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Tubulin 30 Years Later

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Microtubules, together with microfilaments and intermediate filaments, are important and ubiquitous members of the cytoskeleton of eukaryotic cells. The term "microtubule" was first proposed in 1963 by Slautterback (10) who had observed tubular elements in hydrozoan cells, although such elements had by that time been widely observed in spindles of mitotic cells, dendrites and axons of nerve cells, cytoplasm of protozoa, flagella and cilia, etc.. The obvious question arose as to what kind of protein constitutes microtubules.

Since the discovery and purification of actin and myosin from skeletal muscle, it had been believed that an actin-myosin (actomyosin) system was responsible for every kind of cell motility. In fact, actin and myosin had been obtained from diverse cell types including plasmodia of slime mold, amoebae and dividing cells. Flagellar and ciliary movement did not appear to be exceptional; however, many investigators including our laboratory had tried to extract actin, myosin or a complex of actomyosin from sperm flagella without success. In 1963, Gibbons (3) succeeded in extracting the main ATPase protein from *Tetrahymena* cilia after removal of the ciliary membrane with digitonin which he named "dynein" because its basic properties differed from those of myosin.

Gibbons' chemical dissection of cilia made it possible to isolate the outer doublet microtubules from flagellar or ciliary axonemes and subject their component proteins to biochemical and/or biophysical analyses. We, in parallel to Gibbons' group, struggled for a couple of years to characterize this protein in sea urchin sperm flagella, and independently revealed that the protein contained guanosine, not adenosine nucleotides bound to actin; the amino acid composition resembled but was somewhat different from actin; the molecular weight was larger than actin; and its interactions with myosin were different from those of actin. Combined with the our inability to isolate actin from flagella, we concluded that the main constituent of the microtubule was a new "contractile protein" which interacted with dynein in a manner similar to the actomyosin system and proposed to refer it as "tubulin" in 1968 (5, 14). However, it took some time until this name was recognized among biologists.

In the same year, Mazia and Ruby (4) proposed the name "tektin" to cover a wide variety of globular structural proteins including membrane protein and actin. The terms "spactin" or "flactin" had previously been applied to the actin-like protein obtained from sperm flagella (7). The former, tektin, is too general and includes proteins other than tubulin. The latter are too specific and it is now evident that tubulin is distinct from actin. Meanwhile, Taylor's group (1, 9) revealed that a protein binding to colchicine was ubiquitous in cells containing microtubules. The protein, i.e. tubulin, was referred to as the "colchicine-binding protein". Under such circumstances, Stephens (11) who worked with Gibbons on the protein of flagellar microtubules recommended the adoption of the term "tubulin" in 1972 and since then this term has widely been used. "Tektin" has since been assigned to the protein of a certain filament present in the wall of flagellar outer doublet microtubules.

Tubulin is a heterodimer composed of α- and β-tubulin subunits. Success with *in vitro* assembly of brain tubulin to microtubules by Weisenberg in 1972 (13) greatly advanced purification and characterization of this protein. In addition, various microtubule associated proteins (MAPs) and their functions have been discovered. Complete amino acid sequences of α- and β-tubulin were determined by both Edman degradation and nucleotide sequencing in 1981. Microtubles participate in diverse cell functions. Heterogeneity among tubulin molecules is responsible for

the various functions of microtubules. Both α- and β-tubulin can be separated into several subspecies with distinct genes. These genes are expressed in a unique manner during development. Furthermore, post-translational modifications of tubulin (e.g. phosphorylation, tyrosination; acetylation, glycosylation, etc.) alter the function of microtubules *in vivo*. Various specific antibodies have been raised against tubulins and are now commercially available. Together with the development of video-enhanced contrast microscopy and other optical techniques, these antibodies and fluorescently-labeled tubulins have been utilized to detect both temporal and spacial changes of different classes of microtubules in various cell types and to investigate the dynamics of individual microtubules. These features of tubulin research were reviewed 20 years after the naming of tubulin (6). Regarding cell motility, a new motor protein, kinesin, was also found (12) in addition to both axonemal and cytoplasmic dyneins and its role in intracellular transport has been investigated.

During the last decade, further advancement has been made in the fields of tubulin, microtubules and MAPs. A new member of tubulin superfamily, γ-tubulin, was discovered and localized at microtubule organizing centers (8). FtsZ, the major cytoskeletal protein in bacterial cell division, is a homolog of tubulin (Cf. 2). Microtubules are thus not restricted to eukaryotic cells as had been thought, but they are present in prokaryotic cells. More information has been obtained concerning interactions between tubulin or microtubules and MAPs, other proteins or cell components. Both the dynein and kinesin superfamilies have been sequenced and microtubule-dependent movement has been more thoroughly investigated. The structure of tubulin has been determined by the developing technique of electron crystallography. Further improvement in imaging technology has made it possible to follow the dynamic changes of microtubules in living cells more precisely. Development in molecular biological techniques has revealed multiple tubulin genes and allowed elucidation of the mechanisms regulating their expression. However, meetings concentrating on tubulin have not been held often in recent years, as topics concerning tubulin or microtubules are dispersed in all fields of biology.

In honor of the thirtieth anniversary of the naming of tubulin, we held an international symposium entitled "Tubulin 30 Years Later" at the 42nd NIBB (National Institute for Basic Biology) Conference in Okazaki from February 24 through 26, 1999. At this symposium, the leading tubulin researchers discussed their recent progress as well as the future direction of their research program. The following manuscripts are the proceedings from this symposium presented as minireviews.

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