Trends in Parasitology

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Spotlight

Affinity-purified *Plasmodium* tubulin provides a key reagent for antimalarial drug development

Shane G. McInally [1,2] and Scott C. Dawson [3,*]

Hirst *et al.* used a TOG-domain-based affinity-purification approach to reconstitute and define the *in vitro* dynamics of blood-stage *Plasmodium falciparum* αβ-tubulin. This provides a key reagent for defining parasite microtubule (MT) dynamics and for evaluating the efficacy of anti-MT drugs throughout the complex parasite life cycle.

MT polymers form essential networks in eukaryotic cells that include both interphase arrays and mitotic arrays such as the mitotic spindle. The size and shape of cellular MT networks is controlled by the regulatory mechanisms that govern the addition and removal of αβ-tubulin building blocks to the dynamic end of these polar polymers. MTs are intrinsically dynamic and stochastically switch between filament growth and shrinkage during a process known as dynamic instability [1]. Additional control over MT dynamics is conferred by various MT motors and/or MT-associated proteins (MAPs). Traditional biochemical approaches to evaluate *in vitro* tubulin dynamics have primarily relied upon purification of either bovine or porcine brain tubulin as these tissues have an abundance of tubulin compared with other tissues or cell types.

Yet not all tubulin is the same. Often there is significant sequence divergence of tubulin in parasitic protists; for example, α-tubulin from the malaria parasite *P. falciparum* is roughly 20% different from human and 30% different from budding yeast α-tubulin, respectively. This primary sequence divergence, combined with the presence of novel, parasite-specific MAPs, underscores the potential for unique biochemical properties and dynamics of conserved cytoskeletal polymers in parasites. Additionally, many parasites have complex life cycles that involve both essential and novel functions of cytoskeletal polymers, with some parasites even employing unique interphase MT arrays to mediate key aspects of their pathogenesis, including invasion, attachment, and egress from host tissues. The *Toxoplasma* conoid, the subpellicular MT array in trypanosomes, and the ventral disc in *Giardia* are well known examples of these novel MT structures. Dynamic rearrangement of the MT cytoskeleton is also a critical aspect of each stage of the *Plasmodium* life cycle. Notably, precise control over the number and length of the subpellicular MTs in sporozoites is required for infection [2].

Despite the importance of using native tubulin in *in vitro* biochemical studies of parasite MT dynamics, purification of sufficient amounts of active tubulin from parasites or non-model eukaryotes has remained challenging. MTs are excellent candidates for novel antimalarial drugs, yet previous attempts to purify native *P. falciparum* tubulin for *in vitro* reconstitution assays have been unsuccessful. One promising method to extend our understanding of MT dynamics to other eukaryotes couples affinity chromatography with the natural tubulin-binding properties of the TOG domains found in the XMAP215/Dis1 family of MAPs [3]. In a recent study [4], Hirst *et al.* applied this TOG-domain-mediated affinity purification to purify *P. falciparum* tubulin. Here, Hirst *et al.* develop a sequential lysis method coupled with TOG-domain affinity chromatography to purify native tubulin from parasite-infected red blood cells. Importantly, the authors found that, in addition to successfully purifying sufficient tubulin from a clinically relevant stage of the *Plasmodium* life cycle, this tubulin was assembly-competent and displayed *in vitro* dynamic instability.

Direct comparisons between the dynamic behaviors of purified *P. falciparum* and bovine brain tubulin revealed that these intrinsic properties are similar despite the evolutionary distance between these organisms. By contrast, the intrinsic MT dynamics of similarly purified tubulin from two closely related frog species, *Xenopus laevis* and *Xenopus tropicalis,* differ and significantly contribute to their assembly of mitotic spindles of different lengths [5]. Thus, the different types and sizes of MT networks found in blood-stage parasites may be controlled by MAPs or other regulatory factors, rather than intrinsic differences in MT dynamics. Native tubulin from *P. falciparum* also lacks the extensive post-translational modifications (PTMs) found on the different isoforms and isotypes of brain tubulin. These differences highlight the possible adverse effects from studying regulatory or other interactions between MT motors and/or MAPs from more evolutionarily distant eukaryotes using heterologous reconstituted systems. Further investigations should explore how specific regulatory factors contribute to the assembly and maintenance of the two distinct MT networks found in blood-stage parasites, the stable subpellicular MTs and the dynamic spindle MTs.

MTs have been successful targets for cancer therapeutics due to their intrinsic and regulated dynamic properties, as well as their essential roles in cell division, intracellular transport, and cell motility. However, this successful approach has yet to be directly translated for the development of drugs to target parasite-specific tubulins. Hirst *et al.* highlight the promise of TOG-domain-mediated affinity purification of native parasite tubulin for *in vitro* evaluation of drugs targeting MT dynamics. The authors directly use their *in vitro* reconstitution of

P. falciparum to identify molecules that selectively inhibit parasite, rather than host, MT dynamics. Two previously identified herbicides, oryzalin and amiprofos methyl (APM), are used to inhibit the *in vitro* dynamics of MTs purified from either blood-stage parasites or the human kidney cell line, HEK293. Confirming prior work, Hirst *et al.* demonstrate that both herbicides directly inhibit the assembly of parasite MTs by decreasing MT growth velocity. Using their *in vitro* reconstitution system, the authors determined that mammalian MTs were not affected by the same herbicide concentrations that strongly inhibit parasite MT dynamics. Overall, the authors provide the first demonstration that parasite MTs can be selectively targeted by pharmacological agents, providing a promising avenue for future antimalarial or other antiparasite drug discovery.

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Declaration of interests

The authors declare no competing interests.

[1]Department of Biology, Brandeis University, Waltham, MA, USA
[2]Department of Physics, Brandeis University, Waltham, MA, USA
[3]Department of Microbiology and Molecular Genetics, University of California Davis, Davis, CA, USA

*Correspondence:
scdawson@ucdavis.edu (S.C. Dawson).

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