patients (Kaur and Cadenhead, 2010) and that early intervention with traditional antipsychotic drugs may not only be ineffective, but may actually worsen the outcome. This makes longitudinal and mechanistically oriented translational approaches such as that applied by Schobel et al. (2013) especially critical for the design of prevention strategies to prevent psychosis in individuals at high clinical risk for schizophrenia.

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Microtubule Stability in the Axon: New Answers to an Old Mystery

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It has been known for decades that a fraction of neuronal tubulin is insoluble in cold and also resistant to calcium as well as drugs that depolymerize microtubules. In this issue of *Neuron*, Song et al. (2013) suggest that this unusual stability results from the polyamination of tubulin by transglutaminase.

For years, it was routine that each new generation of microtubule researchers would learn to make tubulin preps from bovine or porcine brain (Miller and Wilson, 2010). This would involve regular trips to a slaughterhouse, waiting for the brains to become available so that they could immediately be put on ice and rushed back to the lab, minced, and then put into a blender with cold buffer. From there, the strategy was based on the simple principle that microtubules would disassemble into soluble tubulin in the cold and reassemble into microtubules when the prep was warmed. Through cycles of warming and cooling, the prep would become progressively more enriched for tubulin. For most microtubule labs, those days are gone, because molecular approaches can now accomplish what used to require this tedious procedure. Reminiscing about those earlier days brings to mind a fundamental issue about tubulin and microtubules. The initial huge pellet produced by spinning down the brain homogenate was normally washed down the drain without further consideration—but this pellet actually contained a significant amount of tubulin that was not soluble in the cold. Although this was long known, the cold-stable tubulin fraction was given little attention by most investigators—one notable exception being Scott Brady.

Nearly three decades ago, Brady et al. (1984) compared the biochemical properties of tubulin in the cold-stable fraction with the properties of the temperature-cycled tubulin. Much of the tubulin in the cold-stable fraction was shown to be extremely basic in charge, as assessed by two-dimensional electrophoresis. Not only was this different from the cycling tubulin, but, for the ensuing decades, no tubulin isoform produced by any tubulin gene or posttranslational modification became apparent that could explain this behavior.

From biochemical studies alone, it was impossible to know if the cold-insoluble tubulin represented a physiological

structure or some artifact of the preparation. Brady et al. (1984) proposed that cold-stable tubulin exists as regions of longer microtubules that are otherwise less stable, and subsequent electron microscopic work provided support for this idea (Heidemann et al., 1984; Sahenk and Brady, 1987). Notably, cold stability is not an issue in terms of function, because vertebrate neurons would likely never be challenged with temperatures as cold as an ice bath. Rather, cold stability corresponds to resistance to depolymerization, the microtubule regions being composed of cold-stable tubulin that is resistant to virtually anything else that would normally cause microtubules to disassemble, including calcium, dilution, or exposure to drugs such as nocodazole. The hypothesis was that an especially stable tubulin fraction would serve to preserve the organization of the microtubule array, acting as nucleating elements to ensure that assembly occurred from pre-existing microtubules rather than occurring haphazardly (Brady et al., 1984; Black et al., 1984; Heidemann et al., 1984; Sahenk and Brady, 1987). In addition, a marked increase in the levels of cold-stable tubulin as neurons mature was posited to contribute to a normal decline in morphological plasticity that occurs as axons achieve their adult wiring.

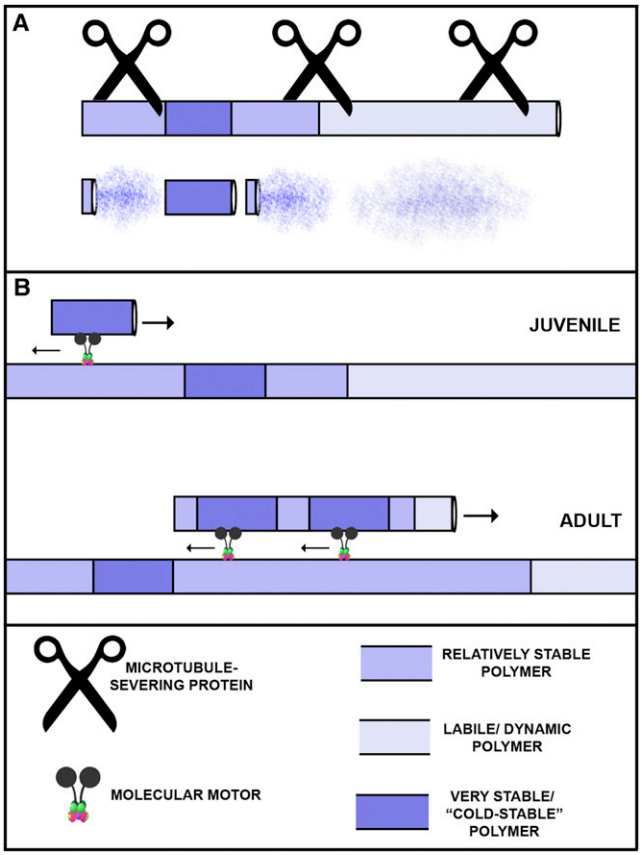

Figure 1. Model for Cold-Stable Regions on Microtubules Acting as Transportable Organizing Complexes

The work of Brady et al. (1984) indicates that a portion of the tubulin in the axon is polyaminated by transglutaminase either before or after assembly of the tubulin into microtubule polymer. They have speculated that certain regions of the microtubule become enriched with this modified tubulin, and, because of this, these regions of the microtubule are especially stable against depolymerization. Posited in this schematic (prepared with the assistance of Aditi Falnikar of Drexel University) is the idea that these regions persist as short microtubule fragments after a bout of severing (A). Then, the fragments engage motor proteins for transportation by a sliding filament mechanism [(B), top]. In adult axons, multiple stable regions may exist along an individual microtubule, raising the possibility that accumulated force by greater numbers of motor proteins may enable longer microtubules to move [(B), bottom].

While the mystery of the cold-stable fraction remained on the back burner for years, the general idea of stable microtubules acting as nucleating elements in the axon became popular. In cultured neurons from newborn and embryonic animals, it was shown that axons contain two sizable populations of microtubules that depolymerize at markedly different rates when exposed to drugs such as nocodazole (Baas and Black, 1990). These two populations, termed stable and labile, were shown to exist as two distinct domains on individual microtubules; the stable domain being toward the minus end of the microtubule and the labile domain being toward the plus end (Baas and Black, 1990; Brown et al., 1993). In drug recovery experiments, it was shown that the labile domains assembled exclusively from the plus ends of the stable microtubules (Baas and Ahmad, 1992).

All of this was good proof of principle in favor of the functional hypothesis advocated by Brady and others, but the relatively stable microtubules in these cultured neurons were not synonymous with the unusually stable microtubules identified in Brady's studies. The relatively stable microtubules in cultured neurons still depolymerized (albeit at a slower rate) in response to drugs, still turned over subunits with the soluble pool as evidenced by incorporation of ectopically expressed tagged tubulins, and did not display the unusual electrophoretic properties of Brady's cold-stable tubulin. Moreover, Black et al. (1984) documented that only about 6% of the tubulin is resistant to cold and calcium when these cultured neurons are homogenized. These observations suggest that neurons contain multiple classes of microtubule polymers that differ in stability. The relatively stable class is presumably rendered less dynamic by cofactors such as STOP and other microtubule-related proteins that function in this manner in other cell types (Slaughter and Black, 2003), whereas the most stable class is unique to neurons and rendered completely nondynamic by a modification of the tubulin itself.

Brady's group has now made significant progress toward solving the mystery of the modification that accounts for the unique properties of cold-stable tubulin. In their new article, they argue that the relevant modification is transglutaminase-catalyzed polyamination (Song et al., 2013). This makes sense because polyamination is known to make proteins more basic, whereas most modifications make proteins more acidic or are neutral, and because polyamination is known to cause proteins to become stable, insoluble, and resistant to proteolysis. In addition, transglutaminase activity is known to increase as neurons mature. However, to date, there has been no evidence showing that brain tubulin is a substrate for this modification that may change microtubule stability.

In the new article, Song et al. (2013) report eight independent lines of biochemical evidence favoring the view that the polyamination of tubulin by transglutaminase contributes to the stabilization of microtubules in neurons. This is fascinating in that the more commonly studied tubulin modifications (acetylation and detyrosination) do not confer stability to microtubules but, rather, accumulate on microtubules that are more stable (Janke and Bulinski, 2011). Thus, polyamination by transglutaminase would be the first identified modification that not only directly confers stability to microtubules but also makes them unusually stable in comparison to other stability classes of microtubules.

Song et al. (2013) present a model in which they posit that the polyamination step can occur on free tubulin, after which modified and unmodified tubulins intermingle during microtubule assembly. Additional modifications may occur on polymerized tubulin. This raises several questions. If the polyaminated tubulin is freely able to incorporate into any newly assembled microtubule polymer, how does a cold-stable fraction become distinguished from a cold-labile fraction? Is there a threshold level that must be incorporated to confer stability? Is the polyaminated tubulin distributed throughout a cold-stable microtubule, or does the polyaminated tubulin simply have to flank otherwise labile regions to make those regions stable as well? Is there any spatial or temporal regulation over the polyamination of tubulin that influences its pattern of incorporation into microtubules?

These new findings on cold-stable microtubules might also have implications for another great mystery of the axonal microtubule array—the nature of microtubule transport. Brady has long favored the idea that the cold-stable regions along axonal microtubules act as "transportable microtubule organizing complexes" (Brady et al., 1984; Sahenk and Brady, 1987). Interestingly, after years of controversy over whether or not axonal microtubules actually move, live-cell imaging on cultured neurons finally revealed that, in the axons of cultured neurons, microtubules unquestionably do move down the axon, but they do so only as very short fragments (Wang and Brown, 2002). Curiously, these mobile microtubules are not only very short, but they are also very stable, undergoing no detectable length changes during bouts of imaging. Mechanistic considerations are most consistent with these short microtubules moving by a sliding filament mechanism rather than as cargo, which is to say that the motor domain of the relevant molecular motor interacts with the short mobile microtubule, whereas the cargo domain interacts with a longer stationary microtubule (Baas and Mozgova, 2012). One possibility is that, when a microtubule is thoroughly chopped by a microtubule-severing protein, what remains are the most stable regions of the microtubule—those enriched with polyaminated tubulin. It may be that it is the unique biochemical properties of polyaminated tubulin that not only provide for great stability of these fragments but also explain how certain motor proteins recognize them and how those motors know to transport them via a sliding filament mechanism. If this is the case, one could also imagine that a much longer microtubule in an adult axon may contain multiple regions that are rich in polyaminated tubulin, thus enabling greater interaction with the relevant motor proteins. If this is the case, perhaps microtubules do not need to be so short in order to be transported in adult axons (Figure 1).

Clearly, there are many issues left on the table, but the latest work by Brady's group has, after nearly three decades, made a much-needed leap toward understanding the nature of the cold-stable tubulin fraction. With rapid progress now underway, it is with some melancholy that many researchers will now remember washing that first pellet down the drain.

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