VISUALIZATION OF THE POLYMERIZATION DYNAMICS OF INDIVIDUAL 25nm-DIAMETER MICROTUBULES IN REAL TIME


* Biology, University of North Carolina, Chapel Hill, N. C. 27514
** Anatomy, Duke University, Durham, N. C. 27710

Microtubules (Mts) of the interphase cytoplasmic Mt complex (CMTC) and the mitotic spindle are dynamic polymers assembled from a cellular pool of tubulin by end-dependent association-dissociation reactions (1,2). Mts have an intrinsic structural polarity, which orients the direction of cell motility. Mts also play a major role in the active translocation of vesicles and chromosomes. Quantitative fluorescence microscopy studies using fluorescently labeled tubulin microinjected into living cells have shown that the great majority of Mts in the CMTC and the spindle exchange rapidly with tubulins in the cellular pool (2,3). Dynamic instability, as proposed by Mitchison and Kirschner, provides the best explanation for this rapid turnover of microtubules (1,2).

Three major temporal phases of Mt polymerization and three major abrupt transitions between these phases characterize the dynamic instability behavior of pure tubulin (Fig. 1). The first temporal phase is termed No Polymer and is a variable period preceding an abrupt transition (nucleation), which results in polymer Elongation. Elongation proceeds steadily for a random period of time before an abrupt transition (catastrophe) takes place, and Rapid Shortening begins. An abrupt transition (termed rescue) from Rapid Shortening to Elongation can occur before the Mt can depolymerize back to the nucleation center.

In order to provide an accurate analysis of this dynamic behavior of microtubules, the assembly of individual polymers must be observed in real time. Hori and Hotani have previously used dark-field microscopy to observe the self-assembly of individual Mt polymers in vitro (4). We have used differential interference contrast (DIC) optical microscopy and digital image processing of video images (5,6) to visualize the dynamics of nucleated polymerization of pure tubulin (7). We used fragments of the stable 9+2 Mt complex of flagellar axonemes as nucleation sites for polymerization at the (+) and (-) ends of Mts, as shown in Fig 2. Table 1 provides a summary of the kinetic parameters measured for the (+) and (-) ends at 11 µM tubulin. We now have a functional assay to determine how Mt-associated proteins (MAPs) and other potential regulators control the extent and rate of Mt assembly in the cell, and how the hydrolysis of GTP ligated to tubulin controls transitions between phases (1,7,8).

9. Supported by GM 24364 (EDS) and GM 28553 (HPE).
FIG. 1.—Phases and transitions of dynamic instability.

FIG. 2.—Polymerization of microtubules from (+) and (-) ends of axonemal fragment. 2a shows sketch of axoneme orientation in 2b. Double-headed arrow indicates direction of maximum contrast in 2b generated by DIC optics. 2c shows kinetics of (+) and (-) end dynamics in 2b. Accuracy of length data in 2c is 0.2 μm. Scale bar in 2b = 2 μm.

TABLE 1.—Dynamic instability of microtubules.

<table>
<thead>
<tr>
<th>POLYMER PHASES:</th>
<th>(+)</th>
<th>(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation</td>
<td>2 μm/min</td>
<td>0.75 μm/min</td>
</tr>
<tr>
<td>Rapid Shortening</td>
<td>25 μm/min</td>
<td>25 μm/min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TRANSITIONS:</th>
<th>FASTER</th>
<th>SLOWER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleation Frequency</td>
<td>0.53 min⁻¹</td>
<td>0.67 min⁻¹</td>
</tr>
<tr>
<td>Catastrophe Frequency</td>
<td>0.01 min⁻¹</td>
<td>0.22 min⁻¹</td>
</tr>
</tbody>
</table>