Microtubules: Sizing Up the GTP Cap

The 'GTP cap' of the microtubule has long been postulated to exist, but a recent experiment gives us the first quantitative measurements of the cap size in the cell.

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Microtubules are cylindrical polymers formed from heterodimers of α-tubulin and β-tubulin. Their assembly and disassembly are essential for cell-cycle progression and differentiation. Microtubule polymerization, structure, and organization are closely regulated by a host of microtubule-associated proteins, but a more basal level of regulation comes from the nucleotide state of tubulin itself. Both α- and β-tubulin must bind GTP in order to polymerize (Figure 1). All evidence suggests that the non-exchangeable nucleotide bound to α-tubulin remains in the GTP state, but the exchangeable nucleotide bound to β-tubulin can be hydrolyzed following polymerization. The central paradigm of microtubule biology is that microtubules are stabilized by a 'GTP cap', a region at the end of a polymerizing microtubule where GTP hydrolysis has not yet occurred. Direct measurement of the size of this cap in cells was never previously possible. In this issue of *Current Biology*, Seetapun et al. [1] leverage recent developments in the field to provide a direct measurement of GTP cap size *in vivo*.

The first suggestion of a GTP cap came more than three decades ago from the observation of a time lag in GTP hydrolysis following tubulin polymerization [2]. Subsequently, microtubules were shown to fall apart completely when broken in the middle [3], indicating that the entire polymer is stabilized by its GTP-containing ends. This basic model explains the phenomenon of dynamic instability, the stochastic switching of microtubules between periods of growth and shrinkage [3]. If the stabilizing GTP cap is lost, the microtubule switches from growth to rapid shrinkage (a 'catastrophe'), and the subsequent gain of a GTP cap would switch the microtubule back to normal growth (a 'rescue'). Insight into the size and nature of the GTP cap is fundamental to microtubule polymerization dynamics, as the size of the cap reports on the interplay of polymerization and GTP hydrolysis rates, and thus to the control of microtubules during cell proliferation and development.

The first attempts to measure the cap size indicated a minimal cap, perhaps as small as a single layer of GTP–tubulin; however, the GTP cap itself was not directly observable (reviewed in [4–7]). This situation changed with the discovery of microtubule end-binding (EB) proteins. These proteins bind preferentially to the end of growing microtubules, forming an extended 'comet', and an immediate hypothesis was that they recognize the GTP cap. This hypothesis has been validated by studies showing that EB proteins bind preferentially to microtubules polymerized with slowly hydrolyzable and non-hydrolyzable GTP analogs [8–10]. In essence, EB1 binding serves as a read-out for the presence of the GTP cap. Making use of these findings, Seetapun et al. [1] used a GFP-tagged EB1 protein to label the growing ends of microtubules in LLCPK1α kidney epithelial cells. By carefully calibrating the fluorescence intensity using GFP–tubulin, the authors measured the brightness of EB1–GFP comets, precisely counting the number of EB1–GFP molecules present in the comet. In sharp contrast to *in vitro* measurements, Seetapun et al. [1] found an average cap size of $\sim$750 tubulin subunits, spread over $\sim$55 rows of tubulin, significantly larger than previous *in vitro* estimates of cap size. Further, they found that the cap region decays exponentially with increasing distance from the tip (illustrated in Figure 1), and that the stabilizing features can be observed more than 1 μm from the microtubule end. Consistent with these findings, the authors also found that depolymerization rates were slower in the cap region, and that stabilizing regions further back from the tip appear to contribute to rescues.

There is no question that EB proteins recognize a stabilizing cap at the microtubule end, but the question remains: what exactly is the nature of this cap region? The hydrolysis of GTP at microtubule ends occurs in at least two steps. The first step is the hydrolysis event, converting GTP to GDP-Pi, and the second step is phosphate release, converting GDP–P_i to GDP within the microtubule lattice. For heterotrimeric G proteins, however, additional transitions occur after GTP binding, namely an isomerization into a hydrolysis-competent state and a catalytic intermediate state [11]. Tubulin may pass through similar transition states on its way from being GTP-bound to GDP-bound. The relative rates of polymerization, hydrolysis and phosphate dissociation will determine the comparative sizes of the GTP, GDP–P_i, and GDP domains at microtubule ends, and indeed of other, still hypothetical nucleotide states. Importantly, EB proteins may distinguish between these different states, an idea proposed in Maurer et al. [10]. In support of this idea, EB proteins bind preferentially to microtubules in the presence of GDP–BeF_3 [8], an analog often considered a mimic of the GDP–P_i state, and EB proteins also prefer one
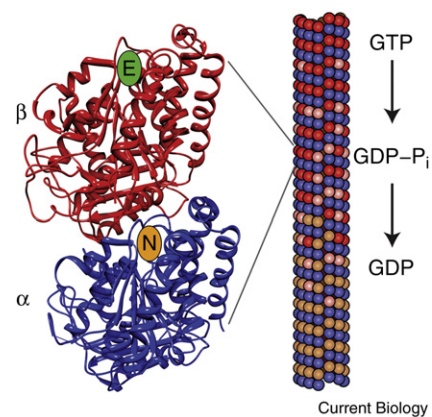

Figure 1. Nucleotide states at the end of a growing microtubule.

The tubulin heterodimer contains two nucleotide sites: a non-exchangeable or N-site in α-tubulin and an exchangeable or E-site in β-tubulin. As the microtubule polymerizes and tubulin is incorporated into the filament, hydrolysis of the E-site GTP and subsequent phosphate release can occur, but the time lag between these events could give rise to three regions in the polymer: a GTP region at the growing tip, a GDP–P_i region where the phosphate has not yet dissociated, and a GDP region in the central portion of the microtubule. GTP–β-tubulin (red), GDP–P_i–β-tubulin (pink) and GDP–β-tubulin (brown).

GTP analog, GTPγS, over the canonical GTP mimic GMPCPP [8]. Ultimately, the GTP cap may need to be viewed as a mosaic of nucleotide and structural states that collectively stabilize the microtubule end. Obviously many questions remain to be answered and the Seetapun *et al.* [1] paper sets the stage for years of future investigation.

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Neuronal Networks: Enhanced Feedback Feeds Forward

Modulatory projection neurons gate neuronal networks, such as those comprising motor central pattern generators; in turn, they receive feedback from the networks they gate. A recent study has shown that, in the crab stomatogastric ganglion, this feedback is also subject to modulation: the enhanced feedback feeds forward through the projection neurons to modify circuit output.

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By now those interested in motor networks are well aware that central pattern generating networks (CPGs), both in invertebrates and in spinal cord and brainstem, feed back on to the modulatory projection pathways that drive and gate them through direct synaptic and neuromodulatory interactions [1–5]. But what if that feedback itself is modulated? That would lead to new levels of complexity, but more importantly, to new levels of control. Blitz and Nusbaum [6], in an apparent first, report a clear example of how such modulation of CPG feedback works and its functional consequences.

Blitz and Nusbaum [6] studied the interaction between two CPG networks in the crab stomatogastric nervous system [7,8]. This bit of the crab central nervous system consists of the stomatogastric ganglion (STG), in which reside two CPGs that control the foregut and associated higher ganglia that provide modulatory input to the STG. One CPG, the pyloric, produces a fast rhythm, controls food particle sorting, and seems continuously active in response to tonic modulatory input from projection neurons; the other produces a slow rhythm (ten times slower than the pyloric rhythm), controls chewing, and must be gated on by projection neurons, which are in turn driven by sensory or other higher order inputs.

These two CPGs interact strongly in the STG, and the linchpin of this interaction is the AB neuron [9,10]. This neuron is the pacemaker of the pyloric CPG and provides pyloric timed inhibitory input to the gastric CPG. This input is onto the STG terminals of the bilaterally paired modulatory projection neurons MCN1 — the focus of the new study [6] — and through this interaction entrains the gastric rhythm and regulates its period. MCN1 then modulates the pyloric period with gastric periodicity [11].

The AB neuron is also the nexus of pyloric feedback to projection neurons that arise in a set of higher ganglia of the stomatogastric nervous system, the bilateral commissural ganglia, including the MCN1s [9,10]. Thus, the AB neuron provides feedback to MCN1s both locally on their terminals in the STG and distally in the higher commissural ganglia where they arise. In the new study [6], the authors focus on a version of the gastric rhythm that is gated by MCN1 and has clear pyloric timed interruptions in each gastric burst (another pair of projection neurons also participates similarly but will not be mentioned further here). This gastric rhythm is evoked by transient stimulation of a modulatory pathway called POC that terminates in the commissural ganglia and causes long-lasting (>20 minutes) activation of MCN1 in the commissural ganglia [6].

The authors [6] observe that when a gastric rhythm was evoked by POC stimulation, AB inhibitory feedback to MCN1 was enhanced in the commissural ganglia but not in the STG. They show convincingly that the time course of this enhancement parallels the duration of POC-evoked gastric rhythm activation, and that this modulation is presynaptic. Similar site specific presynaptic modulation has been observed in the vertebrate central nervous system (for example, [12]). These observations suggest that the POC pathway is responsible for the presynaptic enhancement. The increased AB inhibitory feedback to MCN1 in the commissural ganglia leads it to burst in pyloric time, and parallel pyloric-timed interruptions of gastric bursts are observed [6]. Without enhancement of this feedback synapse, pyloric time bursting is weak or non-existent in MCN1.