Tctp in Neuronal Circuitry Assembly

Cláudio Gouveia Roque[1] and Christine E. Holt[2]

[1] The Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, United States

[2] Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom

SUMMARY

Although *tctp* expression in many areas of the human brain was reported more than 15 years ago, little was known about how it functions in neurons. The early notion that Tctp is primarily expressed in mitotic cells, together with reports suggesting a relative low abundance in the brain [1], has perhaps potentiated this almost complete disregard for the study of Tctp in the context of neuron biology. However, recent evidence has challenged this view, as a number of independent genome-wide profiling studies identified *tctp* mRNA among the most enriched in the axonal compartment across diverse neuronal populations, including embryonic retinal ganglion cells [2-5]. Considering the emerging parallels between axon guidance and cancer cell invasion [6], the axonal expression of cancer-associated *tctp* was suggestive of it holding an unexplored role in the assembly of neuronal circuits. Our study revealed that Tctp is necessary for the accurate and timely development of axon projections during the formation of retinal circuits via its association with the survival machinery of the axon [7]. The findings overall indicate that compromised pro-survival signaling in Tctp-deficient axons results in mitochondrial dysfunction and a subsequent decrease in axonal mitochondrial density. These effects likely translate into a metabolic state inadequate to support the normal guidance and extension processes of a developing axon.

1.INTRODUCTION

1.A Features of Axon Development

The arrangement of retinal neurons in the brain reflects that of the light-sensitive cells in the retina and, ultimately, the visual world. During embryonic development, independent of the birthplace in the retina, retinal ganglion cell (RGC) axons extend in the direction of the optic nerve head, where they collect to exit the eye and form the optic nerve. In vertebrates, once past the midline optic chiasm in the ventral diencephalon, retinal ganglion cell axons grow to the optic tectum, their most prominent synaptic target in the midbrain, and arborize in a topographic array that, in essence, copies the spatial map in the retina onto the brain. Likewise, several other neuronal projections are concurrently established in the embryonic brain; so, how do axons succeed in finding their way?

Observations *in vivo* of developing axonal projections have discovered that their growth is highly directed, with axons navigating along a prescribed trajectory en route to their respective synaptic targets and making very few errors of navigation in the process [8-11]. This remarkable pathfinding fidelity depends on successive spatial signals – guidance cues – presented in the embryonic landscape and integrated by the growth cone, a sensory and motile structure at the tip of developing axons. Axon trajectories are thus seemingly divided into shorter segments in such a way that the effort of navigating towards a distant target is reduced to the simpler task of reaching consecutive intermediate points.

Four evolutionary conserved families of signaling molecules that function as instructive – chemotactic – guidance cues are classically described for their widespread roles in axon guidance: netrins, semaphorins, slits and ephrins. In addition to these *chemical* signals, growth cones are also instructed by cell-cell and cell-matrix *physical* adhesions that provide not only an effective roadmap for navigation but also an essential platform for the protrusive behavior of the growth cone [12, 13]. These contacts can be mediated by members of the integrin, cadherin and, most prominently, immunoglobulin (IgG) superfamilies. Importantly, the actions of these various signals are not mutually exclusive, but rather coordinately act to ensure that axon navigation ensues unerringly. Indeed, the involvement of various instructive signals even along a

short trajectory considerably diminishes the likelihood of guidance errors and promotes the necessary fidelity in the establishment of neuronal connections.

1.B Axonal mRNA Localization – One in Thousands

The synapse underlies one the neuron's most striking features: the axon-dendrite duality, with the inherent cellular and molecular polarization of the nerve cell that it encompasses [14]. Necessarily asymmetric in function, these two compartments receive an independent assortment of organelles, membrane components and molecules from the cell body [15]. Subcellular RNA localization has emerged as a particularly prevalent and cost-efficient mechanism of outsourcing genomic information in these highly polarized cells, where the site of transcription can be far removed from the final destination of the protein [16, 17]. Mechanistically, specific transcripts can be precisely localized to subcellular compartments using the 'address' information harbored in their untranslated regions (UTRs), which function as *cis*-acting platforms for regulatory RNA-binding proteins (RBPs) and small non-coding RNAs [18]. Subsequently, local, 'on-site' synthesis confers both spatial and temporal precision, as the new protein is present only where and when a biological demand for it exists **(Figure 1)**. It is this mRNA-based mechanism that, for example, allows the growth cone to enjoy a certain degree of functional autonomy in its guidance process [16].

Still, it was not until the recent appreciation of the complexity of the axonal transcriptome – several independent genome-wide screens have identified thousands of mRNAs localizing in the axonal compartment of embryonic and adult neuronal cells [2-5] – that the functional significance of this cellular mechanism was fully grasped. Indeed, the prevailing view at the turn of the century was that axons did not synthesize proteins but that instead the proteome in the this compartment was maintained by a constant provision of proteins synthesized in the cell body and transported along the axon [16, 19]. Significant evidence linking local mRNA translation to many aspects of axonal biology has since overwhelmingly dismissed this notion. It is now known that axonal mRNA translation regulates not only growth cone guidance decisions [20-24], where its involvement was originally studied, but also axon elongation [25, 26], axon maintenance and degeneration [3, 27-29], as well as nerve injury and axon regeneration responses [30-32], among other processes [16].

In turn, the impact of local protein synthesis in such diverse cellular mechanisms underlines a crucial aspect of axonal RNA localization: the dynamic nature of the local transcriptome. Indeed, even within the same population of neurons, comparative profiling of two different developmental stages has revealed that axons contain 'age'-specific mRNA pools. For example, mRNAs encoding branching-promoting cytoskeletal proteins and synaptic vesicle proteins, which intuition suggests being irrelevant during the pathfinding stages, are only found in target-arrived axons [4]. It is noteworthy, however, that all of the axonal populations analyzed to date appear to have a common core of transcripts, such as those encoding mitochondrial and ribosomal proteins [16], suggesting that these molecules are implicated in 'everyday' axon upkeep. This also seems to be the case with Tctp: its transcript is ranked among the most enriched in the axonal compartment of diverse embryonic and adult neuronal populations **(Table 1)**, which indicates that Tctp has a constitutive axonal function.

1.C Axon Guidance and Cancer – Shared features

The parallels between the processes of axon guidance and cancer cell invasion hinted that Tctp, a protein associated with malignancy, plays a particularly important role during the wiring of neuronal circuits **(Box 1)**. Indeed, from the continuous changes in motility and adhesion, or the crosstalk with the surrounding environment, the challenges faced by a metastatic cell echo those overcome by a pathfinding growth cone as it navigates through the developing brain.

Curiously, a short incursion into the history of the classical axon guidance molecules reveals an association with cancer pathology dating back to their discovery, suggesting that common signaling pathways operate in both contexts. The *Epha1* gene, for example, was cloned from a carcinoma cell line in 1987 in a screen for novel tyrosine kinase receptors with oncogenic potential, and the first Ephrin ligand was also described by a group working in the context of cancer [33]. Likewise, the *Dcc* gene, the prototypical Netrin-1 receptor, was originally identified as a tumor suppressor in advanced stages of colorectal carcinoma (hence its designation, deleted in colorectal cancer) [34]. Only in the mid-1990s did their association with axon guidance mechanisms begin to be established [35, 36].

However, the links between these processes are perhaps best illustrated by the recent characterization of frequent mutations and copy number variations in classical axon guidance genes in tumors derived from pancreatic ductal adenocarcinoma and liver fluke-associated cholangiocarcinoma patients [37, 38], or the ongoing cancer clinical trials targeting axon guidance molecules. Finally, it is also relevant to note that *tctp* is not the only cancer-associated transcript localizing in developing axons; in fact, the 'cancer' gene ontology (GO) term is among the most significantly enriched in these axonal transcriptomes [4, 5], underlying the cellular and molecular commonalities between both contexts.

1.D Axonal Mitochondria

Since, as a general rule, neuronal cells cannot be replaced throughout the individual's lifetime [39], the preservation of functional neural circuits must necessarily rely on effective axonal protective mechanisms. Classical conjectures supported the view that the process of axonal degeneration ensued from deficient sustenance from the cell body (e.g. as a result of cell body death) [40]. However, it is now well established that the axonal degenerative cascade can be actively promoted by *in situ* death pathways and is counteracted by locally acting and, to some extent, axon-specific pro-survival mechanisms [40]. Moreover, adequate metabolic provision – and hence mitochondria– is pivotal to axonal function, as the demand for energy, metabolites and calcium buffering is particularly elevated at axons terminals (e.g. to support synaptic transmission) [41]. Indeed, many mitochondrial dysfunctions trigger neurodegenerative disorders with prominent axonal phenotypes [42-44], suggesting that axons are indeed particularly vulnerable to mitochondrial compromise. Similarly, a growing axon is dependent on adequate mitochondrial operation, as it requires the continuous provision of energy for its extension in the embryonic brain. It follows that neurons must preserve a damage-prone mitochondrial network to maintain functionality and integrity.

2. TCTP IN NEURONAL CIRCUITRY ASSEMBLY

Given that the identification of *tctp* as a potential candidate of study stemmed from genome-wide profiling screens, we initially sought to validate that its transcripts localize to retinal ganglion cell axons and growth cones at a time when the *Xenopus laevis* retinotectal projection is developing[a]. *In situ* hybridization showed robust *tctp* signal in the optic fiber layer and in the optic nerve head, axon-only structures through where retinal ganglion cell axons navigate to exit the eye. Additionally, in eye explants, *tctp* mRNA signal could be detected in the growth cone of retinal ganglion cell axons. In concordance with *tctp* mRNA axonal localization, Tctp protein was similarly detected in these retinal ganglion cell structures. Ample mRNA and protein signals were also found in the inner and outer plexiform layers, suggestive of localization in the neurites of other retinal neurons, as well as in the photoreceptor layer, populated by light-sensitive neurons, and the ciliary marginal zone, a neurogenic niche in the retina. Significantly, our initial investigations also showed that *tctp* expression is nearly tenfold higher than *actb* in retinal ganglion cell axons as measured by quantitative PCR[b], confirming *tctp* as a highly enriched axonal transcript.

Further analyses revealed that Tctp is implicated in the development of the retinotectal projection **(Figure 2)**. Specifically, Tctp depletion using antisense morpholino oligonucleotides results in splayed projections that fail to innervate the optic tectum at the normal developmental time window **(Figure 2A)**. These effects are not a consequence of extracellularly acting Tctp, as normal retinal ganglion cell axons develop unerringly through a Tctp-deficient optic tract pathway **(Figure 2B)**. Moreover, *in vivo* time-lapse imaging of developing Tctp-depleted retinal axons revealed that their rate of extension was about half of that observed in controls, excluding the possibility that the axonal phenotypes observed are a result of an underlying delay in eye development.

We began our characterization of Tctp axonal mode of action by focusing on mitochondria. This line of investigation unexpectedly arose while examining the

---

[a] The retinotectal projection is formed by the nerve fibers of retinal ganglion cells, which connect the retina to the optic tectum.

[b] *actb* is a well-characterized axon-enriched mRNA [16, 23].

histology of Tctp-depleted retinas for signs of delayed development. Curiously, although the gross stratification of the retina was unaffected, we noted obvious signs of degeneration in the photoreceptor layer of Tctp morphants. The subsequent finding that Tctp expression in these cells is confined to the mitochondria-rich inner segments, together with reports documenting Tctp as part of the mitochondrial proteome [45, 46], suggested a potential link between Tctp and mitochondrial function[c]. These indeed proved to be insightful observations, as Tctp morphant retinas show reduced total ATP levels. Following on this result, we measured a ~20% decline in the membrane potential of mitochondria from Tctp-depleted axons[d], as well as a significant decrease in the number of axonal mitochondria. Importantly, this decrease in axonal mitochondrial density was not accompanied by changes in overall mitochondrial biogenesis or mass, arguing for a phenotype with predominantly axonal repercussions. Indeed, examination of mitochondrial transport dynamics in axons showed that a higher proportion of these organelles move towards the cell body in axons deficient in Tctp than in controls, in line with previous reports showing that dysfunctional mitochondria are selectively 'shipped' to the cell body for repair and/or degradation [47, 48].

How does mitochondrial dysfunction develop from Tctp deficiency? An attractive possibility stemmed from reports linking Tctp to the B-cell lymphoma 2 (Bcl2) family of proteins, which play key mediator roles of mitochondrial integrity and apoptosis [49]. Significantly, embryonic sensory neurons lacking Bcl2, the prototypic member of this family, show reduced axon growth rates [50], a phenotype encountered in Tctp morphants. Particularly well defined is the association between Tctp and myeloid cell leukemia 1 (Mcl1) [51-53], a neuroprotective Bcl2-related pro-survival factor [54], which prompted us to explore whether these two proteins shared a functional relationship in axons. We first showed that axonal Tctp interacts with Mcl1 using a proximity ligation assay, complementing previous biochemical data with an approach that allows the examination of protein-protein interactions with subcellular precision. Second, we looked for signs of unbalanced pro-survival signaling in Tctp-depleted

---

[c] It is relevant to note that photoreceptor degeneration is frequently characterized by bioenergetic decline. For example, mitochondrial dysfunction is reported in age-related macular degeneration.

[d] The mitochondrial membrane potential ($\Delta\Psi_m$) is a parameter directly related to the ability of cells to generate ATP by oxidative phosphorylation and thus serves as cardinal indicator of mitochondrial function.

axons [e][51, 55-58]. Both cleaved Caspase-3 and P53 levels were found to be elevated in axons in the absence of Tctp. Third, consistent with the idea that Tctp works via Mcl1 and the survival machinery to regulate axon development, Mcl1 morphants show similar, albeit milder, axon misprojection phenotypes[f]. Finally, since the N-terminal region of Tctp is required for its pro-survival properties [52, 53], we were able to test whether Tctp pro-survival interactions are a requirement for normal axon development. To this end, we designed a mutated transgene encoding a truncated Tctp protein devoid of pro-survival activity ($Tctp_{40\text{-}172aa}$). $Tctp_{40\text{-}172aa}$ retains Tctp's signature motifs, as well as the interactions domains of several known Tctp-interacting proteins, but lacks those necessary for the association with Mcl1 [52]. Unlike full-length *tctp*, co-delivery of $tctp_{40\text{-}172aa}$ with a *tctp*-targeting morpholino failed to prevent the abnormal development of the retinotectal projection resulting from Tctp deficiency. Collectively, these various findings suggest that Tctp regulates axon development through its association with the survival machinery of the axon **(Figure 3)**.

[e] Tctp stabilizes Mcl1 biological activity, and promotes the degradation of P53, which itself counteracts the pro-survival actions of Mcl1 at the mitochondria. Hence, we speculated that Tctp deficiency resulted in compromised pro-survival signaling.

[f] This milder phenotype may be due to compensation by other members of the Bcl-2 family.

3. SUMMARY AND FUTURE DIRECTIONS

Neurons are highly compartmentalized cells with great energy demands. Given their elongated morphology and unique metabolic requirements, mitochondrial operation needs to be appropriately regulated in these cells to sustain normal neuronal functioning. This assumes particular relevance at distal axon terminals, which require the localized presence of mitochondria to support growth, maintenance, and synaptic transmission [48]. Significantly, our study identified Tctp as a key checkpoint for normal axon development by impacting on axonal mitochondrial homeostasis. Given the importance of maintaining an operational mitochondrial network during axon development and overall neuronal function, it is perhaps not surprising that all axonal populations analyzed to date at the transcriptome level contain a large proportion of mitochondria-related mRNAs [16]. In fact, it has been demonstrated that up to 25% of all proteins synthesized in nerve terminals become associated with mitochondria [59]. Hence, our efforts to characterize Tctp in the context of axon development typify the significant biological investment put into supporting these organelles subcellularly.

Whereas we focused exclusively on examining the role of Tctp in axon development, future work should aim at elucidating its implications in the adult nervous system. Indeed, the decreased Tctp protein expression levels observed in Down syndrome and Alzheimer's disease [60], pathologies associated with mitochondrial dysfunction [41, 61], together with the finding that *tctp* is also among the most abundant transcripts in adult axons, prompt speculation that Tctp holds an important lifelong axonal function. However, given that Tctp is required for the assembly of neural circuitry, temporal control over its knockdown will be a key aspect of any successful approach. This could be achieved by crossing the existing *tctp*-floxed heterozygous mouse line with an inducible, neuron-specific Cre recombinase strain [62, 63]. Considering that proper mitochondrial operation is an imperative of synaptic homeostasis [48], such strategy would, for example, allow one to study Tctp in the context of synaptic function independently of preceding defects in neural circuitry formation.

Box 1: Neuronal connectivity and Cancer Metastasis – Historical parallels?

Historically, the neuroscience field debated two explanatory hypotheses regarding the wiring of the nervous system. The 'resonance theory' explained the developmental patterning of the central nerve tracts on a purely mechanical basis, by schemes of initially non-selective growth that, based on the validity of the connection formed, were later maintained or eliminated [29, 30]. A second framework proposed that selective chemical or electrical forces guided neuronal connections and found initial support in the experiments of John Langley in the late ninetieth century [31]. The extensive studies of Roger Sperry on how regenerating frog retinal ganglion cell axons are arranged when re-innervating their target categorically proved the latter hypothesis [32-34]. In his most dramatic experiment, Sperry rotated the eye 180º on its dorsoventral axis after severing the optic nerve and noted that it lead to the animal having inverted vision; that is, the axons were originating from reversed positions in the eye yet managing to find their appropriate synaptic connections in the brain. He concluded that "the cells and fibers of the brain and cord must carry some kind of individual identification tags, presumably cytochemical in nature, by which they are distinguished one from another almost, in many regions, to the level of the single neuron" [34], a molecular view of the structuring of the nervous system which remains largely unchallenged to date [35].

This idea resonates with the seminal work of Stephen Paget, an English surgeon who published in 1889 what has come to be known as the 'seed and soil' hypothesis, for it embodies an idea quite akin to that implied in Sperry's chemoaffinity postulate. Paget noted, in the process of analysis of more than 900 autopsy records, that tumor metastasis contains an organ-specific, non-random character: "The evidence seems to me irresistible that in cancer of the breast the bones suffer in a special way (...) Some bones suffer more than others; the disease has its seats of election" [242]. From these observations, he equated that metastases depend on certain cancer cells – the 'seeds' – having a specific affinity for the environment of certain organs – the 'soil' – correctly concluding, with sound resemblance to modern day theories of neural circuitry assembly, that only when both 'seed' and 'soil' were compatible would metastasis form [216, 243].

ACKNOWLEDGEMENTS

Our work was supported by Fundação para a Ciência e a Tecnologia [fellowship SFRH/BD/33891/2009 to Cláudio Roque] and a Wellcome Trust Programme Grant [085314/Z/08/Z to Christine Holt]. Cláudio Roque is grateful to the Doctoral Programme in Experimental Biology and Biomedicine as well as the Center for Neuroscience and Cell Biology at University of Coimbra.

REFERENCES

1. Thiele, H., et al., *Expression of the gene and processed pseudogenes encoding the human and rabbit translationally controlled tumour protein (TCTP).* Eur J Biochem, 2000. **267**(17): p. 5473-81.
2. Taylor, A.M., et al., *Axonal mRNA in uninjured and regenerating cortical mammalian axons.* J Neurosci, 2009. **29**(15): p. 4697-707.
3. Andreassi, C., et al., *An NGF-responsive element targets myo-inositol monophosphatase-1 mRNA to sympathetic neuron axons.* Nat Neurosci, 2010. **13**(3): p. 291-301.
4. Zivraj, K.H., et al., *Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs.* J Neurosci, 2010. **30**(46): p. 15464-78.
5. Gumy, L.F., et al., *Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localization.* RNA, 2011. **17**(1): p. 85-98.
6. Harburg, G.C. and L. Hinck, *Navigating breast cancer: axon guidance molecules as breast cancer tumor suppressors and oncogenes.* J Mammary Gland Biol Neoplasia, 2011. **16**(3): p. 257-70.
7. Roque, C.G., et al., *Tumor protein Tctp regulates axon development in the embryonic visual system.* Development, 2016. **143**(7): p. 1134-48.
8. Crossland, W.J., et al., *The specification of the retino-tectal projection in the chick.* J Comp Neurol, 1974. **155**(2): p. 127-64.
9. Lance-Jones, C. and L. Landmesser, *Pathway selection by chick lumbosacral motoneurons during normal development.* Proc R Soc Lond B Biol Sci, 1981. **214**(1194): p. 1-18.
10. Holt, C.E. and W.A. Harris, *Order in the initial retinotectal map in Xenopus: a new technique for labelling growing nerve fibres.* Nature, 1983. **301**(5896): p. 150-2.
11. Raper, J.A., M. Bastiani, and C.S. Goodman, *Pathfinding by neuronal growth cones in grasshopper embryos. I. Divergent choices made by the growth cones of sibling neurons.* J Neurosci, 1983. **3**(1): p. 20-30.
12. Lowery, L.A. and D. Van Vactor, *The trip of the tip: understanding the growth cone machinery.* Nat Rev Mol Cell Biol, 2009. **10**(5): p. 332-43.
13. O'Donnell, M., R.K. Chance, and G.J. Bashaw, *Axon growth and guidance: receptor regulation and signal transduction.* Annu Rev Neurosci, 2009. **32**: p. 383-412.
14. Barnes, A.P. and F. Polleux, *Establishment of axon-dendrite polarity in developing neurons.* Annu Rev Neurosci, 2009. **32**: p. 347-81.
15. Hirokawa, N. and R. Takemura, *Molecular motors and mechanisms of directional transport in neurons.* Nat Rev Neurosci, 2005. **6**(3): p. 201-14.
16. Jung, H., B.C. Yoon, and C.E. Holt, *Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair.* Nat Rev Neurosci, 2012. **13**(5): p. 308-24.
17. Jung, H., et al., *Remote control of gene function by local translation.* Cell, 2014. **157**(1): p. 26-40.
18. Andreassi, C. and A. Riccio, *To localize or not to localize: mRNA fate is in 3'UTR ends.* Trends Cell Biol, 2009. **19**(9): p. 465-74.
19. Lasek, R.J., C. Dabrowski, and R. Nordlander, *Analysis of axoplasmic RNA from invertebrate giant axons.* Nat New Biol, 1973. **244**(136): p. 162-5.
20. Campbell, D.S. and C.E. Holt, *Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation.* Neuron, 2001. **32**(6): p. 1013-26.
21. Brittis, P.A., Q. Lu, and J.G. Flanagan, *Axonal protein synthesis provides a mechanism for localized regulation at an intermediate target.* Cell, 2002. **110**(2): p. 223-35.
22. Wu, K.Y., et al., *Local translation of RhoA regulates growth cone collapse.* Nature, 2005. **436**(7053): p. 1020-4.

23. Leung, K.M., et al., *Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1.* Nat Neurosci, 2006. **9**(10): p. 1247-56.
24. Leung, L.C., et al., *Coupling of NF-protocadherin signaling to axon guidance by cue-induced translation.* Nat Neurosci, 2013. **16**(2): p. 166-73.
25. van Kesteren, R.E., et al., *Local synthesis of actin-binding protein beta-thymosin regulates neurite outgrowth.* J Neurosci, 2006. **26**(1): p. 152-7.
26. Hengst, U., et al., *Axonal elongation triggered by stimulus-induced local translation of a polarity complex protein.* Nat Cell Biol, 2009. **11**(8): p. 1024-30.
27. Cox, L.J., et al., *Intra-axonal translation and retrograde trafficking of CREB promotes neuronal survival.* Nat Cell Biol, 2008. **10**(2): p. 149-59.
28. Yoon, B.C., et al., *Local translation of extranuclear lamin B promotes axon maintenance.* Cell, 2012. **148**(4): p. 752-64.
29. Baleriola, J., et al., *Axonally synthesized ATF4 transmits a neurodegenerative signal across brain regions.* Cell, 2014. **158**(5): p. 1159-72.
30. Verma, P., et al., *Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration.* J Neurosci, 2005. **25**(2): p. 331-42.
31. Ben-Yaakov, K., et al., *Axonal transcription factors signal retrogradely in lesioned peripheral nerve.* EMBO J, 2012. **31**(6): p. 1350-63.
32. Perry, R.B., et al., *Subcellular knockout of importin beta1 perturbs axonal retrograde signaling.* Neuron, 2012. **75**(2): p. 294-305.
33. Pasquale, E.B., *Eph receptors and ephrins in cancer: bidirectional signalling and beyond.* Nat Rev Cancer, 2010. **10**(3): p. 165-80.
34. Mehlen, P., C. Delloye-Bourgeois, and A. Chedotal, *Novel roles for Slits and netrins: axon guidance cues as anticancer targets?* Nat Rev Cancer, 2011. **11**(3): p. 188-97.
35. Tessier-Lavigne, M. and C.S. Goodman, *The molecular biology of axon guidance.* Science, 1996. **274**(5290): p. 1123-33.
36. Serafini, T., et al., *Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system.* Cell, 1996. **87**(6): p. 1001-14.
37. Biankin, A.V., et al., *Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes.* Nature, 2012. **491**(7424): p. 399-405.
38. Ong, C.K., et al., *Exome sequencing of liver fluke-associated cholangiocarcinoma.* Nat Genet, 2012. **44**(6): p. 690-3.
39. Dekkers, M.P., V. Nikoletopoulou, and Y.A. Barde, *Cell biology in neuroscience: Death of developing neurons: new insights and implications for connectivity.* J Cell Biol, 2013. **203**(3): p. 385-93.
40. Pease, S.E. and R.A. Segal, *Preserve and protect: maintaining axons within functional circuits.* Trends Neurosci, 2014. **37**(10): p. 572-82.
41. Friedman, J.R. and J. Nunnari, *Mitochondrial form and function.* Nature, 2014. **505**(7483): p. 335-43.
42. Nunnari, J. and A. Suomalainen, *Mitochondria: in sickness and in health.* Cell, 2012. **148**(6): p. 1145-59.
43. Delettre, C., et al., *Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy.* Nat Genet, 2000. **26**(2): p. 207-10.
44. Alexander, C., et al., *OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28.* Nat Genet, 2000. **26**(2): p. 211-5.
45. Fountoulakis, M., et al., *The rat liver mitochondrial proteins.* Electrophoresis, 2002. **23**(2): p. 311-28.
46. Rezaul, K., et al., *A systematic characterization of mitochondrial proteome from human T leukemia cells.* Mol Cell Proteomics, 2005. **4**(2): p. 169-81.
47. Miller, K.E. and M.P. Sheetz, *Axonal mitochondrial transport and potential are correlated.* J Cell Sci, 2004. **117**(Pt 13): p. 2791-804.
48. Sheng, Z.H. and Q. Cai, *Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration.* Nat Rev Neurosci, 2012. **13**(2): p. 77-93.

49. Czabotar, P.E., et al., *Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy.* Nat Rev Mol Cell Biol, 2014. **15**(1): p. 49-63.
50. Hilton, M., G. Middleton, and A.M. Davies, *Bcl-2 influences axonal growth rate in embryonic sensory neurons.* Curr Biol, 1997. **7**(10): p. 798-800.
51. Liu, H., et al., *Stabilization and enhancement of the antiapoptotic activity of mcl-1 by TCTP.* Mol Cell Biol, 2005. **25**(8): p. 3117-26.
52. Yang, Y., et al., *An N-terminal region of translationally controlled tumor protein is required for its antiapoptotic activity.* Oncogene, 2005. **24**(30): p. 4778-88.
53. Zhang, D., et al., *Physical and functional interaction between myeloid cell leukemia 1 protein (MCL1) and Fortilin. The potential role of MCL1 as a fortilin chaperone.* J Biol Chem, 2002. **277**(40): p. 37430-8.
54. Mori, M., et al., *Expression of apoptosis inhibitor protein Mcl1 linked to neuroprotection in CNS neurons.* Cell Death Differ, 2004. **11**(11): p. 1223-33.
55. Amson, R., et al., *Reciprocal repression between P53 and TCTP.* Nat Med, 2012. **18**(1): p. 91-9.
56. Rho, S.B., et al., *Anti-apoptotic protein TCTP controls the stability of the tumor suppressor p53.* FEBS Lett, 2011. **585**(1): p. 29-35.
57. Vaseva, A.V. and U.M. Moll, *The mitochondrial p53 pathway.* Biochim Biophys Acta, 2009. **1787**(5): p. 414-20.
58. Leu, J.I., et al., *Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex.* Nat Cell Biol, 2004. **6**(5): p. 443-50.
59. Gioio, A.E., et al., *Local synthesis of nuclear-encoded mitochondrial proteins in the presynaptic nerve terminal.* J Neurosci Res, 2001. **64**(5): p. 447-53.
60. Kim, S.H., et al., *Decreased brain histamine-releasing factor protein in patients with Down syndrome and Alzheimer's disease.* Neurosci Lett, 2001. **300**(1): p. 41-4.
61. Pagano, G. and G. Castello, *Oxidative stress and mitochondrial dysfunction in Down syndrome.* Adv Exp Med Biol, 2012. **724**: p. 291-9.
62. Chen, S.H., et al., *A knockout mouse approach reveals that TCTP functions as an essential factor for cell proliferation and survival in a tissue- or cell type-specific manner.* Mol Biol Cell, 2007. **18**(7): p. 2525-32.
63. Susini, L., et al., *TCTP protects from apoptotic cell death by antagonizing bax function.* Cell Death Differ, 2008. **15**(8): p. 1211-20.

FIGURES AND TABLES

Figure 1. Axonal mRNA localization and local protein synthesis

(A) Subcellular targeting of specific mRNAs depends on the recognition of *localization barcodes* by nuclear and cytoplasmic *trans*-acting factors (TAFs), which collectively associate as part of higher-order messenger ribonucleoprotein (mRNP) complexes. Most of the axon-targeting elements that have been identified are situated in the 3'UTR of the mRNAs, and are decoded by various TAFs operating synchronously. Some functionally related transcripts share similar axon-targeting motifs and are regulated by common sets of TAFs, a property that allows these messages to be translated simultaneously with temporal and spatial precision.

(B1) Upon recruiting additional adapter proteins (not depicted), mRNPs are shipped along cytoskeletal tracts by motor-driven active transport mechanisms towards their subcellular destination. Notably, mRNAs are maintained in a translationally dormant state during the assembly and transport phases.

(B2) By modulating the activation of mTORC1 signaling and, in parallel, eliciting changes in the binding affinity of specific TAFs, various local stimuli, including guidance cues, can bring about concerted alterations in gene expression programs.

Figure 2. Tctp is required for axon development in the embryonic visual system

(A) *tctp* knockdown *in vivo* was achieved using an antisense oligonucleotide morpholino (MO) delivered into both dorsal blastomeres of four-cell stage *Xenopus laevis*, which give rise to the entire central nervous system. The retinotectal projection was labeled by intra-ocular delivery of a fluorescent lipophilic dye (DiI) at stage 40, when pioneer axons have completed their stereotyped growth through the optic tract (OT) and reached their target area. Whereas control embryos consistently developed compact axon profiles and had innervated the optic tectum, Tctp deficiency resulted in stunted and splayed projections that lagged in their development. The retinotectal projection is labeled in orange by DiI. Dashed contour delineates the contralateral, dye-filled eye.

(B) Tctp displays IgE-dependent histamine-releasing activity and other cytokine-like extracellular roles. Consequently, it could regulate axon development through its effects in the embryonic brain environment. To test this possibility, we devised an approach that generates embryos deficient in Tctp only in one half of the nervous system. Because the retinotectal projection projects contralaterally (i.e., axons from the left eye extend towards the right side of the brain), this methodology allowed us to probe the effects of a Tctp-deficient optic tract pathway. Overall, normal axons developed unaffected through the Tctp morphant environment, suggesting that the observed axon phenotypes are independent of Tctp acting extracellularly.

Figure 3. Mechanistic insights into the role of Tctp in neuronal circuitry assembly

The normal physiologic scenario is illustrated in (A), whereas the consequences of Tctp deficiency on axon development programs uncovered by our study are shown in (B).

Figure 1. Axonal mRNA localization and local protein synthesis

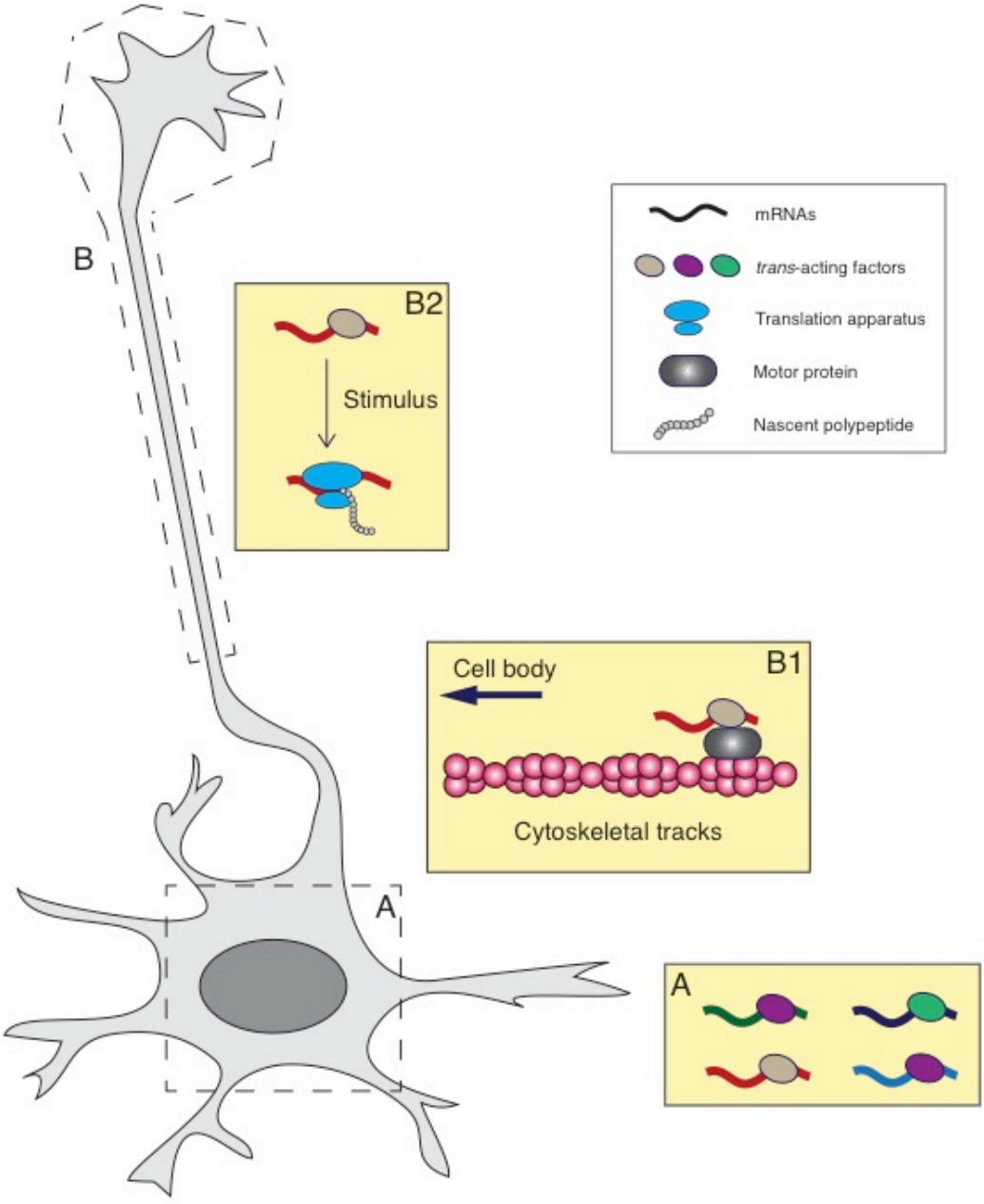

Figure 2. Tctp is required for axon development in the embryonic visual system

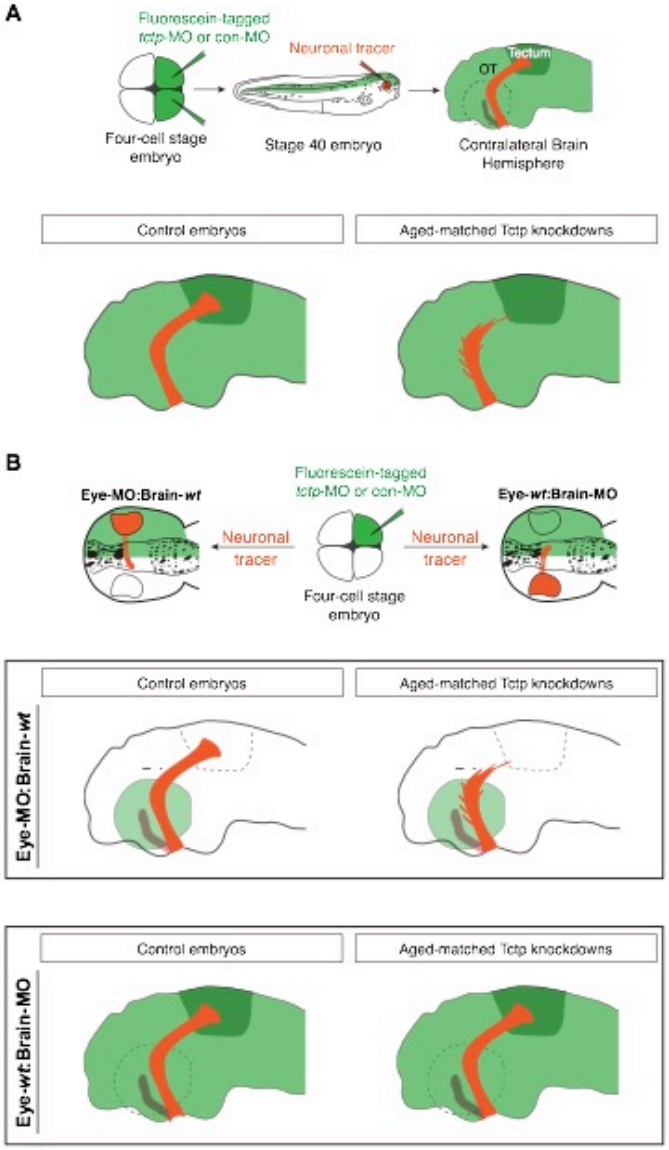

Figure 3. Tctp acts via the survival machinery to promote axon development

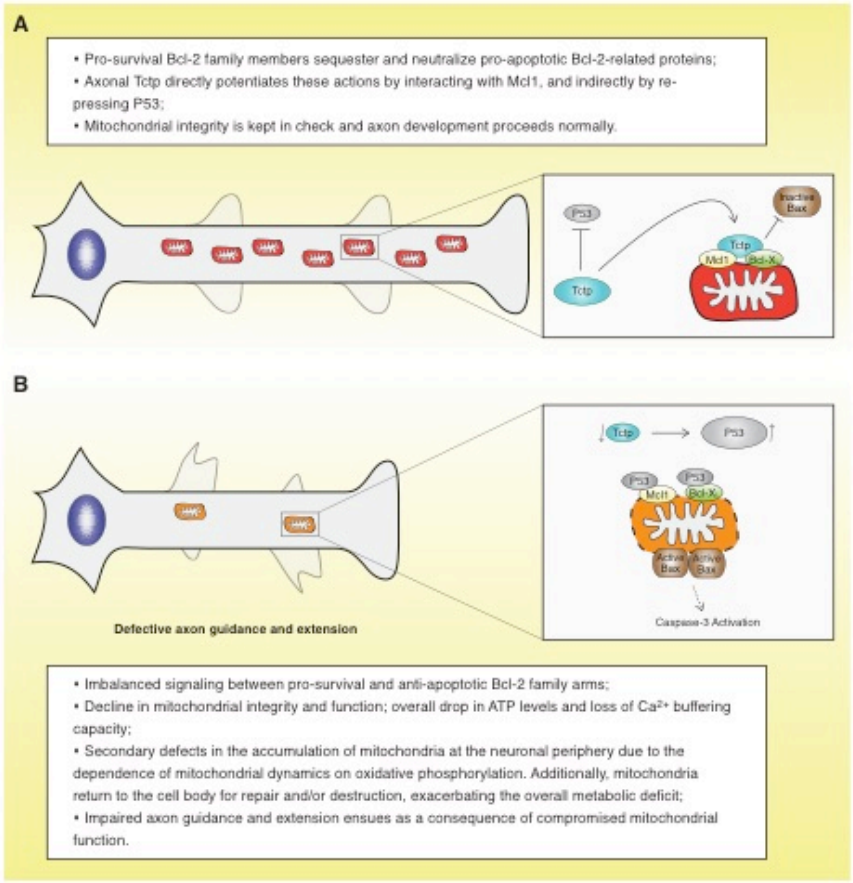

Table 1

Table 1 | *tctp* is a highly enriched axonal mRNA

Neuronal type	Embryonic	Adult	Methodology	Rank	Ref.
Dorsal Root Ganglia (Rat)	✓	✓	Microarray	31st / 69th	[5]
Cortical Neurons (Rat)	Not investigated	✓ (aged in culture)	Microarray	11th	[2]
Sympathetic Neurons (Rat)	✓ (perinatal)	Not investigated	SAGE analysis	5th	[3]
Retinal Ganglion Cells (Frog) [a]	✓[b]	Not investigated	Microarray	83rd / 72nd	[4]
Retinal Ganglion Cells (Mouse) [a]	✓	Not investigated	Microarray	23rd	[4]
Retinal Ganglion Cells (Frog)	✓	Not investigated	RT-qPCR	n.a.[c]	[7]

[a] mRNAs isolated specifically from the growth cone compartment.
[b] Present in 'pathfinding' and 'target-arrived' axons.
[c] 10-fold higher than *actb*.