The functional domain grouping of microtubule associated proteins

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Abbreviations: GO, gene ontology; MT, microtubule; MAP, microtubule associated protein; RIC, replication factor C

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Microtubules (MTs), which play crucial roles in normal cell function, are regulated by MT associated proteins (MAPs). Using a combinatorial approach that includes biochemistry, proteomics and bioinformatics, we have recently identified 270 putative MAPs from Drosophila embryos and characterized some of those required for correct progression through mitosis. Here we identify functional groups of these MAPs using a reciprocal hits sequence alignment technique and assign InterPro functional domains to 28 previously uncharacterized proteins. This approach gives insight into the potential functions of MAPs and how their roles may affect MTs.

Introduction

MTs, a constituent of the eukaryotic cytoskeleton, are intracellular protein polymers composed of α- and β-tubulin heterodimers, which grow and shrink in a highly regulated fashion. Their varied roles within the cell include regulating the division of nuclear material, cell motility and maintaining cell shape and polarity. The precise structure and function of MT populations is governed by MAPs. MAPs were originally identified in mammalian brain tissue extracts as proteins which co-sediment with purified tubulin. Recently, the term MAPs has been applied to any protein that can associate, directly or indirectly, to MTs in vivo or in vitro. Adaptations of early biochemical co-sedimentation methods have been carried out with some success, identifying a selection of proteins, which co-localize with MTs and regulate their organisation. The advance of proteomics techniques has opened up the possibility of identifying hundreds of MAPs from a single tissue.

We recently used a co-sedimentation approach to isolate putative MAPs from blastoderm stage Drosophila embryos, resulting in the identification of 270 proteins, 83 of which were found to be functionally uncharacterized by Gene Ontology (GO) in FlyBase. In that study, we analyzed the putative MAPs using a combination of bioinformatics, biochemistry and cell biology, in order to investigate MAP complexes that function during the cell cycle.

Results and Discussion

To further investigate the potential roles of the 83 uncharacterized proteins and to identify functional domains within the whole set of MAPs, we have carried out sequence alignments using BLAST. Protein sequences were downloaded from FlyBase in FASTA format and each MAP was aligned in turn against every other sequence within our data set. In order to increase the accuracy of homology identification, only hits identified in a reciprocal manner were collected. Reciprocal alignments can be visualized by edges in a network diagram with proteins as nodes (Fig. 1). Of the 270 proteins aligned, 130 gave a reciprocal alignment to another MAP, 28 of which were novel proteins. Functional categories were then assigned to each group of proteins where a common domain or family was found using Pfam (Fig. 1).

Some well-studied groups of proteins, such as kinases, septins and isoforms of actin and their related proteins, align with one another without the inclusion of novel proteins. However, other groups of MAPs do align with previously uncharacterized proteins. For example, the protein CG18190 aligns with EB-1, a previously identified MAP of the +Tip family that binds specifically to the growing ends of MTs (Fig. 1A). As expected, CG18190 has been shown to bind to and localize to MTs in the early embryo, although its precise function remains unclear (Hiro Okhura, personal communication). Dynein Heavy Chain (Dhc64C), a subunit of the MT motor protein complex Dynein, aligns with two novel MAPs, CG3339 and CG9492 (Fig. 1B). In good agreement, a previous study which searched for Drosophila homologues of over 1000 known cytoskeletal proteins also predicted CG3339 and CG9492 to be Dynein-like motor proteins. By demonstrating that they co-sediment with MTs, we support this prediction. Further investigation of these proteins should help elucidate the functional significance of multiple dyneins in the early embryo.

It is worth noting that some well characterized MAPs, such as Lava Lamp and D-TACC align with the group of kinesin-like proteins and one another, but this cannot be attributed to an identifiable common domain. This may be an indication of a novel functional element that exists between the aligning proteins, and...
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could be of interest in the future. Alternatively, it may be a reflection of overall structural similarity within this class of MAPs; for example, all proteins in this group (Fig. 1C) are predicted to possess coiled-coils. These helical secondary structures provide surfaces for protein-protein interactions, and are prevalent in MAPs involved in the regulation of mitosis.

Another interesting cluster is based on homology between proteins containing G-beta repeats (Fig. 1D), a sub-category of WD-40 repeats, which are also known to be involved in protein-protein interactions. While several G-beta repeat-containing proteins, such as the Dynein Intermediate Chain Shortwing, Gbeta13F, Rack1, and Bub3, are known to localize to MT populations, the other proteins in this cluster have not been reported to associate with MTs. Our identification of these proteins as MAPs suggests that the G-beta repeat motif may, like the coiled-coil, occur in many MAPs.

A final aspect of the clustering of proteins that are known to form complexes together. Not surprisingly, our analysis found homology between all TCP-1 subunits; TCP-1 is a tubulin chaperone complex whose constituents have evolved from a single ancestral protein (Fig. 1E). In addition, all subunits of the Replication Factor C (RFC) complex fall within a single cluster (Fig. 1F). This heteropentameric complex loads the sliding clamp, PCNA, around DNA and the sequence similarity of all five subunits lies in their RFC domain, a class of AAA+ ATPase domain. Why this complex might bind to MTs and what effect this might have upon the MTs themselves, has yet to be investigated. However, it is interesting to note that the Pontin-Reptin complex, whose subunits also possess an AAA+ ATPase domain, the Tip49 domain (Fig. 1G), has recently been shown to interact with tubulin and regulate mitotic spindle assembly.

Our biochemical approach, teamed with mass spectrometry and bioinformatics, has elucidated 270 novel MAPs. We have successfully categorized 130 of these into functional groups by reciprocal alignment with e-value less than 1E-03. Of the 83 previously uncharacterized genes encoding divergent subunits of the TCP-1 containing chaperonin. Curr Biol 1994; 109:2977-91.

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Note
Supplementary materials can be found at:
www.landesbioscience.com补充/FisherCIB1-1-Sup.pdf

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