understanding of the evolution of aging in nature.

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Microtubule Recognition: A Curvy Attraction

While many proteins specifically associate with microtubule ends, the mechanisms underlying these associations remain largely undetermined. A new study demonstrates that doublecortin may localize to microtubule tips through preferential binding to regions of microtubule curvature.

Heidi L.H. Malaby and Jason Stumpff*

Neuronal growth, particularly in the developing brain, requires the efficient transport of materials from the cell body through the growing axon [1]. An extensive cytoskeletal network assists in this transportation, composed largely of tracked bundles of directed microtubules [2]. Among the regulators of this system, doublecortin (DCX) plays an essential role in microtubule maintenance early in neuronal migration and differentiation. Mutations in DCX cause double cortex syndrome or lissencephaly, which manifest as epilepsy and mental retardation and are characterized by 'smooth brain' — a lack of gyri in the cerebral cortex [3–5]. These genetic mutations in DCX are concentrated in two domains with ubiquitin-like folds, designated N-DC and C-DC. Not surprisingly, cryogenic electron microscopy of a reconstituted system has shown that at least N-DC can bind directly with the microtubule components α- and β-tubulin [6]. While it has been shown that DCX colocalizes and cosediments with tubulin *in vivo*, [5], tracks the growing ends of microtubules [7], and binds cooperatively to microtubule ends containing 13 protofilaments [6–8], the recognition mechanism of DCX for microtubules has yet to be fully elucidated. In this issue of *Current Biology*, Bechstedt *et al.* provide evidence for a DCX recognition mechanism driven by the structural curvature of microtubules [9].

Currently, limited information is available for the underlying end recognition mechanisms of microtubule tip-associated proteins. High-resolution EM and crystal structures of kinesin-13 [10] and Stu2p [11] bound to α- and β-tubulin display a shear in the orientation of tubulin dimers which is not present in structures of tubulin alone, hinting that these proteins may associate with curved regions of protofilaments. Of the microtubule end-binding proteins characterized physiologically, end-binding protein 1 (EB1) has been shown to selectively recognize the γ-phosphate state of β-tubulin [12]. Since GTP hydrolysis primarily occurs at the growing ends of microtubules, EB1 is effectively localized to polymerizing tips where it functions to increase microtubule nucleation, catastrophes and rescues [12,13]. While EB1 makes direct contact with the nucleotide-binding third helix of β-tubulin, structural studies of DCX do not show a β-tubulin contact in this region, implicating a recognition mechanism independent of nucleotide state [12].

In the current study, Bechstedt *et al.* utilized a single-molecule fluorescence microscopy assay to observe DCX recognition at microtubule ends [9]. The authors first found that DCX and EB1 have distinct kinetic behaviors, quantified in these assays as 'comets', where the length of a microtubule that fluoresces from a bound protein correlates with the protein concentration at the microtubule end. While EB1 comets elongate with an increase in the microtubule nucleation rate from a higher concentration of tubulin, DCX comets remain constant for all growth rates observed (Figure 1A,B). Thus, the DCX binding site at microtubule ends does not change as a function of polymerization rate. EB1 comets have also been observed to shrink upon increases in EB1 concentration

[13,14]. In contrast, DCX comets continued to grow in correlation with DCX concentration until the entire lattice of the microtubule was saturated with fluorescent DCX to form 'light sabers', indicating that DCX does not catalyze the destruction of its own binding site. Lastly, the authors questioned a common recognition mechanism by comparing DCX and EB1 nucleotide sensitivity using several GTP analogs. When the fluorescence intensity of GMPCPP, GTPγS, and GDP-BeF_3 were expressed against GDP fluorescence intensity for DCX and EB1, it was found that DCX binding was only enhanced by GTPγS. However, given that GTPγS also increased the binding of tau and kinesin-1, which primarily associate with the microtubule lattice, this result needs further corroboration to understand the mechanism of these GTPγS heightened binding affinities.

Given the kinetic differences in DCX and EB1 comets and deviating binding enhancements of GTP analogs, the authors tested the possibility that DCX recognizes curved structural features at microtubule ends. Seeded microtubules were grown in the presence of flow, creating longitudinal curves. When DCX was added, it localized to the regions of microtubule bending, both in bulk imaging assays and by tracking individual DCX molecules. Single-molecule quantification showed that individual DCX proteins have a higher affinity for curved areas of microtubules. EB1, in contrast, showed no preference for curvature of microtubules. This finding suggests that DCX might recognize the curved protofilaments often observed at microtubule ends (Figure 1A,B) [15]. In further support of this idea, the authors found that when microtubules were straightened by treating with paclitaxel, DCX localization to microtubules was severely reduced, while EB1 recognition was unaffected (Figure 1C).

While these data strongly support the idea that DCX recognizes the curved ends of growing microtubules *in vitro*, an important question is whether this activity is relevant to the protein's function in neurons. To address this, the authors tested the effects of double cortex syndrome-derived mutations in the N-DC and C-DC domains on curvature binding. Four mutants where found in the C-DC domain that abolished or severely reduced curvature localization. Interestingly, these mutations were found in three out of four corner regions in the domain. The authors elegantly introduced two alanine mutations in residues of the remaining corner, and found that these mutants also strongly decreased curvature recognition. Together, these results implicate loss of microtubule end recognition by DCX as a possible mechanism underlying the pathogenesis of double cortex syndrome in a subset of patients.

In order to substantiate these findings, more detailed structural information will be required. Resolving DCX binding to protofilament curvature at microtubule ends will be a particularly important goal for future studies, and comparing these findings with those of kinesin-13 and Stu2p will be highly informative towards understanding the molecular mechanism of curvature recognition. Given the strong influence of the disease state mutations on microtubule curvature localization, exploring potential drug targets to increase curvature affinity would be enticing. And, as the authors keenly observe, now that nucleotide state and microtubule curvature are both viable options for microtubule end recognition, future studies of dynamic microtubule ends with their plethora of associated proteins must account for multiple modes of attachment and regulation.

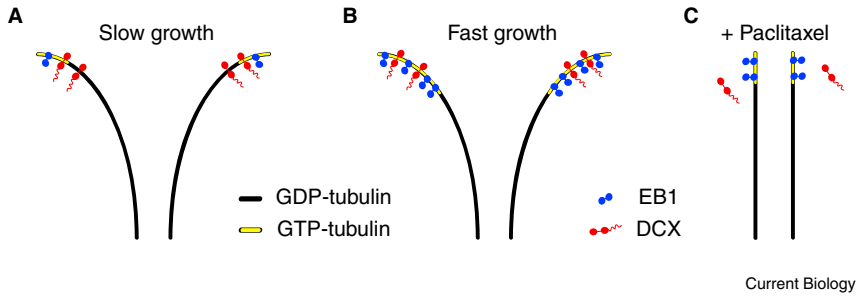

Figure 1. Working model of microtubule end recognition by DCX and EB1.
(A) EB1 binds specifically to GTP-tubulin, while DCX recognizes microtubule curvature. (B) When nucleation occurs quickly, more EB1 is associated with the rapidly growing GTP-tubulin end, while DCX binding does not change. (C) Microtubules treated with paclitaxel are still bound by EB1, but DCX no longer localizes.

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Amygdala: Eyes Wide Open

A new study sheds light on how the amygdala contributes to social interactions by providing direct evidence for specialized visual neurons that selectively respond to seeing the eyes of another individual or making eye contact with them.

Steve W.C. Chang[1,*] and Michael L. Platt[2,3,4,5]

The eyes convey privileged social information in primates [1]. Numerous studies to date have reported a specialized role for the amygdala in processing information about the eyes in primates. In a patient known as S.M., bilateral damage to amygdala caused a deficit in perceiving the emotional states of others by viewing their faces, and this impairment stemmed from reduced exploration of the eyes [2]. A welter of neuroimaging studies has found that viewing the eyes of others strongly activates the amygdala, and this activity is related to the angle subtended by the eyes, typically with greater activation for direct eye contact [3,4]. One study [4] found that a specific region within the right amygdala in humans is specifically engaged during direct eye contact. The importance of the amygdala in signaling information about the eyes of others appears to extend to actively making eye contact: patient S.M. shows difficulty making direct eye contact during social interactions, and instead pays much more attention to the mouths of her social partners [5].

Despite this strong evidence for an important role for the amygdala in sensing and potentially making eye contact with others, however, direct physiological evidence that neurons in amygdala actually respond to eyes has been elusive. As they report in this issue of *Current Biology*, using a more ethological way of testing neuronal selectivity in the amygdala [6], Mosher *et al.* [7] have now shown that there are distinct classes of visual neurons in the amygdala that selectively respond when monkeys view the eyes of other monkeys. Notably, a subset of eye-fixation cells are specialized for detecting when monkeys in movie clips looked at the camera, resulting in eye contact with the subject monkey [7]. These findings suggest the amygdala — a collection of brain nuclei long associated with emotion, punishment, reward, and attention — contains neuronal specializations for processing some of the most important, emotionally-arousing, and meaningful biological stimuli in our environment, namely the eyes of others.

Foraging for Social Information with the Eyes

Foraging is one of the most important and fundamental behaviors controlled by the nervous system. Foraging is usually considered in the context of acquiring food, but the computational principles that guide foraging behavior [8] can be applied more generally, and in particular they offer insights helpful for understanding how the nervous system organizes social behavior [9]. For highly visual animals such as primates and humans, eye movements may be considered behaviors aimed at foraging for valuable information, including information about others — and in primates and humans, such information can be gained from looking at the eyes of conspecifics [1]. The direction of gaze, for example, conveys social information [1], signaling intentions, promoting cooperation and directing the attention of others to important objects and events [10].

Following the gaze of others is an adaptive response to their use in foraging for visual information, and is found in gregarious primates and other social animals [10]. Gaze-following behavior is foundational for joint attention and theory of mind, and may be critical for developing language [10]. These observations invite the hypothesis that the primate brain may have evolved neuronal specializations for detecting and responding to the eyes of others.

Social Behavior and the Amygdala

In their classic 1939 paper, Klüver and Bucy [11] described the behavioral deficits caused by bilateral temporal lobectomy in rhesus macaques. Monkeys with amygdalectomy showed profound social impairments. They tended to lose social rank and were often excluded from their troops, sometimes leading to their deaths [12]. Many of these social impairments were attributable to loss of amygdala functions. In addition to social impairments, amygdala lesions are also associated with alterations in nonsocial behaviors, including loss of fear in response to physical threats (for example, from a snake or human), especially in novel and unfamiliar settings [13]. Thus, social processing is just one of many operations carried out by the amygdala that appear to orient the organism to critical objects and events in the environment.

Though often discussed as if it were a single, homogeneous nucleus, the amygdala is actually a heterogeneous region composed of multiple nuclei that receive inputs from all sensory modalities, with particularly strong visual inputs in primates [14]. The amygdala is ideally situated anatomically to integrate multiple sources of information from both central and peripheral sources [14]. This may allow the amygdala to compute the state of the brain with respect to the rest of the body. The amygdala is also involved in learning and decision-making: neuronal activity