ANALYSIS OF THE MECHANISM OF MICROTUBULE DYNAMIC INSTABILITY USING A UV MICROBEAM TO SEVER ELONGATING MICROTUBULES

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Microtubules polymerized in vitro from tubulin purified free of microtubule-associated proteins exhibit dynamic instability (1,2,3). Free microtubule ends exist in persistent phases of elongation or rapid shortening with infrequent, but, abrupt transitions between these phases. The abrupt transition from elongation to rapid shortening is termed catastrophe and the abrupt transition from rapid shortening to elongation is termed rescue. A microtubule is an asymmetrical structure. The plus end grows faster than the minus end. The frequency of catastrophe of the plus end is somewhat greater than the minus end, while the frequency of rescue of the plus end is much lower than for the minus end (4).

The mechanism of catastrophe is controversial, but for both the plus and minus microtubule ends, catastrophe is thought to be dependent on GTP hydrolysis. Microtubule elongation occurs by the association of tubulin-GTP subunits to the growing end. Sometime after incorporation into an elongating microtubule end, the GTP is hydrolyzed to GDP, yielding a core of tubulin-GDP capped by tubulin-GTP ("GTP-cap"). The "GTP-cap" model of dynamic instability proposes that tubulin-GTP microtubule ends are relatively stable and elongate. In contrast, tubulin-GDP microtubule ends are labile and rapidly shorten. A catastrophe is proposed to occur by the loss of tubulin-GTP from an elongating microtubule end, while rescue occurs when a rapidly shortening end is re-capped with tubulin-GTP.

We have tested this GTP-cap hypothesis by constructing a UV microbeam to sever the elongating end from individual microtubules. This instrument permits UV irradiation of regions of individual microtubules and near simultaneous, real-time, visualization of the assembly dynamics of these 25 mm diameter microtubules using high resolution differential interference contrast (DIC) light microscopy, video contrast enhancement and digital image processing (5). The UV microbeam apparatus was constructed on a custom built polarization microscope configured for DIC microscopy using a Zeiss Ultratfluar 100X/0.85NA quartz objective as the condenser (6). The image of the UV source, a 200W mercury burner, was projected onto a 0.2 x 0.4 mm mirror positioned at the condenser field diaphragm plane. The Ultratfluar lens projected a 2 um wide slit onto the specimen plane in focus with the visible light DIC image of the specimen. The mirror was slid into the light path just before UV irradiation and pulled out of the light path for the observation of microtubule ends. Three sec UV irradiations were sufficient to sever the microtubules. Fragments of sea urchin flagella axonemes were used to nucleate the growth of plus and minus microtubule ends from purified porcine brain tubulin (3,4). The assembly dynamics of 16 um tubulin preparations were viewed at 21-22°C in 10 um thick chambers made from glass and quartz coverslips.

When the distal tip of an elongating plus end microtubule was cut off, the severed plus end always rapidly shortened back towards the axoneme. This occurred for cut sites at any position along the length of a microtubule. Cutting 0.5 um from the end of a 15 um long microtubule was seen to produce rapid shortening of the severed plus end all the way back to the axoneme. These results showed that some form of "stabilizing cap" like the proposed "GTP-cap" governs the instability at the plus end of an elongating microtubule.

When the distal tip of an elongating minus end microtubule was cut-off,
no rapid shortening occurred. Instead, the severed minus end began
elongating, at a rate similar to an unsevered minus end. This result
occurred for cut sites at any position along the length of a microtubule.
These results indicate that instability at the plus and minus ends of
microtubules can be distinctly different: severed plus ends are labile while
severed minus ends are relatively stable.

It might be argued that the UV irradiation could have preferentially
cauterized or irreversibly cross-linked the tubulin-GDP subunits exposed at
the severed minus ends. However, these possibilities seem unlikely for three
reasons. First, the severed minus end elongated after UV irradiation.
Second, the rate of elongation of severed minus ends was similar to the rate
of elongation of unsevered minus ends. Third, spontaneous rapid shortening,
which happened following UV irradiation and subsequent elongation, occurred
over several micron distances without hesitation at the site where the
microtubule was initially severed. If UV irradiation stabilizes the tubulin-
GDP subunits at the severed minus end, this stabilization must be transient
and allow the efficient addition of tubulin-GTP subunits.

The previously proposed GTP-cap models (7) do not account for this
differential instability between the plus and minus microtubule ends seen in
our UV microbeam studies. It is possible that catastrophe is not a one, but
a two step process. First, the cap must be lost. Second, a structural
transformation must then occur at the microtubule end before rapid
dissociation of tubulin subunits takes place. Rapid shortening occurs as
this structural transformation propagates back toward the nucleation center.
At the plus end, this transformation occurs quickly whenever the cap is
lost. At the minus end, this transformation occurs relatively slowly in
comparison to the rate of recapping of the end. Rescue initially involves
re-transformation back to the normal tubulin lattice configuration and
subsequent recapping of the ends. Re-transformation at the plus end is
relatively unfavorable in comparison to the minus end, thus yielding the
much lower frequencies of rescue observed at the plus end in comparison to
the minus end. (8)

References

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