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# Direct Optical Microscopic Observation of the Microtubule Polymerization Intermediate Sheet Structure in the Presence of Gas7

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The process of microtubule elongation is thought to consist of two stages—formation of a tubulin sheet structure and its closure into a tube. However, real-time observation of this process has been difficult. Here, by utilizing phospho-tau binding protein Gas7 (growth-arrest-specific protein 7), we visualized the polymer transformation process by dark-field microscopy. Upon elongation, thin and flexible structures, often similar to a curved hook, appeared at the end of microtubules. Electron microscopic observations supported the idea that these flexible structures are tubulin sheets. They maintained their length until they gradually became thick and rigid beginning in the central portion, resulting in straight microtubules. In the absence of Gas7, the sheet-like structure was rarely observed; moreover, when observed, it was fragile and engaged in typical dynamic instability. With Gas7, no catastrophe was observed. These results suggest that Gas7 enhances microtubule polymerization by stabilizing sheet intermediates and is a useful tool for analyzing microtubule transformation.



*Keywords:* microtubule polymerization; Gas7; MAPs; intermediate sheet; dark-field microscopy; Pin1

## Introduction

Microtubules, which are the polar, hollow, and cylindrical polymers of α-/β-tubulin dimers (tubulin protomers), are the essential components of the cytoskeleton. Microtubules exhibit distinct assembly–disassembly dynamics called dynamic instability.[1,2] Individual microtubules are growing slowly or shortening rapidly, with stochastic switching between these phases.[3,4] In addition, microtubule elongation sometimes pauses, which is known to be of considerable duration.[5] This behavior characterizes microtubule dynamics, and various protein factors are involved in the physiological regulation of dynamic instability.[2,6] An important aspect of this mechanism is the hydrolysis of β-tubulin-associated GTP, which occurs upon polymerization. The hydrolysis is not tightly coupled to

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Abbreviations used: Gas7, growth-arrest-specific protein 7; MAPs, microtubule-associated proteins; EM, electron microscopy.

the binding of the tubulin protomer to the polymer. Rather, it occurs after protomer addition with a slight delay such that a small cluster of GTP-bound tubulin molecules remains at the growing end of the microtubule. This cluster, the "GTP cap," serves to protect the unstable polymer lattice consisting of GDP-bound tubulin against depolymerization.[1]

The tubulin protofilaments composed of GTP-bound tubulin and those of GDP-bound tubulin are thought to have different conformations: the protofilaments observed in a depolymerizing condition showed highly curved and ring-like appearances, probably corresponding to the GDP-protofilament conformation,[7] whereas the protofilament sheets that bound a GTP analog, GMPCPP, showed a much lesser amount of curvature.[8,9] Further straightening would be needed to make these curved protofilaments into a straight conformation observed in microtubules, which are probably stabilized by the lateral contacts between neighboring protofilaments.

Elongation of microtubules is thought to be based on the growth of tubulin sheets consisting of laterally associated protofilaments. Sheet growth is followed by subsequent closure into the microtubule cylinder.[10,11] Because the sheet structures observed at the end of the microtubules in an assembly condition were slightly curved outwardly,[11,12] straightening of the protofilaments would be needed in this process. Once the microtubule is formed, lateral interaction between the neighboring protofilaments could stabilize the straight conformation.

The abovementioned model including formation of a sheet and its closure into a microtubule structure is based mostly on electron microscopic observations of microtubules that are fixed during polymerization. However, real-time observations of this dynamic process under a light microscope have been difficult, probably because the sheets formed at the end of the growing microtubules are short and unstable and have a lower contrast than microtubules. To observe the process by light microscope, we utilized a novel phospho-tau binding protein, Gas7 (growth-arrest-specific protein 7), which accelerates microtubule polymerization (H.A. *et al.*, unpublished data). Gas7 is expressed preferentially when cell growth is arrested, and it was previously shown to be necessary for neurite formation.[13] We polymerized a crude tubulin preparation including tau, in the presence of Gas7, and observed the process by dark-field microscopy.

By analyzing the microtubule formation process in the presence of Gas7, we (1) visualized the structural conversion of sheets into microtubules, (2) determined, using a technique incorporating electron microscopy (EM), that the basic properties of the present precursor are comparable with those of the sheet as originally described,[11] and (3) found that the effects of Gas7 are to maintain the sheet structure and to suppress catastrophe. In Discussion, we propose a novel mechanism for the Gas7-dependent maintenance of the sheet structure and its role in microtubule dynamics.

## Results

### Gas7 promotes microtubule polymerization

To visualize the process of microtubule elongation, we examined the effects of Gas7 on microtubule polymerization. We used the tubulin preparation containing microtubule-associated proteins (MAPs), first because it was reported that tubulin-containing MAPs show a higher degree of sheet formation than PC-column-purified tubulin,[14] which could make it easier to observe the process of sheet formation and closure into a microtubule. In addition, these samples contain various physiological factors required for microtubule polymerization, including multiple phosphorylated forms of tau[15] (Supplemental Fig. 1), with which Gas7 interacts (H. A. *et al.*, unpublished data). First, bulk microtubule polymerization from 1.0 mg/ml of crude tubulin preparation was assayed using light-scattering measurements at 350 nm (Fig. 1a). In the presence of Gas7, the level of light scattering decreased slightly during the first 1.5 min of incubation at 37 °C but then increased in a sigmoidal manner, indicating microtubule polymerization. The reason for the initial decrease is unknown but could be the consequence of the disappearance of some types of polymeric forms. At concentrations of Gas7 ranging from 0 to 4.3 μM, the initial rate of increase and the final level of light scattering became higher with increasing Gas7 concentrations. At concentrations above 4.3 μM, however, the initial rate of increase and the final level of light scattering tended to decline (data not shown). Thus, Gas7 promotes polymerization of microtubules *in vitro*, an effect that was observed for concentrations of Gas7 up to approximately 4.3 μM.

To define the role of Gas7 in the polymerization processes described above, we examined microtubule polymerization using dark-field microscopy. In the absence of Gas7, a small number of microtubules appeared 10 min after the temperature increased (control microtubules; Fig. 1b). All the microtubules were straight and looked rigid, as observed previously[16] (Fig. 1c; also see Supplemental Movie 1). In the background and along the microtubules, many small particles, which probably consist of various MAPs and unpolymerized tubulin, were observed.

In the presence of Gas7, numerous filamentous polymers appeared only 3 min after increasing the temperature (Fig. 1d). Although the typical length of these filaments (3 to 5 μm) was similar to that observed in the control samples formed in the absence of Gas7, the filaments looked much more flexible than typical microtubules; the ends of these filaments were often curved (Fig. 1e), waving vigorously back and forth (Supplemental Movies 2 and 3). The lengths of the flexible parts were often more than 1 μm, and the signal intensities associated with them were weaker than those of the control microtubules. As time advanced, the