Microtubule dynamics: characterizing a candidate 'conformation' mutant

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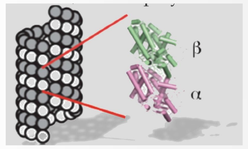
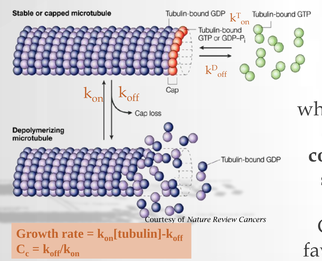
Abstract: The microtubule (MT) cytoskeleton is essential in eukaryotic cell trafficking and organization. Our lab is interested in understanding the role that αβ-tubulin's conformational change play in MT dynamics. To do so, in this work, we purified an αβ-tubulin mutant that had been previously characterized genetically to study its polymerization dynamics *in vitro*. We found that although they elongated somewhat slower than wild-type MTs, the mutant MTs were very stable: little to no catastrophe was observed and the mutant polymerized more robustly than wild-type at comparable concentration. Because the mutated site is distant from any MT assembly interfaces, our observations may indicate that the mutant more readily adopts the straight αβ-tubulin conformation, the form which favors MT polymerization.

Introduction

Microtubules (MT) are hollow cylindrical polymers of αβ-tubulin heterodimers. Both *in vivo* and *in vitro*, MTs switch frequently between growing and shrinking , a behavior known as **dynamic instability** that is necessary for proper cell function.



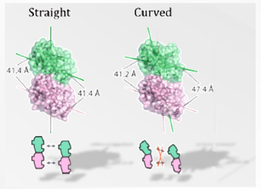
[Figure 1] Structure of αβ-tubulin



[Figure 2] Dynamic instability of MT

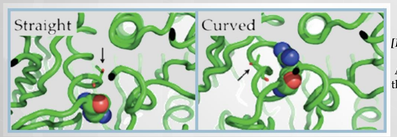
The rate of MT growth depends on the on and off rates at the ends. The tubulin concentration at which the on and off rates are equal is termed the **critical concentration.** In addition to subunits concentration, MT dynamics also depends on GTP-hydrolysis. A GTP cap favors growth, but if it is lost, then depolymerization ensues

The mechanism of the switch from growing to shrinking, or **catastrophe,** and the role that **αβ-tubulin's conformational change** plays in MT dynamics are still very poorly understood; this is mostly due to the lack of routine access to recombinant αβ-tubulins. In this work, we will focus on tubulin's conformations..



[Figure 3] Straight and Curved Conformations of αβ-tubulin
The straight form favors MT polymerization since it optimizes lateral interactions of subunits in the MT lattice.

Earlier genetic screenings in yeast have described a single-point **mutated αβ-tubulin (T238A)** that appears to hyperstabilize the MT lattice in yeast. Located in the β-monomer, this mutation reduces the viability of yeast cells. The molecular mechanism of this has never been studied. Our initial goals are to purify this mutant and characterize its dynamics.



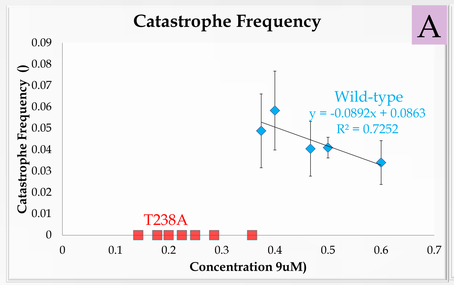
[Figure 4] Location of T238A αβ-tubulin mutant
Arrows indicate position of the T238A mutation in the β-monomer.

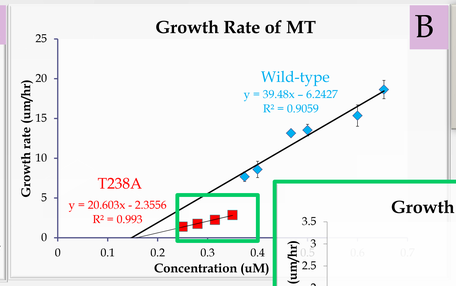
Hypothesis

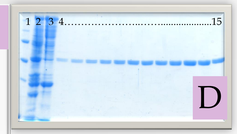
Recently, the lab has successfully purified the mutant T238A from yeast. We **hypothesized** that this mutant might stabilize the MT lattice by favoring the straight conformation of αβ-tubulin. If true, then the mutant MTs should exhibit polymerization dynamics that differs from wild-type. Measuring the polymerization dynamics of the mutant MTs therefore has the potential to provide new insight into the relationship between MT dynamics and conformational change in αβ-tubulin.

Results

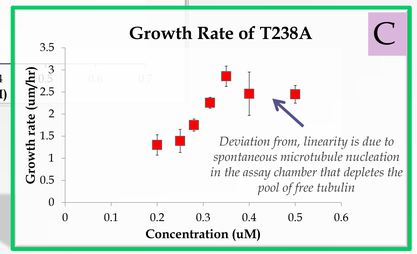
The polymerization dynamics of mutant MTs differed significantly from those of wild-type. Mutant MTs exhibit little to no catastrophe *[Fig. A]*, assemble at lower concentrations than wild-type *[Fig. B]*, .and also form spontaneously at lower concentration than wild-type *[Fig. C]*.

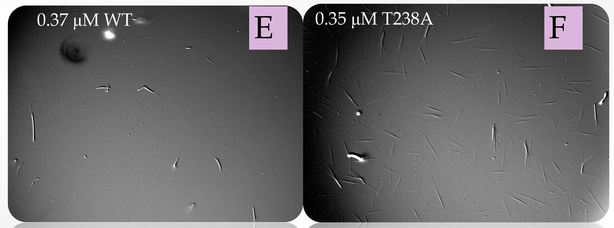


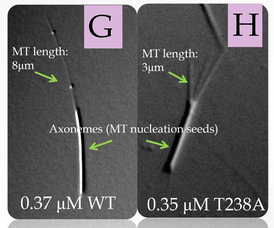




[Fig. D] SDS-PAGE gel of T238A purification







At roughly the same concentration, MTs formed from T238A mutants elongate slower *[Fig. B]* but more robustly than wild-type *{Fig. E & F]*. Due to the slower growth rate, the mutant-formed MT are shorter than wild-type *[Fig. G & H]*. And despite the difference in the apparent k_{on} and k_{off}, both the mutants and wild-type have similar critical concentration (Wild-type: 0.15 uM vs. T238A = 0.114 uM).

Discussion

The finding that T238A MTs have different polymerization dynamics suggests that the mutation is altering one or more important properties of αβ-tubulin. The lack of catastrophe might indicate that the mutation, which is roughly 40Å from the site of GTPase, reduces the rate of GTP-hydrolysis in the MT. The altered concentration dependence of the elongation rate suggests that the end structure of mutant MTs may differ from that of wild-type. These changes may indicate that the mutant favors the 'straight' conformation of αβ-tubulin, as we hypothesized.

Future Directions

From this work, we have begun the first mechanistic characterization of a candidate 'conformation' mutant of αβ-tubulin. The next step in the project will be to identify the biochemical and/or structural changes that occur in response to the mutation. Since MT polymerization is temperature-sensitive, we will also look into the mutants' dynamics under cold temperature. The results of these studies will help improving our understanding of MT dynamics.

Methods:

We overexpressed and purified the mutant in yeast cells. Using a flow chamber, we templated MT growth near the surface of the coverslip. Then individual MT was imaged using time-lapse differential interference microscopy. This method allowed us to directly measure the growth rate and catastrophe frequency of each individual MT.

Acknowledgement:

The Rice Lab (L. Rice, F.A. Piedra, L. Downes, X. Ye, E. Geyer, S. Munyoki, S. Majumdar), **QP-SURF Coordinators** (N. Street, V. Powell), **SMU Advisor** (S. Vik)

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