Topic Introduction

Isolation and Analysis of Microtubules and Associated Proteins

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Microtubules, microtubule-associated proteins (MAPs), and motor proteins are essential components of all eukaryotic cells. They are all involved in mitosis and in the movement of organelles, proteins, and vesicles in cells. MAPs act as structural elements of the microtubule component of the cytoskeleton, whereas molecular motors propel cargo along microtubule tracks or translocate microtubules in the cytoplasm. This introduction provides an overview of procedures developed by many labs to isolate microtubules from cell homogenates, purify tubulin, MAPs, and motor proteins from microtubules preparations, and analyze kinesin and cytoplasmic dynein activity by video-enhanced differential interference contrast microscopy and fluorescence microscopy. These ingenious microscope-based assays, which were developed to determine the motility characteristics of kinesin and dynein, reveal, in clear and dramatic fashion, the activity of these amazing nanomachines in real time.

INTRODUCTION

Microtubules are long, tubular polymers of indefinite length having an overall diameter of 25 nm, a hollow core 15 nm in diameter, and a wall thickness of 5 nm. They are composed of a subunit protein called tubulin, which exists in its native form as a heterodimer composed of two polypeptides called α - and β -tubulin. Each tubulin is the product of a distinct gene, and all eukaryotes contain at least one gene for each α - and β -tubulin. The subunit for assembly of a microtubule is the tubulin dimer, and under appropriate conditions, the figure-eight-shaped dimers self-assemble in a head-to-tail fashion, producing a helical polymer with the long axis of the heterodimer parallel to the long axis of the microtubule. Associated with microtubules are other classes of proteins: Microtubule-associated proteins (MAPs; Sloboda et al. 1975) and motor proteins, specifically kinesin and dynein. These proteins vary widely in molecular mass (e.g., 275,000–300,000 for high-molecular-weight MAPs; 50,000–60,000 for tau MAPs; and 1,500,000 for dynein) and have various functions. Some MAPs stabilize the assembled polymer, whereas others mediate the interaction of microtubules with other components of the cytoskeleton. Motor proteins power the directional movements of membrane-bound organelles, proteins, and vesicles along microtubules. Some MAPs and motor proteins are cell type-specific, whereas others are common to different cell types.

ISOLATION OF MICROTUBULES

All eukaryotic cells assemble microtubules, as they are the main structural components of the mitotic apparatus. However, the richest source of tubulin is terminally differentiated—and hence nondividing—neuronal tissue, because the axons and dendrites of neurons are full of assembled microtubules

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that can be readily depolymerized by cold treatment. Microtubules are capable of assembly and disassembly in the cell, usually (but not always) in a cell cycle-dependent manner. Weisenberg (1972) first published the conditions for promoting microtubule assembly in extracts of neuronal tissue. The procedure is based on the ability of the investigator to control the dimer-polymer distribution of tubulin by varying the temperature of the extract (see Protocol: **Isolation of Microtubules by Assembly/Disassembly Methods** [Sloboda 2014a]). The inclusion of glycerol in the buffer can promote microtubule assembly by disrupting the hydration shell around the tubulin dimers. Glycerol also reduces endogenous activity that inhibits microtubule assembly (Farrell 1982).

Another compound that stabilizes microtubule polymers is paclitaxel, originally commercialized by Bristol-Myers Squibb. It is referred to as Taxol in older literature—the name given to the compound when it was first isolated from the bark of the Pacific yew (*Taxus brevifolia*) by Kepler et al. (1969). Paclitaxel can be used to enhance the isolation of microtubules, especially from sources that contain lower levels of tubulin (see Protocol: **Isolation of Microtubules and Microtubule-Associated Proteins Using Paclitaxel** [Sloboda 2014b]).

ISOLATION AND ANALYSIS OF MAPS AND MOTOR PROTEINS

Many MAPs associate stoichiometrically with microtubules assembled in vitro, an observation that led to the coining of the term MAP (Sloboda et al. 1975). Once microtubules have been isolated, various biochemical techniques can be used to separate MAPs from tubulin. For example, paclitaxel-stabilized microtubules can be separated into tubulin and MAPs in the presence of salt (see Protocol: **Isolation of Microtubules and Microtubule-Associated Proteins Using Paclitaxel** [Sloboda 2014b]). Tubulin and MAPs can also be separated using ion exchange column chromatography (see Protocol: **Separation of Tubulin and Microtubule-Associated Proteins by Ion Exchange Chromatography** [Sloboda 2014c]).

Like MAPs, motor proteins are closely associated with microtubules. Kinesin and dynein can be isolated from preparations of microtubules by taking advantage of their nucleotide-dependent microtubule binding properties (see Protocol: **Isolation of Microtubule-Based Motor Proteins by ATP Release from Paclitaxel-Stabilized Microtubules** [Sloboda 2014d]). The activity of isolated kinesin and dynein or of motor proteins in cell lysates can be observed using a variety of imaging techniques, including video-enhanced DIC microscopy and fluorescence microscopy (see Protocol: **Observation of Microtubule-Based Motor Protein Activity** [Sloboda 2014e]).

ADDITIONAL APPROACHES

Investigators have developed ingenious uses for, modifications of, and variations in the basic purification schemes and motility assays introduced above. Using genome sequence information, the source of MAPs or motor proteins for purification need not be a tissue homogenate, but rather a cloned gene expressed in bacteria, baculovirus, or some other genetic expression system. Because the behavior of microtubules (see Protocol: **Observation of Microtubule-Based Motor Protein Activity** [Sloboda 2014e]) reveals information about the motor activity of the enzyme being studied, genetic approaches enable specific mutations to be created in the proteins, and then the readout of the resulting enzyme activity can be analyzed via a motor assay. One example is recent work by Cochran et al. (2012) in which the switch 1 motif of kinesin-1 was mutated so that the activity of the engineered kinesin was dependent on Mn^{2+} instead of Mg^{2+} ions. The characteristics of the mutant form of the enzyme were analyzed by a number of approaches, among them a microtubule motor assay.

The basic principles underlying these motility assays also lend themselves to whole-cell analyses (Cai et al. 2010; Veigel and Schmidt 2011; Guilford and Bloodgood 2013). For example, Laib et al. (2009) have measured the activity of intracellular motors (both kinesin and dynein) in *Chlamydomonas* cells. These authors used a laser trap to position microspheres on the outer surface of the flagellar membrane of living cells; the microspheres bind to a transmembrane protein called FMG-1B.

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This protein is moved in the plane of the flagellar membrane via interactions with motor proteins present in the flagella. Knowing the force profile of the laser trap, these authors were able to estimate the number of plus-end- and minus-end-directed motors acting on FMG-1B in wild-type cells, as well as in cells from various mutant lines. Finally, the ability to produce transgenic organisms expressing GFP-labeled fusion proteins (Signor et al. 1999; Engel et al. 2009a) allows the motility of subcellular particles and proteins to be observed directly in the cytoplasm of living cells. Subcellular particle movements, such as those described in the preceding two references, are often coupled to observation by total internal reflection microscopy (Engel et al. 2009b).

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