Doublecortin (DCX), a microtubule-associated protein, is essential for neuronal migration, although a clear mechanistic understanding of this requirement remains elusive. In this issue of *Developmental Cell*, Bechstedt and Brouhard (2012) report that DCX relies on cooperative binding and an affinity for growing microtubules to nucleate and stabilize 13-protofilament microtubules.

The long-distance migration of immature neurons from the ventricular zone to the cortical region of the brain, where they organize into the six-layered structure of the cerebral cortex, is a crucial step during development of the central nervous system in mammals. Genetic lesions that disrupt neuronal migration and lead to severe neurodevelopmental disorders underscore the complexity and importance of this process (Kato and Dobyns, 2003). Neuronal migration depends on tight control of the microtubule and actin cytoskeletons. Therefore, it is of no surprise that mutations in cytoskeletal regulators, such as doublecortin (DCX), are among those that inhibit normal migration (Kato and Dobyns, 2003). DCX is a neuronal-specific modulator of the microtubule cytoskeleton, and mutations in the X-linked DCX gene give rise to type 1 lissencephaly (“smooth brain”) or a milder condition called subcortical laminar heterotrophy (Francis et al., 1999; Gleeson et al., 1999; Kato and Dobyns, 2003). Patients with these syndromes are characterized by an abnormally thick cerebral cortex lacking the normal folds (hence the alternative name double cortex syndrome) and are afflicted with severe mental retardation and epilepsy (Kato and Dobyns, 2003). While evidence pointing to a role for DCX in controlling neuronal microtubules is strong, the molecular mechanism that underlies DCX function is still a subject of intense investigation. A study by Bechstedt and Brouhard (2012), published in this issue of *Developmental Cell*, provides new insights into how DCX promotes the formation and stabilization of 13-protofilament microtubules that may have important implications for its role in neuronal migration.

Neurons typically migrate by first extending a leading process in the direction of movement and then translocating the nucleus and cell body behind it (Figure 1A). These cellular movements rely on spatially and temporally controlled remodeling of the cytoskeleton (Conde and Cáceres, 2009). Existing evidence indicates that DCX may contribute to this control by modulating the microtubule cytoskeleton within the leading processes of migrating neuroblasts. DCX concentrates on microtubules within the leading process (Figure 1A), and loss of its function leads to excessive neurite branching, a problem indicative of defects in maintaining proper leading process morphology (Conde and Cáceres, 2009). Although a number of studies have addressed how DCX may be modifying the microtubule cytoskeleton to promote neuronal migration, this has not been an entirely straightforward question to answer.

DCX is capable of binding, nucleating, bundling, and “measuring the thickness” of microtubules. The DCX protein directly interacts with microtubules via tandem globular domains called doublecortin domains. The majority of the missense mutations associated with lissencephaly, due to loss of DCX function, result in amino acid substitutions within the doublecortin domains, suggesting that interaction of DCX with microtubules is critical to its function during neuronal development (Kato and Dobyns, 2003). DCX has been proposed to function by stabilizing microtubules, possibly by crosslinking tubulin dimers within a single microtubule or by bundling multiple microtubules together (Conde and Cáceres, 2009; Moores et al., 2004). DCX also specifically promotes the formation of microtubules with a 13-protofilament composition, suggesting that the protein can somehow dictate or “measure” protofilament number (Moores et al., 2004). These data support the idea that DCX functions as a microtubule nucleator, which could be an important role given that microtubules in the leading process may be nucleated far from the centrosome, the major microtubule organizing center, and in a region that does not contain detectable γ-tubulin, the canonical microtubule nucleator (Baas and Joshi, 1992). The question of how DCX, which does not form oligomers in solution, can template microtubule growth and control protofilament number is an intriguing one, especially when considering that microtubules in cells predominantly contain 13-protofilaments, whereas those grown in vitro contain variable protofilament compositions. Recent structural studies suggest that this preference may be related to the unique DCX binding site at the vertex of four tubulin dimers, a region that will change dimensions depending on the thickness of the microtubule (Fourniol et al., 2010). Interestingly, this binding site is shared with proteins of the end-binding (EB) family, which have a high affinity for growing microtubule ends and can also promote the formation of 13-protofilament microtubules (Maurer et al., 2012; Vitre et al., 2008). While DCX is clearly capable of modulating microtubules in a number of ways, the mechanisms it uses to carry out these functions remain unclear.

To address how DCX functions at the molecular level, Bechstedt and Brouhard (2012) used high-resolution total internal reflection fluorescence microscopy to...
investigate the behavior of purified DCX on microtubules in a reconstituted system. In contrast to ensembles of DCX, the authors found that single DCX molecules display no preference for protofilament number. At low concentrations, DCX appeared to bind and diffuse along the lattice of both 13- and 14-protofilament microtubules. However, as the protein concentration was increased, DCX displayed cooperative microtubule binding, a significantly reduced rate of microtubule dissociation, and a clear preference for 13-protofilament microtubules. To determine whether cooperative binding might be related to the developmental defects caused by DCX mutations, Bechstedt and Brouhard carried out quantitative comparisons between purified wild-type DCX and a panel of 15 patient-derived DCX-missense mutants. Although each of the mutants tested was capable of binding microtubules in some capacity, each also displayed a reduction in cooperative binding. The loss of cooperativity scaled roughly with the severity of the neuronal developmental defects associated with each missense mutant. In other words, mutations that cause the most severe cortical band thickening in patients also exhibited the largest reduction in cooperative microtubule binding. Additionally, Bechstedt and Brouhard demonstrated that, like EB-family proteins, DCX molecules track the tips of growing microtubules, suggesting that DCX has an increased affinity for structural intermediates that occur during microtubule assembly. Perhaps cooperative microtubule binding and an affinity for microtubule tips can help to explain how DCX stabilizes microtubules to promote neuronal migration (Figure 1B).

Certainly one of the next challenges toward understanding how DCX functions at a molecular level in cells will be to integrate single molecule and structural data with functional studies of DCX during neuronal migration. For example, does cooperative binding facilitate the stabilization of individual microtubules or the formation of bundles in migrating neurons? Can DCX utilize both its preference for microtubule ends and its unique binding site at tubulin dimer intersections to nucleate microtubules within the leading process? Does DCX actually specify protofilament number in vivo, or is its preference for 13-protofilament microtubules simply a by-product of its unique binding site? Experiments to address how DCX affects the nucleation, dynamics, and structure of microtubules within neurons and whether it localizes specifically to growing microtubule tips in these cells will thus be quite informative. The recent work of Bechstedt and Brouhard makes a significant contribution to our understanding of the mechanisms underlying the complex behavior of DCX on microtubules, but there is indeed still much to be learned about how these activities translate to control of neuronal development.

REFERENCES


