effect of TERRA on telomerase remains to be tested *in vivo*. It is worth noting that the NMD effector UPF1 interacts with the DNA polymerase δ, suggesting additional links between NMD, TERRA and telomere replication[7] (Fig. 1a).

A crucial question is how TERRA is specifically associated with telomeric chromatin. *A priori*, two non-exclusive modes can be envisaged: interaction with telomeric proteins and hybridization with single-stranded telomeric DNA (Fig. 1b). Although any telomere-bound protein should now be examined for their putative ability to tether TERRA to telomeres, the ability of nucleolin and of heterogeneous nuclear ribonucleoproteins (hnRNPs) to bind to both telomeric RNA and DNA makes them attractive candidates[14]. Alternatively, TERRA may be involved in intermolecular G4 with either the G-tail or the displaced strand of the D-loops formed at the base of t-loops. Support for this hypothesis comes from the finding that a 22mer oligonucleotide composed of UUAGGG repeats can fold into G4 structures *in vitro*[15]. However, intermolecular G4 involving DNA–RNA hybrids has not yet been reported. Another interesting possibility involves co-transcriptional pairing of TERRA to the C-rich strand DNA templates. Indeed, such structures, named G-loops, can be formed when G-rich DNA templates, including telomeric DNA, are transcribed *in vitro* (reviewed in ref. 2; Fig. 1b). Interestingly, such DNA–RNA duplexes are highly recombinogenic and various factors associated with RNA processing (such as THO/TREX and ASF2/SF2) assist in their resolution, thereby preventing genomic instability[2]. Hence, it may be anticipated that not only NMD, but also other RNA-processing pathways are important in TERRA turnover and telomere association.

Recent studies have shown that transcription occurs throughout the genome (including repetitive DNA regions), that a substantial portion of polyadenylated transcripts is non-coding, and that PolII is associated with silent regions[16–18]. In view of these findings, the observation that telomeres are transcribed is not so surprising. Thus, it is possible that TERRA is merely the product of a general mechanism governing repetitive DNA transcription, without an essential function at telomeres. On the other hand, given the results discussed here[7,8], TERRA may prove to be a key component of the telomere machinery. Functional organization of telomeres should now be revisited and, undoubtedly, future studies will reveal intriguing new connections between telomere protection, epigenetic regulation and RNA metabolism.

The finding of RNA molecules at telomeres is not without clinical relevance in the treatment of cancer, as it may contribute to the telomeric alterations accompanying malignant transformation[8]. Thus, TERRA may be a valuable target for anti-cancer agents directed against telomeres. In this regard, it will be of great interest to evaluate the capacity of known DNA G4 ligands to bind to equivalent structures in TERRA.

Last but not least, TERRA may be part of the transmissible information determining telomere state. Consistent with recent work showing that RNA can transmit epigenetic information between generations[19], one can imagine that TERRA belongs to the telomeric heritage that we received from our parents.

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Outsourcing CREB translation to axons to survive

Andrew C. Lin and Christine E. Holt

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 survival[2]. 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

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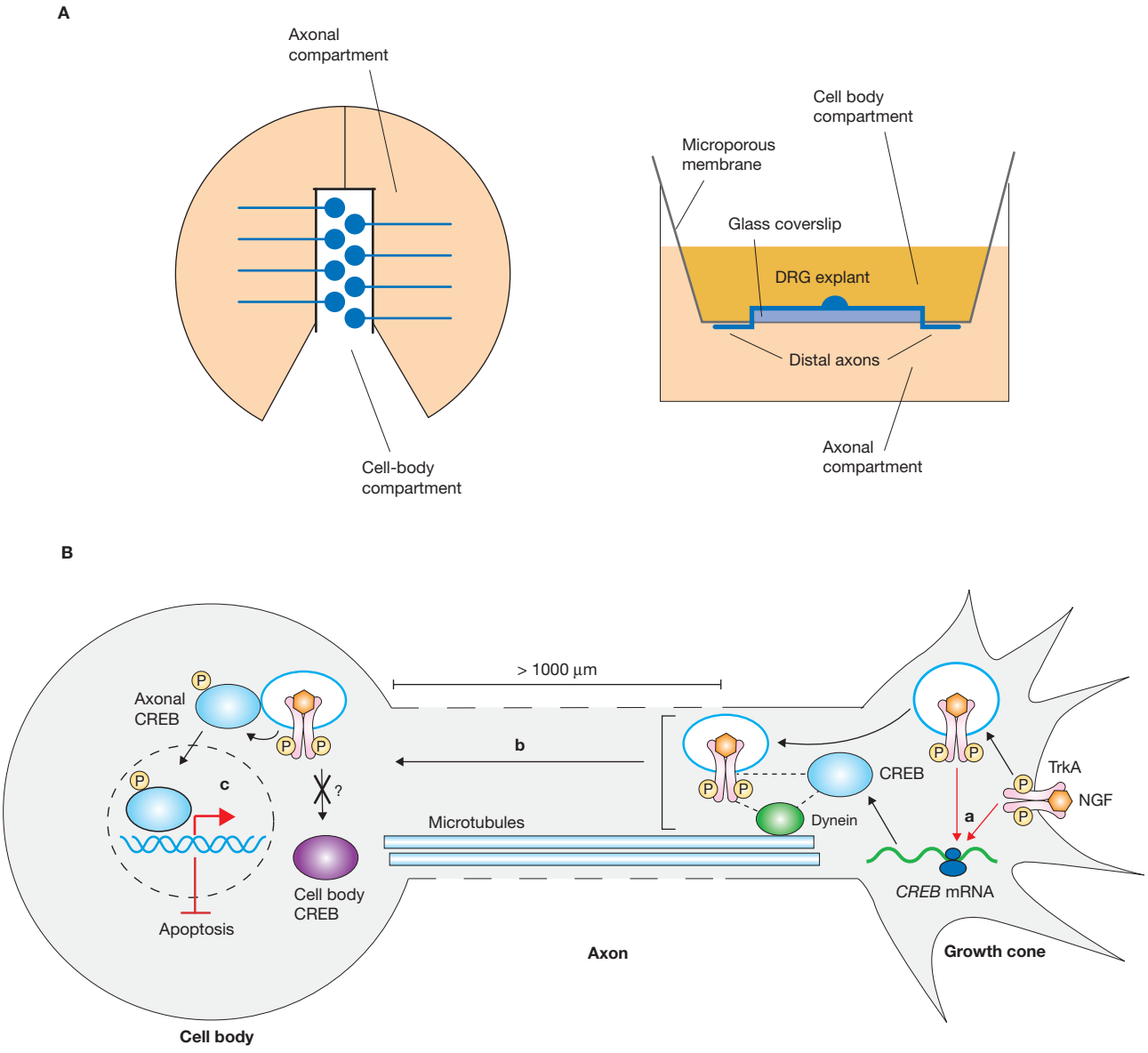

Figure 1 Compartmentalized cultures reveal axonal synthesis and retrograde transport of CREB. (**A**) Schematic representations of compartmentalized culture systems. Experimental treatments can be applied exclusively to cell bodies or axons using a Campenot chamber, in which neurons plated in the cell 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]).

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 plasticity[4] and axon guidance[5], 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 RhoA[8] is required for growth cone responses to guidance cues. In dendrites, CREB is known to be locally translated and transported to the nucleus[9], 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 axons[3]. 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 photoconversion, 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 body[11]. 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 heterodimers[2], 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 synapses[12]. 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 study[3] 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 dendrites[9], or perhaps even pre-synaptic axons[13,14], may have a special role in regulating gene expression — a function previously attributed to cell body CREB[2]. Finally, given that local synthesis of the nuclear transport-associated β-importins occurs in injured DRG axons[15], local synthesis of transcription factors may be important for axonal regeneration.

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Facing up to Mena: Tes(ting) times for EVH1 domains

J. Victor Small

Ena/VASP family members have a common binding interface on their EVH1 domains for FPPPP motifs in partner proteins. The finding that Tes, a protein lacking FPPPP motifs, competes specifically for the FPPPP ligand site on Mena, provides new insights into the differential regulation of Ena/VASP proteins.

The protrusion of lamellipodia and filopodia at the front of migrating cells functions to seed new adhesion sites that, in turn, support further cycles of protrusion and, eventually, the translocation of the trailing cell body. In rapidly migrating cells, phases of protrusion dominate over retractions and adhesions formed at the front turn over within 1–2 min; in slower cells, retractions are more frequent and adhesions more persistent. The efficiency of cell migration and, conversely, the degree of anchorage, therefore depends on the coordination between the protrusion and adhesion machineries. Likely coordinators are the Ena/VASP family members, Mena, VASP and Evl[1–3], which localize to the tips of protruding lamellipodia and filopodia as well as to adhesion foci. *In vitro*, Ena/VASP proteins potentiate actin polymerization and crosslink actin filaments into bundles through tetramization of their C-terminal, F-actin binding domains.

The function of Ena/VASP proteins *in vivo* is controversial[4] but it is already apparent that Ena/VASP proteins collaborate in some way with nucleators of actin-filament assembly to generate cytoplasmic protrusions. VASP potentiates filopodia formation and binds formins[5,6], and Mena binds the Abl-interacting protein, Abi[7], the core component of the WAVE complex at the tips of lamellipodia. Neither formins nor WAVE-complex components have been localized in substrate adhesions, suggesting that once filaments have been initiated to form these sites, Ena/VASP proteins continue independently to promote actin-filament elongation.

But what are the functions of the individual Ena/VASP family members and how are their activities regulated? Genetic deletions in mice have confirmed the requirement of Ena/VASP proteins in actin-based processes, particularly in neurogenesis[3,4] but the mild deficiencies seen suggest overlapping roles of the family members. Vertebrate Ena/VASP proteins are phosphorylated on a conserved N-terminal site (corresponding to Ser 157 in VASP), which influences F-actin binding and F-actin polymerizing activity, and on other non-conserved sites that could endow differential functions[3]; however, mutation of these latter sites has no effect on subcellular localization[2], and distinguishing activities have not been detected. In addition, *Drosophila melanogaster* Ena is tyrosine-phosphorylated at several sites, whereas vertebrate VASP bears no tyrosine phosphorylation sites; yet VASP can largely restore Ena function *in vivo*. Thus, the different patterns of of Ena/VASP phosphorylation are not yet interpretable in terms of functional variations.

Ena/VASP proteins also seem to use similar domains to associate with common partner proteins: they share common N-terminal EVH1 (Ena/VASP homology) and C-terminal EVH2 domains that flank a proline -rich region of variable length (Fig. 1). The C-terminal domain binds F- and G-actin and is responsible for tetramerization of the molecule whereas the polyproline-rich region binds profilin, SH3 and WW-domain containing proteins. Until now the EVH1 domains

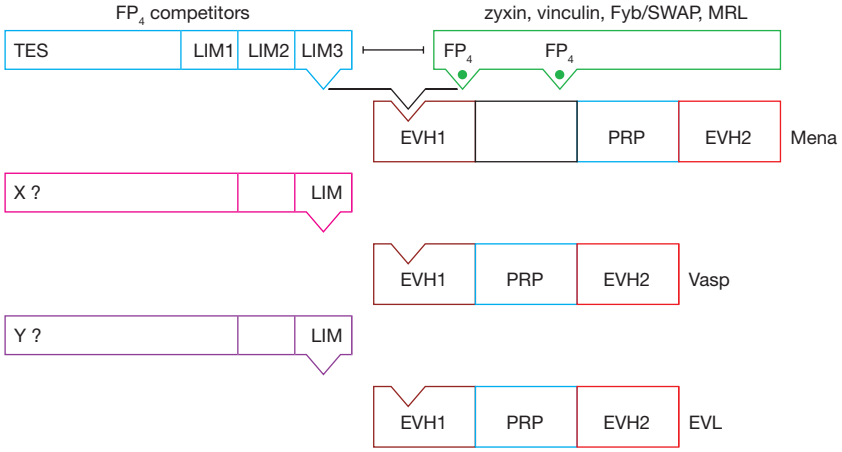

Figure 1 Competition for EVH1 domains of vertebrate Ena/VASP proteins between LIM domain-containing proteins and proteins containing FP4 motifs. The binding of Tes LIM 3 exclusively to the EVH1 domain of Mena suggests that other LIM domain-containing proteins (X and Y) specific for VASP and EVL may also exist.

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