Dendrite development: a surprising origin

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Neurons extend elaborate dendrites studded with spines. Unexpectedly, this cellular sculpting is regulated by the origin recognition complex—the core machinery for initiating DNA replication.

The circuitry of the brain arises from the complex geometry of neurons. For most neurons, synapses form over an elongated and highly branched dendritic arbor whose precise construction enables highly compartmentalized signaling to be integrated in time and space (Hausser et al., 2000). This Brobdingnagian feat of cellular growth and specialization happens during a short period of development after neurons exit the cell cycle and occurs with amazing stereotypy for given subclasses of neurons (Jan and Jan, 2003). Because of their importance in orchestrating neuronal connectivity and signaling, the mechanisms and molecules controlling dendrite morphology have received considerable attention.

Quite understandably, studies to date have focused on the cytoskeletal changes and signaling events that determine how dendrites achieve their shape (Jan and Jan, 2003). As dendrites grow, branches are thought to arise from extended filopodia, which are stabilized by making synaptic contact with nearby axons (Niell et al., 2004). As the dendrites continue their growth, actin-rich membrane protrusions appear over their surface, which contact axons and differentiate into micron-sized mushroom-shaped spines (specialized compartments housing neurotransmitter receptors and other requisite machinery for postsynaptic signaling; Segal, 2005). Emerging from this basic script have been a cast of molecular characters including growth factors, actin regulatory proteins, postsynaptic density proteins, and transcription factors, whose presence onstage seems wholly justified and satisfying. Now, barging from behind the curtain to interrupt this comfortable dialog is a most peculiar Puck of a protein. In the current issue, a paper from the laboratory of Louis Reichardt identifies the origin recognition complex (ORC) as a molecular mischief maker regulating the development of dendrites and spines (see Huang et al. on page 527 of this issue).

Huang et al. (2005) began their unlikely journey by noting the expression of ORC core subunits in adult brain and postmitotic hippocampal neurons (a rather surprising finding given the usual role of the ORC in initiating DNA replication during mitosis; Bell, 2002). More surprising still was the localization of endogenous Orc3 and expressed GFP fusions of Orc2, Orc3, and Orc5 outside the nucleus in the neuronal cytoplasm. Double labeling immunocytochemistry showed that these ORC subunits were restricted to the cell body and dendrites and were absent from axons, pointing to a dendritic function. Fractionation of brain lysates revealed an enrichment of the ORC subunits Orc3–5 in microsome and synaptosome membrane fractions, suggesting a membrane-associated function. To explore this further, Huang et al. (2005) performed loss-of-function experiments in hippocampal neurons using RNA interference against ORC subunits and found that depletion of either Orc3 or Orc5 caused a marked reduction in the number of dendritic branches and overall dendritic length.

What is the origin recognition complex (ORC)? "Origin" in this case refers to the origins of DNA replication present in all eukaryotic chromosomes (Bell, 2002). The mammalian ORC is a hexameric protein complex composed of four core subunits (Orc2–5) and two peripheral subunits (Orc1 and Orc6) that together participate in initiating DNA replication during G1-S transition (Bell and Stillman, 1992; Bell, 2002; Fig. 1 A). Among these subunits, three (Orc1, Orc4, and Orc5) belong to the AAA family of ATPases. The ORC initiates the assembly of a prereplication complex in part by binding the accessory proteins CDC-6 and CDT-1, which are essential for coating the DNA with MCM proteins, a requisite step in replication. In addition, the ORC prevents replication reinitiation to ensure single copy genome duplication. Importantly, the function of the ORC is tightly regulated during the cell cycle by cyclin-dependent kinase (Cdk) activity and by ATPase-dependent changes in subunit conformation and modification (Bell, 2002).

One might logically ask how the nuclear function of the ORC in binding DNA and regulating replication initiation could relate to a cytosolic function in dendrite growth and morphogenesis. In fact, recent studies in nonneuronal cells indicate an expanding repertoire of cellular functions for ORC subunits. For example, Orc6 localizes to the spindle midzone during mitosis where it participates in cytokinesis (Prasanth et al., 2002; Chesnokov et al., 2003), whereas Orc2 associates with centrosomes and centromeres and is required for chromosome segregation following replication (Prasanth et al., 2004; Fig. 1 B). Thus, ORC subunits can regulate cell morphological changes in a manner seemingly independent of a role in replication. Consistent with this notion Huang et al. (2005) found that whereas ORC subunits are expressed in adult brain, proteins required for DNA replication downstream of ORC, including

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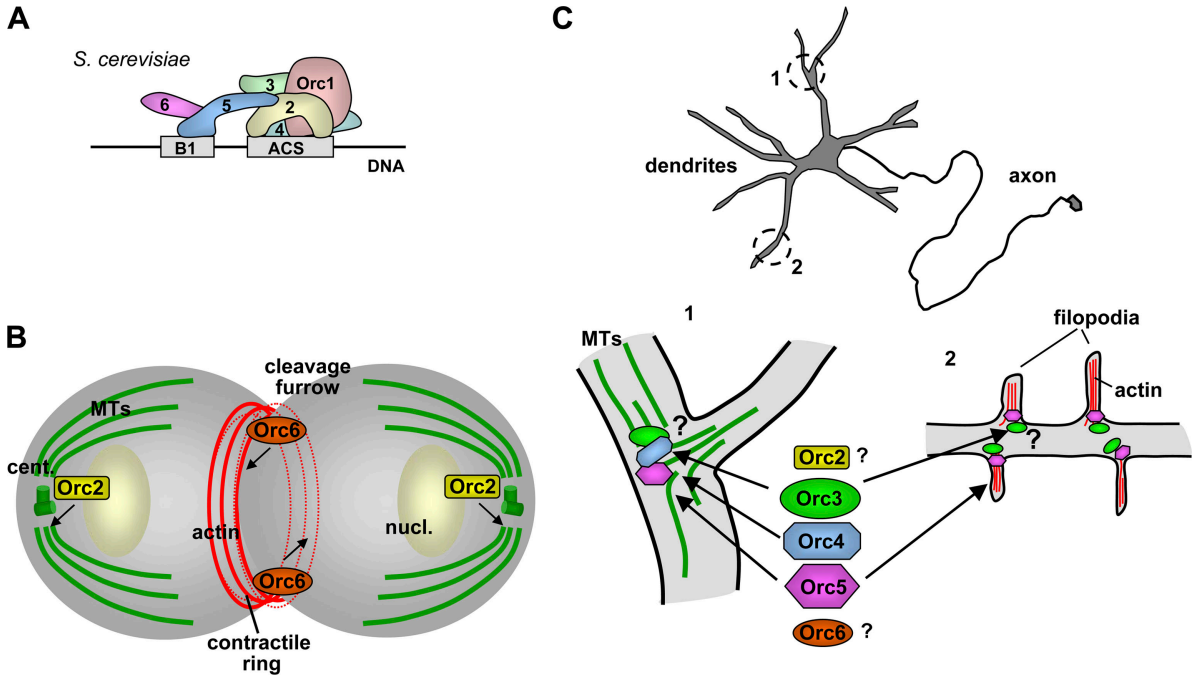

Figure 1. **Proposed role for ORC subunits in dendrite development.** (A) The ORC is best known as a heterohexameric protein complex composed of six subunits (Orc1–6) that acts to initiate DNA replication by binding origin replication sites and recruiting the replication machinery. In *Saccharomyces cerevisiae*, the ORC recognizes the 11-bp autonomous replicating sequence (ARS) consensus sequence (ACS) and other nearby DNA sequences that are less well-defined including B1 element sequences. (B) Nonnuclear functions of the ORC. ORC subunits also associate with centrosomes (Orc2) and the cleavage furrow during cytokinesis (Orc6), suggesting roles in cytoskeletal organization and cell shape changes. (C) In postmitotic neurons, ORC subunits (Orc3–5) localize to the dendrites and fractionate with membranes. Although the precise mechanisms are not known, RNAi-mediated depletion of Orc3–5 leads to decreased dendrite branching (1), reduced dendrite growth (2), and a lack of dendritic filopodia (2), perhaps through local regulation of microtubules (1) or the actin cytoskeleton (2). See text for details. Centr, centrosome; nucl, nucleus; MT, microtubule.

CDC-6 and MCM proteins 2, 4, and 6, are either not expressed or expressed at very low levels.

What about later events in the development of dendrites such as the formation of synapses? Initial studies showed that the *Drosophila* latheo gene, which encodes the fly Orc3, is required for proper development and transmission at neuromuscular synapses (Pinto et al., 1999; Rohrbough et al., 1999), suggesting a role in synapse development. In the current study, Reichardt and colleagues found that RNAi-mediated knockdown of either Orc3 or Orc5 results in a profound loss of dendritic spines on mammalian hippocampal neurons. This loss of spines was not accompanied by a change in spine morphology or a failure to accumulate the postsynaptic density protein PSD-95, suggesting that ORC loss of function perturbs an early step of spine formation without affecting maturation. Such selectivity is unlike many actin-regulatory and postsynaptic density proteins, which typically influence both spine initiation and maturation (Terry-Lorenzo et al., 2005). Consistent with a role for the ORC in spine initiation, neurons in which Orc3 expression was suppressed by RNAi exhibited fewer dendritic filopodia (structures thought to be the precursors of dendritic spines) as well as a decrease in EGFP–Mena puncta, an early marker for nascent filopodia.

So what is the mechanism of ORC-mediated dendrite branching and spinogenesis? In large part, this remains an open question. One clue provided by Huang et al. (2005) is that expression of point mutants of Orc4 predicted to disrupt ATP binding and hydrolysis (E157Q and K71A in the Walker A and Walker B motifs, respectively) increased the elaboration of dendritic branches in hippocampal neurons. These data suggest that the ATPase activity of Orc4 suppresses ORC-mediated dendritic branching, perhaps by changing ORC subunit conformation or altering the association of ORC subunits with as yet unknown regulatory proteins. Due to the chronic nature of the manipulations used by Huang et al. (2005), it is not yet possible to discern whether Orc subunits engage directly, locally, and acutely with mechanisms of dendrite and spine growth (e.g., association with the cytoskeleton, local signaling molecules, or membrane trafficking mechanisms) or whether the observed effect is far downstream of more general and perhaps global changes in cell gene expression, protein levels, or metabolism.

It is tempting to postulate the existence of a direct link between ORC subunits and the actin cytoskeleton, given the well known role for actin dynamics in spine morphogenesis and dendrite patterning. In this regard, it is interesting to note that Orc6 localizes to the cell membrane and cleavage furrow during mitosis and has thereby been implicated in cytokinesis (Prasanth et al., 2002; Chesnokov et al., 2003), a process requiring the assembly and constriction of a circumferential array of actin filaments and myosin-2 (Glotzer, 2005; Fig. 1 B). On the other hand, Orc2 localizes to centrosomes throughout the cell cycle (Prasanth et al., 2004), suggesting a role in microtubule organization that, if operating in dendrites, could direct for membrane transport to accommodate localized dendrite growth

or branching (Horton and Ehlers, 2004; Fig. 1 B). Indeed, Huang et al. (2005) observed an atypical "loose" organization of microtubules in Orc3-depleted neurons. It will be interesting to determine whether mechanisms by which ORC subunits influence cytokinesis and centrosome organization are coopted in neurons to mediate their unique morphological requirements (Fig. 1 C).

The molecular machinery of mitosis has a long and illustrious history in cell biology. Recent years have seen the surprising emergence of many key cell cycle components in the quintessential postmitotic cell—the neuron. For example, the anaphase promoting complex (APC), a multisubunit ubiquitin ligase well known for its role in targeting mitotic cyclins and other cell cycle regulatory proteins for proteasomal degradation, has been found to regulate axon outgrowth and synapse development in neurons (Juo and Kaplan, 2004; Konishi et al., 2004; van Roessel et al., 2004), the latter effect through its ubiquitination of the synaptic scaffold protein liprin-α–SYD-2 (van Roessel et al., 2004). Polo-like kinases (Plk's) are also best known as key regulators of the cell cycle, yet Plk2–SNK mediates the loss of dendritic spines by phosphorylating spine-associated Rap–GAP activating protein (SPAR), a key regulator of actin dynamics in spines (Pak and Sheng, 2003). The results of Huang et al. (2005) now add the origin recognition complex to the growing list of Janus-faced cell cycle molecules regulating neuronal development and plasticity.

More study is clearly needed. In particular, we have very little knowledge about where the ORC resides in dendrites and spines at a subcellular level. Further, much remains to be determined about the mechanisms downstream of the ORC, including such basics as the relevant binding partners and whether the effects of the ORC are far removed or proximate to the ultimate effectors of dendrite growth. Do different ORC subunits mediate distinct aspects of dendrite growth and differentiation? Do the observed effects of ORC subunits on morphology translate into functional effects on synaptic transmission? Do known mechanisms regulating ORC function in replication initiation, such as Cdks, likewise influence dendrite growth via the ORC? Are there additional unknown nuclear functions of the ORC in neurons? Such mechanistic studies will be requisite for determining how the ORC orchestrates the development of dendrites.

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