Undercut combined with a vertex in the x-y image plane constrains the MT. The coverglass maintains high quality imaging and trapping while the low profile of the optical tweezers and microscopy barriers prevent interference with the tightly focused laser beam. The engineered microtubule, as shown in (A), presents on a longer scale is clearly not a step. Rather, this event is revealed to be a growth-phase shortening event, as shown in (B). Note that the gtp cap remains intact, never being lost during growth-phase shortening events. Subsequently, the microtubule polymer continues growth, as shown in (C). Catastrophe occurs after 10 sec, as shown in (D), at which point the gtp cap has less than 3 subunits on average.

Material and Methods

Optical Tweezers

Microtubule Polymerization at the Nanoscale: Highly Variable, But No Oligomeric Steps

Results

Microtubule Growth Rates Over a Range of Forces

A. Experimental record of MT polymerization. The MT is attached to a trap at the nanoscale. The MT is observed by optical microscopy with a trap positioned at 35 nm from the MT's end. The MT is growing at a rate of 3.7 nm/s. The MT is observed to change direction at a rate of 0.2 events/min.

B. Histogram of forces where MT growth pauses for at least 5 seconds.

Microtubule Growth is Highly Variable, But Does Not Involve Oligomeric Steps

Recently stepwise growth of MTs was reported, and steps were attributed to addition of tubulin oligomers (Schek and Hunt, 2005). Since our data shows no evidence of this, we investigated if apparent “steps” might be the result of data processing. Since the MT shortens, we analyzed the trap measurements with 27 Hz video detection we filtered and sampled our data at 27 Hz. This allowed us to capture the shortening phase of MTs at 27 Hz. In addition, we observed no evidence for a stepwise growth model. Rather, the MT growth rates are highly variable, but do not involve the addition of tubulin oligomers.

Conclusions

These data are not compatible with a model of MT polymerization which posits a simple first order growth rate. Such a model does not predict the frequent shortening or persistent changes in growth rate. Therefore, a model posing an evolving rate originating in the structure at the tip and gtp-state of the distal subunits is more reasonable. We show here that such a model qualitatively and quantitatively reproduces the observed phenomena.

A substantial body of work has argued that the GTP-cap is very small, either at most a single layer (Vanderkooi et al., 1999; Panda et al., 2002; Stewart et al., 2002), or a single layer plus a short gtp tail (Obriens and Voter, 1987). If this is the case, our data is very hard to reconcile with the GTP-cap hypothesis, since the entire GTP-cap would be frequently lost during growth-phase shortening events. However, measuring the size of the GTP-cap is very challenging, and after carefully reconsidering the arguments and data, we find that the resolution is probably in some cases not high enough. If this is the case, our data is not incompatible with the GTP-cap hypothesis. However, if the GTP-cap is larger than a single layer of tubulin subunits, or even a few layers, our data are not compatible with the GTP-cap hypothesis. If this is the case, our data is not compatible with the GTP-cap hypothesis.