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Outsourcing CREB translation to axons to survive

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Abstract

Nerve growth factor induces sensory neuron survival via retrograde signalling from the axon to the cell body. Local translation of the transcription factor CREB in the axon, followed by its transport to the nucleus, is involved in this process.

During development, the nervous system often produces more neurons than it eventually requires. These neurons then compete for limiting amounts of ‘survival’ factors, or neurotrophins, and those that do not get enough die; for example, dorsal root ganglion (DRG) sensory neurons compete for nerve growth factor (NGF) released by the cells they target. It is generally thought that binding of NGF to TrkA (its receptor) induces the phosphorylation and endocytosis of TrkA and the retrograde transport of ‘signalling endosomes’ carrying NGF–pTrkA complexes together with downstream effectors such as the MAP kinase Erk5 (ref. 1). When they reach the cell body, these signalling endosomes induce the phosphorylation and activation of cAMP response element binding protein (CREB), a transcription factor that promotes DRG neuron survival². This model has assumed that a pre-existing pool of CREB in the cell body is phosphorylated following NGF signalling. On page 149 of this issue, Cox *et al.*³ report that NGF induces the local translation of CREB in axons and that, surprisingly, this axonally synthesized pool of CREB is required for accumulation of phosphorylated CREB (pCREB) in the nucleus and ultimately cell survival, most likely because axonal CREB is itself the source of nuclear pCREB.

Because of their polarized nature, neurons face a communication problem between the cellular centre (where protein synthesis usually takes place) and its often distant periphery (where signals are sent and received). Previous work has shown that local translation is critical for synaptic plasticity⁴ and axon guidance⁵, partly to allow distal ends of the neuron to respond autonomously to important stimuli; for example, in axons, local translation of β -actin^{6,7} and RhoA⁸ is required for growth cone responses to guidance cues. In dendrites, CREB is known to be locally translated and transported to the nucleus⁹, but the functional significance of this observation has been unclear. Cox *et al.*³ provide the first evidence that CREB is locally translated in axons, and demonstrates a novel role for axonal translation and retrograde transport of transcription factors in neuronal survival.

Growing neurons in compartmentalized chambers allowed the authors not only to observe the effect of applying different experimental treatments to the cell body or the axon, but also to isolate RNA and proteins from only the cell body or axon for further biochemical analysis (Fig. 1A). Such chambers have been used previously to demonstrate that NGF application to axons only is sufficient for DRG neuronal survival *in vitro*¹⁰. Cox *et al.*³ show that axon-

only application of protein-synthesis inhibitors blocks the survival-promoting effects of axonally supplied NGF. In contrast, applying the same inhibitors only to the cell body does not affect the survival-promoting effects of NGF. Moreover, they show that NGF induces axonal phosphorylation of the translation regulator eIF-4EBP, indicating global activation of translation.

Using these compartmentalized chambers, the authors show that CREB protein is locally translated in axons³. They show that *CREB* mRNA is present in DRG axons and that withdrawing NGF leads to the almost complete loss of axonal CREB protein, which is maintained in the presence of NGF. Critically, protein-synthesis inhibitors prevent the NGF-induced maintenance of axonal CREB, implying that NGF promotes the translation of *CREB* mRNA in the axon. Cox *et al.*³ confirmed these results in axons severed from their cell bodies, ruling out anterograde transport of CREB protein. To confirm that NGF regulates axonal translation of CREB, the authors transfected DRG cultures with a myristoylated, destabilized EGFP tagged with the *CREB* 3'UTR. This modified EGFP exhibits a reduced diffusional mobility and half-life, increasing the probability that the EGFP signal observed corresponds to the site of protein synthesis. Axons transfected with this construct showed EGFP puncta that disappeared after NGF was withdrawn and reappeared when NGF was added back.

What is the function of axonal translation of CREB? One hint came from the almost complete disappearance of axonal CREB after NGF withdrawal. This disappearance does not involve proteasomal degradation, but does require microtubules, suggesting that the CREB protein might be transported out of the axon. In fact, after NGF withdrawal, CREB signal declined first in the distal portion of the axon and then in the proximal portion. To confirm the retrograde transport of axonal CREB protein, Cox *et al.*³ used a photoconvertible reporter, Dendra, tagged to the coding sequence of CREB. Dendra is irreversibly converted from green to red fluorescence after blue or UV illumination. The authors photoconverted Dendra or Dendra-CREB in the distal tip of axons and followed the diffusion of red Dendra away from the site of conversion. Dendra alone moved at the rate of diffusion, but Dendra-CREB moved significantly faster, suggesting active transport. Importantly, dynein and microtubule inhibitors reduced Dendra-CREB movement to the rate of diffusion. Twenty minutes after photo-conversion, red Dendra-CREB was observed in the nucleus, whereas red Dendra alone was not. These results indicate that after its synthesis in the axons, CREB undergoes active retrograde transport to the nucleus.

Finally, Cox *et al.*³ show that this axonally synthesized and retrogradely transported CREB is required to mediate NGF-induced neuronal survival. Here, they used the compartmentalized chambers again and took advantage of the previous finding that axonal application of small interfering RNA (siRNA) does not affect mRNAs in the cell body¹¹. Axon-only treatment with *CREB* siRNA successfully decreased axonal levels of *CREB* mRNA and protein but, crucially, did not affect cell-body levels of CREB. Cox *et al.*³ found that axon-only *CREB* siRNA was sufficient to block the processes associated with axon-only application of NGF, such as nuclear accumulation of phosphorylated CREB, CRE-dependent transcription and most importantly, neuronal survival. In contrast, axon-only *CREB* siRNA did not affect the accumulation of phosphorylated TrkA and Erk5 (pTrkA and pErk5) in cell bodies, indicating that the NGF-pTrkA signalling endosomes still arrived at the cell body, but were somehow unable to phosphorylate the large pool of CREB pre-existing there.

These findings suggest that axonally derived CREB is itself the source of nuclear pCREB (Fig. 1B). Cox *et al.*³ also show that axonal CREB colocalizes with pTrkA and pErk5, suggesting that the newly translated CREB protein remains associated with the signalling

endosome as it is trafficked back to the cell body. This physical proximity of the newly synthesized CREB to pTrkA and pErk5 may make it preferentially available for phosphorylation. Conversely, the pre-existing cell-body CREB may be sequestered or otherwise unavailable for phosphorylation by signalling endosomes. Alternatively, axonally derived CREB may have unique post-translational modifications, or lack thereof, that make it especially amenable to phosphorylation or efficient at activating CRE-dependent transcription. As basal levels of nuclear pCREB are very low, a small axonal contribution could strongly affect CRE-dependent transcription.

It remains possible that axonal CREB is not the sole source of pCREB, but instead functions as an adaptor or ‘catalyst’ for phosphorylation of cell-body CREB. CREB forms homo- and heterodimers², so axonally derived CREB associated with signalling endosomes might recruit cell-body CREB to be phosphorylated. Future studies may determine what proportion of nuclear pCREB is axonally synthesized, and whether axonal *CREB* mRNA is sufficient to mediate neuronal survival in the absence of cell-body *CREB* mRNA.

These findings highlight a puzzle implicit in many studies on local translation: why is local translation required when there is already plenty of the desired protein around? For example, local translation of β -*actin* mRNA in axons is required for attractive growth-cone turning^{6,7}, even though pre-existing actin is one of the most abundant proteins in the cell. Similarly, blocking local translation of *sensorin* mRNA in *Aplysia* neurons inhibits synapse formation, but does not affect the total amount of sensorin protein at synapses¹². One answer is ‘location, location, location’: synthesis of actin in a small concentrated volume might aid actin filament nucleation, whereas synthesis of CREB near signalling endosomes may increase its local concentration for phosphorylation. It is also possible that post-translational modifications differentially regulate ‘old’ and ‘new’ proteins. These hypotheses are not mutually exclusive, and require further study.

This study³ also raises the exciting possibility that distal synthesis and retrograde transport of transcription factors may be a general mechanism for retrograde communication in neurons. For example, in axon guidance, this mechanism could change the expression profile of guidance-cue receptors after growth cones pass intermediate targets, or could induce synaptogenic genes when growth cones arrive at their final destination. In synaptic plasticity, distally synthesized CREB in post-synaptic dendrites⁹, or perhaps even pre-synaptic axons^{13,14}, may have a special role in regulating gene expression — a function previously attributed to cell body CREB². Finally, given that local synthesis of the nuclear transport-associated β -importins occurs in injured DRG axons¹⁵, local synthesis of transcription factors may be important for axonal regeneration.

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Figure 1.

Compartmentalized cultures reveal axonal synthesis and retrograde transport of CREB. **(a)** Schematic representations of compartmentalized culture systems. Experimental treatments can be applied exclusively to cell bodies or axons using a Campenot chamber, in which neurons plated in the cell body compartment extend axons through a fluidically sealed barrier to an axonal compartment isolated from the cell bodies (left). RNA and proteins can be isolated exclusively from cell bodies or axons using a modified Boyden chamber, in which DRG explants are plated on one side of a microporous membrane and axons grow through to the other side (pore diameter permits only passage of axons), allowing cell bodies or axons to be removed separately by scraping (right). **(B)** Schematic representation of a model for axonal synthesis and retrograde transport of CREB. **(a)** Activated TrkA receptor undergoes endocytosis and activates local synthesis of CREB through an unknown mechanism. **(b)** Locally synthesized CREB is transported to the cell body along microtubules in a complex with the NGF-pTrkA signalling endosome. **(c)** At the cell body,

axonally synthesized CREB is most likely the source of nuclear pCREB, possibly because axonal CREB, but not cell-body CREB, is in close enough proximity to the signalling endosome to be efficiently phosphorylated (adapted from Cox *et al.*³).