

## Neuronal polarity: consolidation for growth, growth for consolidation

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**Axonal elongation involves the coordinated assembly and activation of molecular pathways in response to environmental signals. Neurons sense the environment at growth cones to decide on further growth, arborization or consolidation. The work by Ana Mingorance-Le Meur and Timothy P. O'Connor highlights the important role of neurite shaft dynamics in the consolidation process.**

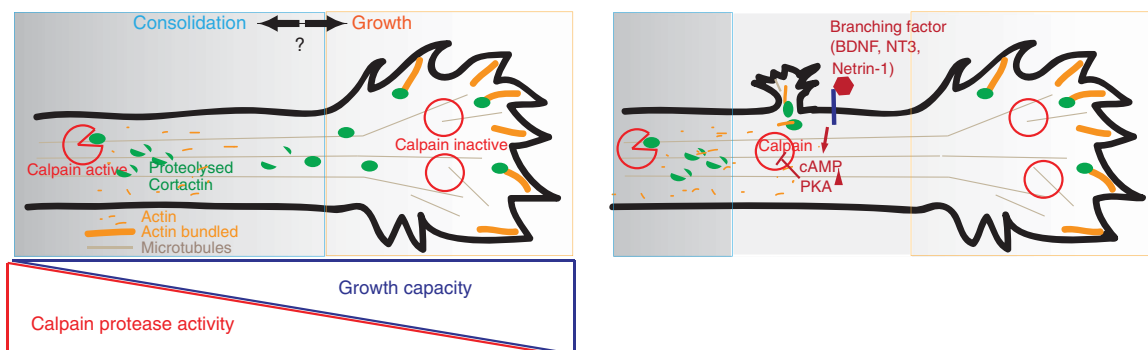
The establishment of functional neuronal circuits requires a large number of precisely coordinated events, with the initial sprouting of neurites from the neuronal cell body being rather crucial. Neurites eventually develop into axons or dendrites, gaining the ability to convert chemical information into electrical signals. However, there is a transitional stage between these two events whereby the first neurites acquire some basic architectural and molecular features of axons or dendrites, yet their main aim is to grow to the territory where they will send or receive information, respectively.

Studies on cortical development demonstrate that newborn neurons sprout two initial, very dynamic neurites, which guide their radial or tangential migration towards their final cortical layer position. Then, neurites need to acquire either axonal or dendritic identity, an event occurring early with the appearance of the first two neurites (Calderon de Anda *et al.*, 2008), followed by a commitment phase, in which growth promoting molecules become restricted to one neurite only, the future axon (Wiggin *et al.*, 2005). This period is critical in brain development, as it changes a neuron from a motile cell, with actively extending and retracting neurites into a stationary cell, in which its unique axon steadily grows towards its eventual target.

A common feature of the advancing axon is the capacity to sense growth, positive or negative environmental cues and act accordingly, by advancing further, retracting or turning. Sensing is largely confined to the growth cones, which locally transduce those signals to the cytoskeleton, mitochondria and membrane reservoirs.

In addition, at specific places along its trajectory, the axon can receive information for branching, which can occur through growth cone splitting or by the appearance of collateral branching spots along the shaft located at the rear of the growth cone (Dent *et al.*, 2003). This brings into light that the neurite shaft must be, except for the peculiar sites of collateral branching, insensitive/irresponsive to the growth cues that elicit a growth response at the growth cone. Failure to do so would result in aberrant branching. In addition, the neurite shaft behind the advancing growth cone must contain all the machinery for the transformation of a dynamic bi-dimensional structure, the growth cone, into the more stable cylindrical neurite as growth proceeds and thus determines the consolidation of the growth. Consolidation may be seen as a simple distal–proximal passive mechanism, whereby the molecules supporting dynamic exploratory and advancing movement at the growth cone become gradually diluted out as one moves towards the cell body. This type of ‘default’ mechanism may be sufficient to activate the machinery to prevent branching and to help making a cylinder. On the other hand, one cannot exclude an ‘active’ mechanism for neurite consolidation. Despite the tremendous importance that neurite consolidation plays in the stabilization in axonal elongation and collateral branching, the different mechanisms governing these processes are far from complete. This confers great relevance to the work of Mingorance Le-Meur and O'Connor in this issue of *The EMBO Journal*.

The main conclusion of the paper is that the restriction of the protease activity of calpain to the neurite shaft in developing neurons is a key event in the stabilization of these extensions. The election of calpains as potential regulators of consolidation was based on previous observations that calpains regulate actin dynamics and concentrate in the rear end of migrating cells, repressing the formation of protrusions. In agreement to their predictions, the activity of calpains, visualized using fodrin proteo-



**Figure 1** Schematic representation of the balance between active growth and consolidation in extending neurites.

lysis as read-out, was restricted to the neurite shaft. Furthermore, inhibition of calpain activity increases the number of actin patches along the neurite shaft. These actin patches may represent sites of high actin turnover, required for localized growth, which is supported by life imaging experiments showing that the inhibition of calpain activity triggers filopodia and protrusion formation at the neurite shaft. The specificity of calpain activity on the process of consolidation/branching is suggested by the observation that calpain activity changes do not affect neurite length. Moreover, the overexpression of a protease dead mutant of calpain increased branching in contrast to the ectopic expression of the full-length calpain-2, which reduced branching capacity. Importantly, inhibition of calpain *in vivo* increased neurite branching in immature and mature neurons, adding biological significance to the *in vitro* data. Furthermore, exposing neurons to ligands known to induce branching inhibits calpain activity when added to neurons *in vitro*, which was blocked by excess of calpain-2. One mechanism by which calpains may prevent branching is through the proteolysis of cortactin, an actin-binding protein that activates the actin-nucleating complex of Arp2/3. Hence, it appears that neurite shaft calpain activity keeps cortactin at basal levels. As regulation of cortactin levels by basal proteolysis is energetically demanding and will need, eventually, to be turned off at the neurite-growth cone interphase, to

allow advance, and along the shaft, to allow branching, consolidation needs to be seen as a key active process of axonal growth, required to keep neurites stable and ready to become dynamic at the same time.

In all, the paper by Mingorance and O'Connor greatly contributes to the field of neuronal differentiation through the study of neurite stabilization. Their data in the context of basic aspects of axonal growth are illustrated in the accompanying figure (Figure 1). The current work leaves a number of important questions unanswered: how do activities regulating growth cone dynamics become repressed towards the neurite shaft? Is calpain activity in the shaft due to de-repression following the dilution of growth cone growing activities (retrograde) or is it because of a cell body-originated trafficking pathway (anterograde), unable to enter (or to be retained) at the growth cone? Do molecules with a presumed role in axonal growth or polarization (Wiggin *et al*, 2005) act in growth cones (pushing molecules) or in the shaft (consolidation molecules)? Clearly, the opening of venues of research is a natural consequence of novel data and therefore we envision that this work will serve as an inspiration to many, to finely dissect the molecular basis of neurite consolidation and how this contributes to axonal growth and branching, in health and pathology. The venues for the future, opened by this work are therefore multiple and exciting.

## References

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