our in vitro findings are consistent with axonal localization of P bodies in sensory neurons and suggest yet another important distinction related to translation control between axons in the CNS and PNS.

The discovery, identification and study of P bodies have been greatly aided by the recognition that autoantibodies from humans with autoimmune disorders can specifically label these RNA granules. The first such marker, GW182, was identified as an autoantibody from a woman with a peripheral sensory neuropathy [10]. Subsequent studies have found additional patients harboring autoantibodies that specifically label P bodies and at least 33% of these patients have peripheral neuropathies, many of which are painful [5]. This suggests an important role of P bodies in the PNS, however, surprisingly, the presence of P bodies has not been evaluated in the PNS until now. Based on our findings and the existing clinical features of autoimmunity patients with autoantibodies reactive to proteins that localize to P bodies, we speculate that P bodies might play an important role in PNS plasticity and maintenance. This notion is consistent with a growing recognition of the key role that RNA granules play in neurodegenerative disorders [19] and represents a first step toward exploring the biology of P bodies in the PNS.

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References

1. Aizer A, Brody Y, Ler LW, Sonenberg N, Singer RH, Shav-Tal Y. The dynamics of mammalian P body transport, assembly, and disassembly in vivo. *Mol Biol Cell*. 2008; 19:4154–4166. [PubMed: 18653466]

2. Andrei MA, Ingelfinger D, Heintzmann R, Achsel T, Rivera-Pomar R, Lührmann R. A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies. *RNA*. 2005; 11:717–727. [PubMed: 15840819]
3. Balagopal V, Parker R. Polysomes, P bodies and stress granules: states and fates of eukaryotic mRNAs. *Curr Opin Cell Biol*. 2009; 21:403–408. [PubMed: 19394210]
4. Barbee SA, Estes PS, Cziko AM, Hillebrand J, Luedeman RA, Collier JM, Johnson N, Howlett IC, Geng C, Ueda R, Brand AH, Newbury SF, Wilhelm JE, Levine RB, Nakamura A, Parker R, Ramaswami M. Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. *Neuron*. 2006; 52:997–1009. [PubMed: 17178403]
5. Bhanji RA, Eystathiou T, Chan EK, Bloch DB, Fritzler MJ. Clinical and serological features of patients with autoantibodies to GW/P bodies. *Clin Immunol*. 2007; 125:247–256. [PubMed: 17870671]
6. Collier J, Parker R. General translational repression by activators of mRNA decapping. *Cell*. 2005; 122:875–886. [PubMed: 16179257]
7. Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. Translational control of long-lasting synaptic plasticity and memory. *Neuron*. 2009; 61:10–26. [PubMed: 19146809]
8. Cougot N, Bhattacharyya SN, Tapia-Arancibia L, Bordonné R, Filipowicz W, Bertrand E, Rage F. Dendrites of mammalian neurons contain specialized P-body-like structures that respond to neuronal activation. *J Neurosci*. 2008; 28:13793–13804. [PubMed: 19091970]
9. Decker CJ, Parker R. P-bodies and stress granules: possible roles in the control of translation and mRNA degradation. *Cold Spring Harb Perspect Biol*. 2012; 4:a012286. [PubMed: 22763747]
10. Eystathiou T, Chan EK, Tenenbaum SA, Keene JD, Griffith K, Fritzler MJ. A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles. *Mol Biol Cell*. 2002; 13:1338–1351. [PubMed: 11950943]
11. Feng Y, Gutekunst CA, Eberhart DE, Yi H, Warren ST, Hersch SM. Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. *J Neurosci*. 1997; 17:1539–1547. [PubMed: 9030614]
12. Kiebler MA, Hemraj I, Verkade P, Kohrmann M, Fortes P, Marion RM, Ortin J, Dotti CG. The mammalian staufen protein localizes to the somatodendritic domain of cultured hippocampal neurons: implications for its involvement in mRNA transport. *J Neurosci*. 1999; 19:288–297. [PubMed: 9870958]
13. Li Q, Lau A, Morris TJ, Guo L, Fordyce CB, Stanley EF. A syntaxin 1, α 1, and N-type calcium channel complex at a presynaptic nerve terminal: analysis by quantitative immunocolocalization. *J Neurosci*. 2004; 24:4070–4081. [PubMed: 15102922]
14. Melemedjian OK, Asiedu MN, Tillu DV, Peebles KA, Yan J, Ertz N, Dussor GO, Price TJ. IL-6- and NGF-induced rapid control of protein synthesis and nociceptive plasticity via convergent signaling to the eIF4F complex. *J Neurosci*. 2010; 30:15113–15123. [PubMed: 21068317]
15. Melemedjian OK, Asiedu MN, Tillu DV, Sanoja R, Yan J, Lark A, Khoutorsky A, Johnson J, Peebles KA, Lepow T, Sonenberg N, Dussor G, Price TJ. Targeting adenosine monophosphate-activated protein kinase (AMPK) in preclinical models reveals a potential mechanism for the treatment of neuropathic pain. *Mol Pain*. 2011; 7:70. [PubMed: 21936900]
16. Melemedjian OK, Khoutorsky A, Sorge RE, Yan J, Asiedu MN, Valdez A, Ghosh S, Dussor G, Mogil JS, Sonenberg N, Price TJ. mTORC1 inhibition induces pain via IRS-1-dependent feedback activation of ERK. *Pain*. 2013; 154:1080–1091. [PubMed: 23607966]
17. Nissan T, Rajyaguru P, She M, Song H, Parker R. Decapping activators in *Saccharomyces cerevisiae* act by multiple mechanisms. *Mol Cell*. 2010; 39:773–783. [PubMed: 20832728]
18. Pilkington GR, Parker R. Pat1 contains distinct functional domains that promote P-body assembly and activation of decapping. *Mol Cell Biol*. 2008; 28:1298–1312. [PubMed: 18086885]
19. Ramaswami M, Taylor JP, Parker R. Altered ribostasis: RNA-protein granules in degenerative disorders. *Cell*. 2013; 154:727–736. [PubMed: 23953108]
20. Schwartz DC, Parker R. mRNA decapping in yeast requires dissociation of the cap binding protein, eukaryotic translation initiation factor 4E. *Mol Cell Biol*. 2000; 20:7933–7942. [PubMed: 11027264]

21. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell*. 2009; 136:731–745. [PubMed: 19239892]
22. Tang SJ, Meulemans D, Vazquez L, Colaco N, Schuman E. A role for a rat homolog of staufen in the transport of RNA to neuronal dendrites. *Neuron*. 2001; 32:463–475. [PubMed: 11709157]
23. Tharun S, Parker R. Targeting an mRNA for decapping: displacement of translation factors and association of the Lsm1p-7p complex on deadenylated yeast mRNAs. *Mol Cell*. 2001; 8:1075–1083. [PubMed: 11741542]
24. Tillu DV, Melemedjian OK, Asiedu MN, Qu N, De Felice M, Dussor G, Price TJ. Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain. *Mol Pain*. 2012; 8:5. [PubMed: 22269797]
25. Zeitelhofer M, Karra D, Macchi P, Tolino M, Thomas S, Schwarz M, Kiebler M, Dahm R. Dynamic interaction between P-bodies and transport ribonucleoprotein particles in dendrites of mature hippocampal neurons. *J Neurosci*. 2008; 28:7555–7562. [PubMed: 18650333]

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.

Fig. 2.

IL6 and NGF decrease while AMPK activators increase P body formation in TG neurons: Dcp2. Representative micrographs of TG neurons co-treated with IL-6 (50 ng/ml) and NGF (20 ng/ml) causing a reduction in Dcp2-labeled puncta. In contrast treatment with metformin (20 mM), A769662 (200 μ M) and rapamycin (100 nM) resulted in an increased number of Dcp2-labeled puncta. The positive PDM values were used to generate a heat map image that visualizes intensity of Dcp2 expressed within neurons. ICQ% values are shown for each experimental condition to quantify Dcp2 intensity in neurons. Scale bar is 30 μ m. * p <0.05, ** p <0.01 and *** p < 0.001. n = 10 per condition.

Fig. 3. IL6 and NGF decrease while AMPK activators increase P body formation in TG neurons: Rck. Representative micrographs of TG neurons co-treated with IL-6 (50 ng/ml) and NGF (20 ng/ml) causing a reduction in Rck-labeled puncta. In contrast treatment with metformin (20 mM), A769662 (200 μ M) resulted in an increased number of Rck-labeled puncta. Rapamycin (100 nM) had no effect. The positive PDM values were used to generate a heat map image that visualizes intensity of Rck expressed within neurons. ICQ% values are shown for each experimental condition to quantify Rck intensity in neurons. Scale bar is 30 μ m. ** $p < 0.01$ and *** $p < 0.001$. $n = 10$ per condition.

Fig. 4. Robust regulation of P bodies in axons. Representative micrographs of TG neurons and their axons treated with vehicle, co-treated with IL-6 (50 ng/ml) and NGF(20ng/ml) or treated with metformin (20 mM). Positive (+) PDM images show axonal localization of P bodies in vehicle treated axons and a paucity of Dcp2 labeled puncta in NGF and IL6 treated culture. Metformin increases Dcp2 labeling in TG axons. Scale bar=30 μ m.