Cell motility

New jobs for dynein ATPases

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STUDY of the molecular basis of cytoplasmic motile systems requires the use of an appropriate *in vitro* model. Although various models are available that retain different levels of the *in vivo* functional and regulatory mechanisms, one of the most useful for studying the elementary properties of a motile system has been direct observation, in the light microscope, of individual protein filaments gliding over the surface of a glass slide coated with the appropriate energy-transducing protein¹. Study of this phenomenon using purified dynein and brain microtubules is described by Bryce Paschal *et al.* on page 672 of this issue², as well as by other reports that have appeared during the past two months³⁻⁶. These data provide the long-awaited validation of cytoplasmic dyneins as cytoplasmic motors distinct from the axonemal dyneins of cilia and eukaryotic flagella⁷, and demonstrate that the motile repertoire of dynein is significantly more elaborate than believed hitherto.

The diversity of the numerous microtubule-associated proteins has become highly confusing to the non-specialist. A helpful clarification can be obtained by grouping them into classes and families (see table), analogous to the general scheme proposed for actin-binding proteins in an earlier News and Views article⁸ by Tom Pollard. Because many forms of cytoplasmic microtubules exist in a state of dynamic equilibrium⁹, the study of the skeletal (non-energy-transducing) class of microtubule-associated proteins has generally focused on their ability to co-polymerize with tubulin *in vitro* and to stabilize the resultant tubules. Little is known about their physiological function in the cell.

The members of the energy-transducing class of microtubule-associated proteins are easier to study by virtue of their ATPase activity and of the various *in vitro* motility models. An abundance of new evidence indicates that most of the complex bidirectional movements of vesicles and organelles that characterize a living cell are driven by one of two molecular motors interacting with a microtubular track. Cytoplasmic microtubules are dynamic, polarized structures, with relatively rapid polymerization of tubulin occurring at their 'plus' end and concurrent slower polymerization or depolymerization at their 'minus' end. In most systems, including nerve axons, the plus end of a microtubule corresponds to its distal end (that further removed from the cell centre) and the minus end to its proximal end, with the same polarities applying also

to the more stable tubules of flagellar axonemes. The tubule motor responsible for distally directed vesicle movement in most cells seems to be kinesin, an ATPase that was first isolated from nerve axons and shown to produce both unidirectional movement of latex beads along a microtubule and gliding of microtubules on glass¹. The motor responsible for proximally directed movement, dynein ATPase, was first characterized from

There remains some uncertainty about the premise of a separate protein motor for each direction of transport along microtubules. Most notably lacking is an explanation for how latex beads coated with a dynein-like protein from the giant amoeba *Reticulomyxa* can mimic the bidirectional motion of the *in vivo* vesicular transport on microtubules in reticulopodia of this organism¹⁴. Nevertheless, the basic premise appears likely to be valid. In flagellar axonemes, where dynein produces sliding between pairs of tubules it does so by forming a structurally stable bond to one tubule and a dynamic cross-bridging attachment to the other. In the retrograde transport of cytoplasmic vesicles, the corresponding structural

Characteristics of microtubule-associated proteins

Class	Family	Source	Subunit <i>M_r</i> (K)	Structure	<i>In vitro</i> movement
Energy-transducing	Dyneins	Cilia/flagella	>400	2–3 globular ATPase domains joined by slender stems	Proximal
		Brain	>400		Proximal
	?	<i>Reticulomyxa</i>	>400		Bidirectional
	Kinesins	Brain Egg	110 134	Rod with branches at one end and small fork at the other	Distal Distal
Skeletal	MAP 1	Brain	~350	Thin filament projecting from tubule	None
	MAP 2	Brain	~270	Thin filament projecting from tubule	None
	Tau	Brain	55–62	Globular, heterogeneous in size	None
	Tektin	Cilia/flagella	45–55	Similar to intermediate filaments	None

Microtubule-associated proteins (MAPs) are grouped into classes by their function and subdivided into families according to their physical and enzymatic properties. This grouping is arbitrary and will probably be modified as more data become available. Dynein from brain was formerly called MAP 1C. Subunit M_r gives the relative molecular mass of the principal heavy polypeptide subunit; in many cases additional smaller subunits are also present. Right-hand column: 'proximal' indicates movement of an attached structure toward the proximal (-) end of microtubule, corresponding to retrograde axonal transport; 'distal' indicates movement of an attached structure toward the distal (+) end of microtubule, corresponding to anterograde axonal transport.

ciliary axonemes as long as 20 years ago, and its structural and enzymatic properties are relatively well known¹⁰, although as mentioned above, a functionally active form of cytoplasmic dynein was reported only recently^{3,4}. The properties of the axonemal dyneins that constitute the 'outer arms' of the microtubules of cilia and sperm flagella are particularly well known¹⁰; a well-defined structure seen in the electron microscope, very large polypeptide subunits of $M_r > 400,000$ susceptible to vanadate-mediated photolysis; and high sensitivity of ATPase activity and motility to inhibition by vanadate. The present paper by Paschal *et al.*² demonstrates that the gliding microtubule procedure can be used to assay the function of outer-arm dynein from sperm flagella, and this provides a basis for probing functional domains of dynein in a manner similar to that successfully exploited with myosin^{11,12}. Not surprisingly, the direction of force generated by axonemal dyneins, which has been determined by observations on the sliding disintegration of trypsin-treated cilia, is proximal¹³, the same as that of cytoplasmic dynein from brain⁴.

attachment of the dynein presumably has to be to the membrane of the vesicle, and is possibly mediated by membrane-bound tubulin¹⁵. Outstanding questions concern the regulation of the motors and how vesicles become attached to the correct motor. Regardless of how the answers to these questions turn out, it is now evident that dynein fulfils a greater variety of jobs in the cytoplasm than has previously been realized. □

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