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STRUCTURE AND UTILIZATION OF TUBULIN ISOTYPES

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BACKGROUND

Understanding how cells establish differentiated internal structures and overall morphologies is one of the major goals of cell biology. During the 1970s, significant strides were made toward this goal with the discovery and biochemical characterization of the major protein components of the cytoskeleton (Goldman et al 1976). These discoveries have allowed the problem of cell structure to be recast, at least in part, in terms of the properties of the cytostructural proteins: How do eukaryotic cells, working

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with a set of highly conserved proteins, create the strikingly different supramolecular and cellular structures that are found even within unicellular organisms?

This question is acutely evident for tubulin, the structural subunit protein of microtubules. Tubulin, a heterodimer of two related 50,000-dalton proteins, α and β, self-associates to form the cylindrical wall of hollow microtubule filaments. Microtubules are employed for a number of diverse functions in eukaryotes including flagellar motility, chromosome segregation, intracellular transport, and the generation and maintenance of cellular morphology. Correlated with this diversity is a corresponding variation in the properties of microtubules, which differ in stability, morphology, and biochemical composition in different cells and organelles. The functions of microtubules depend largely on the regulation of their assembly and their interaction with other cellular components through specific microtubule associated proteins (MAPs). In principle, alterations in the properties of tubulin could directly affect the assembly and binding properties of microtubules. This idea, first articulated as the multitubulin hypothesis, was based on biochemical differences observed among tubulins isolated from single species (Fulton & Simpson 1976; Stephens 1978).

The discovery that tubulins are encoded by small, heterogeneous multigene families in most organisms established a molecular basis for evaluation of the multitubulin hypothesis (Cleveland et al 1980). Indeed, the realization that multigene families represent a common mode of genetic organization in eukaryotes has raised a question of general significance: Why do organisms maintain several copies of genes that encode closely related proteins?

For tubulin, the answer seems to lie in two independent but related aspects of gene function: regulation of gene expression and the structures of the encoded gene products. Although genetic studies in single celled organisms have shown that neither regulatory nor structural multiplicity of tubulin genes is absolutely essential for cell growth or division, the major theme that has emerged from these studies is that multiple tubulin genes may play a major role in microtubule biology. Multiple α and β tubulin genes are subject to differential regulation during development and differentiation in numerous species, which allows organisms to establish multiple, and often complex, programs for tubulin expression in different cells and tissues. The sequences of some 75 tubulin genes or their products from 12 different organisms have now been examined, and a firm picture of the structure and utilization of tubulin isotypes in several species has developed. In vertebrates, we now know that 6 distinct classes of β tubulin isotypes are encoded by a family of genes that has been strikingly conserved throughout vertebrate evolution. Still, the possible functions of isotypic

variation remain enigmatic. A mounting body of evidence based on analysis of tubulin mutations, gene disruption in fungi, gene transfection in mammalian cells, and direct visualization of isotype distribution in cellular microtubule systems has not revealed any obvious indication that tubulin isotypes contribute to the functions of microtubules, but documents instead a remarkable plasticity of microtubules.

Tubulin also undergoes posttranslational modifications that increase the biochemical complexity of cellular tubulin pools. The detyrosination and acetylation of α tubulin both occur primarily on preformed microtubules, while the reverse reactions occur in the soluble tubulin pool, establishing a dynamic asymmetry in tubulin chemistry that is closely linked to microtubule assembly in cells. The development of antibodies capable of discriminating modified forms of tubulin has led to a rapid and exciting elucidation of an important correlation between tubulin posttranslational modification and the differentiation of microtubules within cells. These experiments have demonstrated that α tubulin modification is associated with stable microtubules present not only in classically stable structures, such as flagella and the midbody, but also in a subset of the cytoplasmic microtubules of a wide variety of cells. These discoveries, coupled with a greater understanding of cellular microtubule dynamics, have established new ways to examine the mechanisms by which microtubules are associated with morphogenetic events in eukaryotes.

In this chapter I summarize what has been learned in the last few years regarding the role of tubulin isotypes, their structure and expression, and their chemical modifications in the regulation of cellular microtubule systems. Because of the rapid pace of discovery in this field, it is neither possible nor necessary to present a comprehensive review of the molecular biology and cell biology of tubulin in a single chapter. Several excellent volumes are available that cover general aspects of microtubule biology and biochemistry, and the molecular biology of cytoskeletal proteins (Roberts & Hyams 1979; Dustin 1984; Borisy et al 1984). The early work describing tubulin heterogeneity and gene structure has been reviewed previously (Cowan & Dudley 1983; Cleveland 1983; Cleveland & Sullivan 1985); as has the evolution and function of tubulin genes (Raff 1984; Raff et al 1987).

TUBULIN STRUCTURE

The functional monomer for microtubule assembly is a dimer of two related tubulin polypeptides, α and β. While the biochemistry of tubulin assembly has been well studied, a firm picture of the structural and functional organization of tubulin is just beginning to emerge. Each polypeptide

chain possesses a single binding site for GTP, which results in a stoichiometry of 2 mol GTP/mol tubulin. GTP hydrolysis at the exchangeable site on β tubulin is associated with tubulin polymerization and appears to play an important role in regulating microtubule assembly (for a review see Kirschner & Mitchison 1986). A combination of biochemical analysis and sequence comparisons with other nucleotide binding proteins has established some of the regions important for GTP binding in α and β tubulin (underlined in Figure 1 for β tubulin) (Sternlicht et al 1987; Hesse et al 1987; Little & Ludueña 1987). These sites are found in regions of high homology between α and β tubulin and have been stringently conserved throughout evolution.

Analysis of tubulin by proteolytic digestion has revealed some of the large scale features of the dimer: Both α and β are organized in two domains separated by a protease sensitive site near residue 339 for α and residue 281 for β tubulin (Mandelkow et al 1985; Sackett & Wolfe 1986). In addition, analysis of tubulin assembly after limited proteolysis has demonstrated an involvement of the carboxyl terminal residues of α and β tubulin in the mechanism or regulation of microtubule assembly and possibly MAP binding (Serrano et al 1984; Sackett et al 1985; Littauer et al 1986). The carboxyl-terminal region of both α and β tubulin is a major variable region among different tubulin gene products, which has fueled speculation that tubulin genes encode functionally distinct proteins. However, documentation of a specific role for this site in vivo has so far proven uniformly elusive (see below).

TUBULIN GENES IN VERTEBRATES

The isolation of cDNA copies of α and β tubulin mRNAs from the chicken quickly led to the discovery that nearly all species possess multiple genes encoding each of the polypeptides that comprise the tubulin dimer (Cleveland et al 1980). Vertebrates possess the most abundant tubulin gene families in all phyla, ranging from 7–8 genomic segments that possess detectable homology to cloned α and β tubulin sequences in the chicken to the 15–20 segments for each subunit found in mammalian species. The general properties of these gene families have been reviewed previously (Cowan & Dudley 1983; Cleveland & Sullivan 1985). Here we examine the structures and the expression of the tubulin polypeptides encoded by these multigene families, and their distribution within cellular microtubule systems.

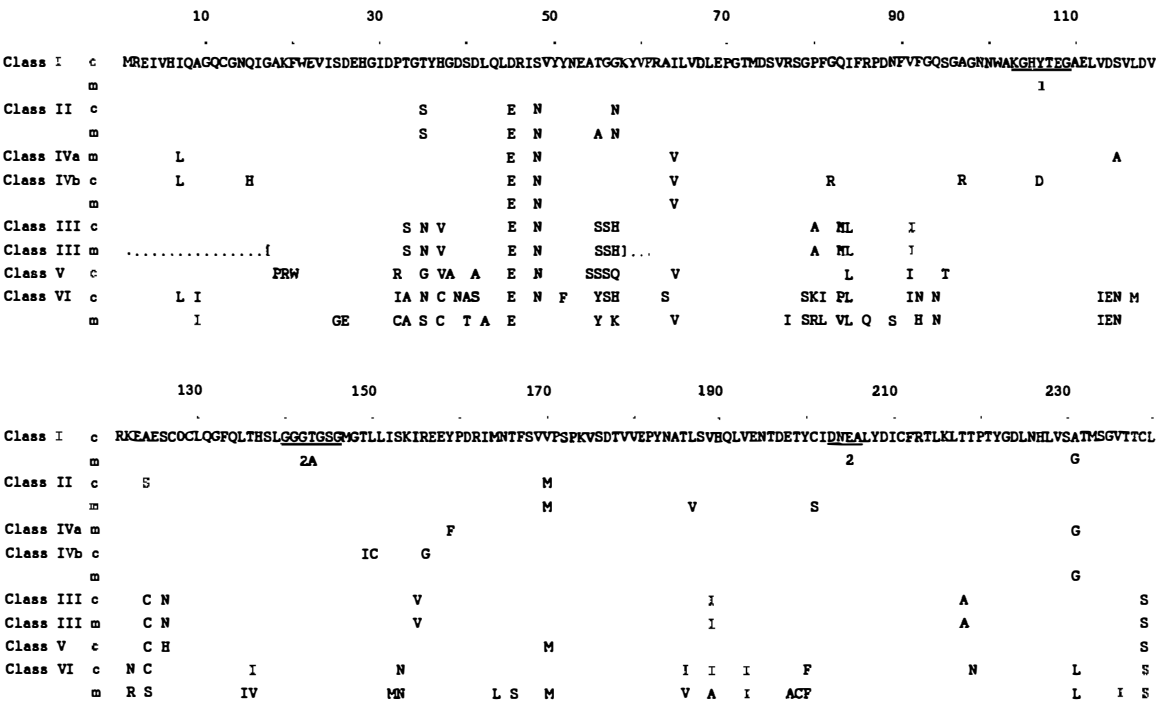
β Tubulin Genes: Six isotope classes encoded by orthologous gene families in birds and mammals

A complete, or nearly complete, characterization of the complement of functional β tubulin genes in three vertebrate species has been

accomplished. For the chicken, Cleveland and coworkers determined the sequence and patterns of expression of each of seven detectable genomic β tubulin sequences or their products, revealing a set of seven distinct encoded polypeptide sequences (Lopata et al 1983; Havercroft & Cleveland 1984; Sullivan & Cleveland 1984; Sullivan et al 1985, 1986a,b; Murphy et al 1987; Monteiro & Cleveland 1988). In mammals, where an abundance of pseudogenes confounds the direct determination of functional genomic sequences, Cowan's laboratory pursued exhaustive cDNA cloning from the mouse; their study revealed five functional β tubulin genes (Lewis et al 1985a; Wang et al 1986), while a combination of genomic (Lee et al 1983, 1984; Lewis et al 1985b) and cDNA (Hall et al 1983; Wang et al 1986; Sullivan & Cleveland 1986; S. Sarkar, N. J. Cowan, unpublished observations cited in Lewis et al 1987) sequence analysis demonstrated six functional human β tubulin genes. In conjunction with important data from other laboratories (Bond et al 1984; Farmer et al 1984; Little & Ludueña 1985; Krauhs et al 1981), these efforts have defined the vertebrate β tubulin gene family as a set of seven functionally distinct genes that each encode a structurally unique β tubulin polypeptide isotype. The conservation of structure and regulatory functions among these genes in different species allowed the identification of six major classes of β tubulin polypeptide isotypes (Sullivan & Cleveland 1986; Wang et al 1986; Lopata & Cleveland 1987; Monteiro & Cleveland 1988). In order to simplify the primary nomenclature they are designated class I–VI β tubulin isotypes in this review (see Table 1).

The sequences of each of the vertebrate β tubulin isotypes determined in the chicken and in mammals are shown in Figure 1. Amino acid sequence substitutions have been accepted at nearly 20% of the amino acid residue positions, but regions of distinctly clustered substitutions, as well as regions of stringent conservation, are evident. The major variable regions in β tubulin are found in the carboxyl terminus beyond residue 430 (Hall et al 1983); amino acid substitutions, deletions, and insertions are accepted at 100% of the final 15 residues and in a secondary region between residues 30–100 (Sullivan & Cleveland 1986), corresponding roughly to exons 2 and 3. Regions implicated in GTP binding (underlined in Figure 1) are found within conserved portions of the molecule (Sternlicht et al 1987). The amino acid sequence divergence between different β tubulin isotypes within a single species ranges from 4–16% (Wang et al 1986; Montiero & Cleveland 1988).

In contrast (shown in Figure 1), for four of the five isotypic classes for which comparative data are available, isotype-specific sequences within variable regions, as well as other sites, are highly conserved between species. Orthologous proteins show complete conservation between mam-



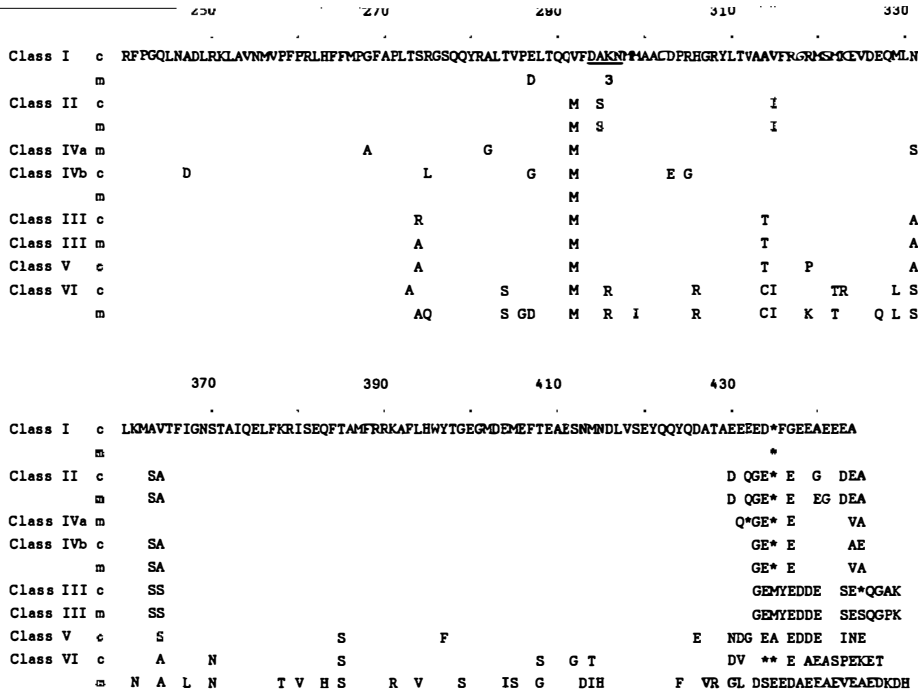


Figure 1 Comparison of the sequences of the six vertebrate β tubulin isotypes. The sequence of chicken cβ7 is shown at the top (Class I). For the sequences below, only those amino acid residues that differ from the Class I isotype are displayed. For each isotypic class, the sequences of both the chicken and mammalian isotypes are shown where available. Regions thought to be involved in GTP binding are underlined and numbered according to Sternlicht et al (1987). Isotype classes are grouped according to the major (Classes I, II, and IV) and minor (Classes III, V, and VI) sequence classes discussed in the text. Sequences are: Class I, cβ7 (Montiero & Cleveland 1988) and mβ5 (Lewis et al 1985b); Class II, cβ2 (Valenzuela et al 1981), mβ2 (residues 125–445; Lewis et al 1985b) and porcine β(A) (residues 1–124; Krauhs et al 1981); Class III, cβ4 (Sullivan & Cleveland 1984), hβ4 (residues 62–450; Sullivan & Cleveland 1986), and bovine β2 (residues 20–58; Little & Ludueña 1985); Class IVa, mβ4 (Lewis et al 1985b); Class IVb, cβ3 (Sullivan et al 1986b), mβ3 (Wang et al 1986); Class V, cβ5 (Sullivan et al 1986a); Class VI, cβ6 (Murphy et al 1987), mβ1 (Wang et al 1986). This figure was prepared using the program PUBLISH of the University of Wisconsin Genetics Computer Group software package.

Table 1 Properties of the vertebrate β tubulin isotype classes[a]

Isotype	Gene/ protein	C-terminal isotype defining sequence	Expression
Class I	cβ7 mβ5 rbt. 3 hβ1	EEEEDFGEEAEEEA	Constitutive; many tissues
Class II	cβ1/cβ2 mβ2 rbt. 1 hβ2 porc. βA bov. β1	DEQGEFEEEGEEDEA EG	Major neuronal; many tissues
Class III	cβ4 hβ4 porc. βB bov. β2	EEEGEMYEDDEEESEQGAK EEEGEMYEDDEEESESQGPK	Minor neuronal; neuron specific
Class IVa	mβ4 rbt. 2 h5β	EEGEFEEEAEEEVA	Major neural; brain specific
Class IVb	cβ3 mβ3 hβ2	EEEGEFEEEAEEEAE EEEGEFEEEAEEEVA	Major testes; many tissues
Class V	cβ5	NDGEEEAFEDDEEEINE	Minor constitutive; absent from neurons
Class VI	cβ6 mβ1	DVEEYEEAEASPEKET GLEDSEEDAEEAEVEAEDKDH	Major erythrocyte/platelets; hematopoiesis specific

[a] The source of the sequences is as in Figure 1 with the addition of: rbt. 1, rbt. 2, and rbt. 3 (Farmer et al 1984); porcine βB (Krauhs et al 1981); bovine β1 (Little & Ludueña 1985).

malian species and 97–99% between birds and mammals. Class VI isotype, a highly divergent hematopoiesis specific β tubulin characterized by Murphy & Wallis (1983, 1985) is an exception; it differs by 18% between chicken and mouse (Villasante et al 1986; Murphy et al 1987). A mammalian counterpart for the class V β tubulin found in the chicken has not been isolated, but data from experiments with isotype-specific antibodies, described below, indicate that a homologous protein is present in mouse cells (Lopata & Cleveland 1987).

Differences in the organization of the avian and mammalian β tubulin gene families are seen in the class II and IV isotype genes. In the chicken, two genes (cβ1 and cβ2) encode polypeptides that differ by only two amino acid residues but are expressed differently during development and

differentiation (Sullivan et al 1985). These genes appeared to be related by a gene conversion mechanism and are both classified as class II. Similarly, in mammals two genes encode very similar class IV polypeptides (IVa and IVb) with different programs of expression (Wang et al 1986; Sullivan & Cleveland 1986). Both genes are designated class IV on the basis of similarity in variable region sequences of the carboxyl terminus. The chicken lacks a class IVa isotype.

The expression of isotypic classes of β tubulin is both complex and highly conserved (see Table 1; Farmer et al 1984, 1986; Havercroft & Cleveland 1984; Griffin et al 1985; Lewis et al 1985b; Wang et al 1986; Ginzburg et al 1985; Denoulet et al 1986). The class I gene encodes an abundant, ubiquitously expressed isotype, while class II represents a major neural β tubulin isotype associated with neural differentiation and regeneration (Hoffman et al 1988). Class III is a minor neuronal polypeptide, apparently specific to chordates (Little & Ludueña 1985; Sullivan et al 1984). The class IVa gene is expressed abundantly in neural tissue in later stages of development in the rat and the mouse, while the closely related class IVb gene is expressed as the major testes β tubulin as well as a minor component of a variety of tissues. Class V gene expression has only been examined in the chicken, where it was found in all tissues and cell types examined except for neurons (Sullivan et al 1986a). Finally, the class VI isotype is expressed only in cells of the hematopoietic lineage, where it comprises the major β tubulin of marginal band microtubules (Murphy et al 1986; Lewis et al 1987). Only the class III, IVa, and VI genes show simple patterns of tissue specific expression. The remaining isotypes are coexpressed in variable quantities in many cell types, and all cells appear to express multiple isotypes.

Two groups of sequences can be discerned among the 6 β tubulin isotypes. The sequences of isotypic classes I, II, and IV are closely related, differing by only 2–4%, while isotype classes III, V, and VI are more divergent, differing at 8–16% of amino acid positions. The expression of class I, II, and IV isotypes is relatively abundant, while class III and V isotypes are less abundantly expressed. The hematopoeitic class VI protein is the most abundant β tubulin in the differentiated cells in which it is found. While the divergent group isotypes show no obvious overall relatedness, they all share a Cys_{239}-Ser_{239} substitution, normally a conserved site, and except for a mammalian β tubulin gene, mβ1, an Ala_{124}-Cys_{124} substitution. In the chicken, where the expression of each isotype has been examined, all cell types express at least one divergent isotype, perhaps reflecting a requirement for representatives of both groups of β tubulin isotypes in vertebrate cells (Sullivan et al 1986a).

Function of β Tubulin Isotypes: The multitubulin hypothesis tested

In order to analyze the role of the carboxyl terminus of β tubulin in subunit utilization and microtubule assembly, Solomon's laboratory examined the function of altered β tubulins by transfection into mouse 3T3 cells. Bond et al (1986) constructed a chimeric gene, the first three-fourths of which was the amino-terminal portion of the chicken class II β tubulin and the last one-fourth of which was the carboxyl-terminal portion of the highly divergent *Saccharomyces cerevisiae* β tubulin gene. The chimeric protein was detected with a yeast-specific antibody after transfection and was found to be codistributed with endogenous tubulin in all microtubules while the cells continued to grow and divide normally. This experiment demonstrated that mammalian microtubules are remarkably adaptable to structural divergence in the carboxyl-terminal region of β tubulin. However, a series of chimeric yeast/chicken proteins involving other regions of β tubulin showed severely impaired function, which indicates limitations in the adaptability of the system (Fridovich-Keil et al 1987).

These experiments with chimeric yeast: vertebrate tubulin genes documented the plasticity of mammalian microtubules, but did not address the question of how endogenous β tubulin isotypes are utilized in a normal cellular context. Seeking to answer this question, the laboratories of Cleveland and Cowan have now developed antibodies specific for each isotypic class of β tubulin using isotype-derived bacterial fusion proteins (Lewis et al 1987) or synthetic peptides (Lopata & Cleveland 1987). In appropriately controlled experiments, both groups observed that all β tubulin isotypes present in a cell assemble freely into all detectable classes of microtubules, with roughly equivalent efficiencies. Microtubules are thus copolymers of available β tubulin isotypes in the cell. Further, examination of the isotype content of fibroblasts from chicken, mouse, and monkey revealed widely different isotypic compositions of the tubulin pool, an observation that is difficult to reconcile with the idea that isotypes confer even subtle functional properties on microtubule systems (Lopata & Cleveland 1987). In a second experiment, the divergent class VI cDNA was transfected into fibroblasts in order to determine how this highly specialized gene product behaves in an inappropriate cellular context. In two independent experiments (Lewis et al 1987; Joshi et al 1987), the divergent isotype was freely incorporated into all classes of microtubules with no gross effects on the phenotype of the transfected cells. Indeed, during normal hematopoeisis, the class VI isotype participates in all microtubule functions (Murphy et al 1986; Lewis et al 1987). If tubulin isotypes have specific functions, these experiments document that they must be subtle indeed.

Nevertheless, evidence for functional differentiation of tubulin isotypes is beginning to emerge. Murphy and his associates studied the assembly of chicken erythrocyte tubulin (comprised of 95% class VI β tubulin) and found it to assemble more efficiently than brain tubulin (Murphy & Wallis 1985, 1986). Although in vitro experiments established that class VI β tubulin is not required, at least not in abundance, for establishment of a marginal band in chicken erythrocytes (Swan & Solomon 1984), Joshi et al (1987) showed that, in vivo, the class IV and VI β tubulins of chicken erythrocytes are differentially susceptible to cold-induced depolymerization. Differences in the ability of tubulins to bind small ligands suggest that their biochemical properties are, indeed, distinct (Ludueña et al 1985; Little & Ludueña 1985; Banerjee & Ludueña 1987).

But are β tubulin isotypes distinguished within the microtubule systems of cells? Phosphorylation of a unique isoelectric variant of β tubulin has been observed during neurite outgrowth in neuroblastoma cells, which documents a cellular mechanism that can distinguish among β tubulin isotypes (Gard & Kirschner 1985). Further, by quantitation of isotypes in the polymerized and unpolymerized tubulin pools of PC12 (pheochromocytoma) cells undergoing nerve growth factor (NGF)-induced differentiation and neurite extension, Joshi & Cleveland (1988) detected that isotype classes I and II were more efficiently recruited into polymers independent of the quantitative composition of the complex β tubulin pool. These differences in the physical and biochemical properties β tubulin isotypes, coupled with the observed conservation of vertebrate β tubulins, suggest that cells may, in fact, be able to distinguish among β tubulin isotypes by some mechanisms. However, elucidating such mechanisms and any role they play in microtubule systems promises to challenge our abilities to resolve the properties of cellular microtubules.

α Tubulin Genes: Regulatory compartments for tubulin synthesis

The biology of the α tubulin genes of vertebrates is superficially similar to that of the β tubulin genes, with the important difference that the mammalian and avian gene families show almost no concordance in terms of conserved, orthologous genes. Again, the most extensive data derive from the mouse, where at least seven genes encode six polypeptide isotypes (Lewis et al 1985b; Villasante et al 1986; Hecht et al 1988), and from the chicken, where five (perhaps six) functional genes encode as many different polypeptides (Valenzuela et al 1981; Pratt et al 1987; Pratt & Cleveland 1988). The mammalian genes are highly conserved within the order; except for a single highly divergent mouse testes-specific gene, orthologous genes corresponding to each of the five mouse α tubulins have been identified in

at least one other species. On the contrary, only one chicken α tubulin gene, cα1, appears to correspond in sequence or expression to a possible mammalian counterpart, represented by mα1. The remaining chicken α tubulins show a surprising degree of heterogeneity compared to the mammalian family, with no specific relationship either to mammalian α tubulins or to each other. Like the β tubulins, each gene possesses a unique pattern of expression that leads to a complex situation of coexpression in most, if not all, cell types (Villasante et al 1986; Pratt & Cleveland 1988). These data are summarized in Table 2.

Comparison of the sequences of vertebrate α tubulins (see Villasante et al 1986; Pratt & Cleveland 1987) reveals that, like the β tubulins, amino acid substitutions are largely clustered at the carboxyl terminus and in an amino-terminal region between residues 35 and 60. It is notable that the presence of carboxyl-terminal tyrosine on α tubulin, the substrate for posttranslational modification, is quite variable. As discussed below, α tubulin undergoes a modification involving cyclic removal and addition of C-terminal tyrosine. All but two of the mammalian α tubulin isotypes, but only two of six chicken α tubulin isotypes, possess encoded C-terminal tyrosine. Since there is significant divergence between the carboxyl-terminal sequences of the different α tubulin isotypes, it is possible that individual gene products differ in their ability to participate in this modification cycle. Nevertheless, it appears that the abundant α tubulin isotype expressed in most cells is accurately represented by a consensus α tubulin sequence (Valenzuela et al 1981; Ponstingle et al 1981).

The expression of the vertebrate α tubulin genes suggests that regulatory compartmentalization is one of the key functions of these multigene families. Analysis of the expression of two α tubulins in the rat, Tα1 and T26, reveals that Tα1 is highly expressed during periods of neurite outgrowth, both during ontogeny and following injury (Miller et al 1987). In contrast, T26 is expressed constitutively at low levels throughout development and is not perturbed by neuronal injury. These results suggest that the gene represented by rat Tα1 is linked to a global program of neuronal gene expression. Since the proteins encoded by these two genes differ by only a single amino acid, it seems unlikely that functional differences can distinguish the two polypeptides. Tubulin expression in the testes also reveals a highly differentiated program of gene expression. In mammals, two testes-specific genes have been identified, one of which is similar (95%) to the consensus α tubulin sequence, while another, confined in expression to haploid spermatids, shows only 70% homology to other α tubulin isotypes (Distell et al 1984; Hecht et al 1988). Using an antipeptide antibody directed against this sequence, this unusual α tubulin has been detected in all microtubule structures of the spermatid and was shown to be absent

Table 2 Vertebrate α tubulin genes: C-terminal sequences and expression

Gene[a]	C-terminal sequence	Encoded Tyr	Expression
mα1, hbα1, rα1, chα2, porc. α	DYEEVGVDSVEGEGEEEGEEY	Yes	Major neural/neural development; many tissues
mα2, hkα1, rαT26, chα1	DYEEVGVDSVEGEGEEEGEEY	Yes	Major neural/constitutive; many tissues
mα6, chα3	DYEEVGADSAEGDDEGEEY	Yes	Minor constitutive; many tissues
mα3/7, h2α	DYEEVGVDSVEAEAEEGEEY	Yes	Major testes; testes specific
mα4, hα44, mfα	DYEEVGIDSYEDEDEGEE	No	Minor constitutive; many tissues; untranslated in testes
mα(testes)	GYEEVGMGSVEAEGEEEDRNTSCCIMFSSSIGNR	No	Testes/haploid specific
cα1	DYEEVGVDSVEGEGEEEGEEY	Yes	Major brain; many tissues
cα2	DYDEEATDLFEDENEAGS	No	Testis specific
cα3	DYEEVGRNSADGGEFEE	No	Minor constitutive
cα5	DYEEVGLDSYEDEEEGEE	No	Minor; many tissues
cα8	DYEEVGTDSMDGEDEGEEY	Yes	Minor constitutive; many tissues
cα4	DYEEVGTDSFEDENDEE	No	?

[a] Sequences are from mouse: mα1, mα2 (Lewis et al 1985); mα3, mα4, mα6, mα7 (Villasante et al 1986); mα(testes) is pRDαTT1 (Hecht et al 1988); human: hbα1, hkα1 (Cowan et al 1983; Hall & Cowan 1985); h2α, hα44 (Villasante et al 1986); Chinese hamster: chα1, chα2, chα3 (Elliot et al 1986); rat: rα1 (Lemischka et al 1981; Ginzburg et al 1981); rαT26 (Ginzburg et al 1986); pig: proc. α (Ponstingl et al 1981); chicken: cα1 (Valenzuela et al 1981); cα2 (Pratt et al 1987); cα3, cα4, cα5, cα8 (Pratt & Cleveland 1988).

from brain tubulin (Hecht et al 1988). A third testes-specific transcript derives from a gene that is ubiquitously expressed elsewhere, but is differentially spliced in testes to yield an mRNA that lacks a 5′ AUG initiator codon and is presumably incapable of translation (Dobner et al 1987; Villasante et al 1986). None of the testes-specific isotypes show particular homology to a chicken testes-specific α tubulin gene, cα2.

INVERTEBRATE TUBULIN GENE FAMILIES

Analysis of tubulin gene family structure and expression in invertebrate species has revealed a wide diversity in the numbers and patterns of expression of tubulin genes. By application of classical and molecular genetics, the functions of tubulin isotypes have been directly tested in organisms such as yeasts, *Aspergillus*, and *Drosophila*, and has revealed that a multiplicity of genes is not essential for the basic functions of microtubules, namely mitosis and meiosis. Nevertheless, multiple tubulin genes provide regulatory diversity for tubulin synthesis in a number of species.

Drosophila

In *Drosophila*, a complex metazoan, tubulin gene function has been examined using classical and molecular genetics. *Drosophila* possesses four genes for α tubulin and four for β tubulin, which reveal patterns of both constitutive and developmentally regulated expression (reviewed by Raff 1984). The four α tubulin genes have been isolated and sequenced (Theurkauf et al 1986). Two constitutive genes, α1 and α3, encode α tubulins very similar to the mammalian consensus α tubulin. A pupal and adult testes isotype, α2, differs by 4.6% from α1, while α4 encodes a highly divergent maternal α tubulin isotype that shares only 67% homology with α1. The sequences of three of the four β tubulin genes have now been determined; the constitutive β1 gene and the testes-specific β2 share 95% homology with each other and with the vertebrate class I, II, and IV β tubulin isotypes (Rudolph et al 1987; Michiels et al 1987). In contrast, the quantitatively minor, developmentally regulated β3 gene differs at 12% of amino acid residues. These results clearly demonstrate that the genes encoding the major α and β tubulin isotypes, represented by the major neural isotypes in vertebrates, have been strongly conserved throughout metazoan evolution. For β tubulin, they further suggest that the divergence of the major and minor isotype classes may have preceded the divergence of the insect and vertebrate lines (Rudolph et al 1987).

Genetic and biochemical analysis of the testes specific β2 gene in *Drosophila* quickly demonstrated that β2 is not, as might have been anticipated,

a specific axonemal β tubulin, but rather is the major β tubulin of postmitotic spermatocytes (Kemphues et al 1979, 1982). Mutations that affect the stability (Kemphues et al 1983) or the assembly properties (Raff & Fuller 1984; Fuller et al 1987) of the $\beta 2$ isotype affect all microtubule systems in the postmitotic spermatid. The recessive nature of $\beta 2$ mutations that disrupt microtubule structure in homozygous fly spermatids further documents the remarkable flexibility in microtubule assembly since microtubules of heterozygotes, made from 50% "bad" β tubulin subunits, are fully functional. These data provided the impetus for the proposal made by Raff (1984) that multiple tubulin genes exist primarily to fulfill regulatory requirements, allowing eukaryotes to coordinate tubulin synthesis with multiple programs of gene expression in development and differentiation.

An important question not answered by these experiments is whether the $\beta 2$ isotype confers some subtle, specialized properties on axonemal microtubules in addition to their well documented multifunctionality. As for the vertebrate β tubulin isotype classes, the specific sequence of the *Drosophila melanogaster* $\beta 2$ gene has been conserved in a related species, *D. hydei*, which suggests that the unique structure of the protein may be under positive selection (Michiels et al 1987). The availability of P-element mediated gene transfer strategies in *Drosophila* should allow both the regulatory function and the potential structural uniqueness of this gene to be tested directly in the near future.

Physarum

The myxomycete *Physarum* polycephalum has emerged as a useful model system for analysis of tubulin gene and isotype utilization during cellular differentiation, largely through the work of Gull, Dove and their coworkers (Burland et al 1983; Schedl et al 1984a,b; Gull et al 1986). *Physarum* possesses four α tubulin and three β tubulin genes that are differentially expressed in the two cell types that constitute the two major phases of the *Physarum* life cycle: the free living myxamoeba and the syncytial plasmodium. Surprisingly, the complexity of tubulin isotypes in the plasmodium, which possess only intranuclear mitotic spindle microtubules, is greater than that seen in the myxamoeba, which possess cytoplasmic and centriolar microtubules as well. Two β tubulins expressed in myxamoebae comigrate as a single electrophoretic isoform, $\beta 1$, and appear to be very similar in sequence (Singhofer-Wowra et al 1986). By contrast, the $\beta 2$ isotype encoded by the major plasmodial β tubulin is almost 20% divergent from this sequence (Burland et al 1988). Of the two α tubulin isotypes resolved by two dimensional electrophoresis, cDNA and direct protein sequence analysis demonstrates that myxamoebal $\alpha 1$ tubulin isotypes,

probably a mixture of three different genes, are highly homologous while the plasmodium specific α2 isotype is more divergent (Singhofer-Wowra & Little 1987; Krammer et al 1985; Birkett et al 1985; Monteiro & Cox 1987a). Not surprisingly, all available plasmodial isotypes are present in the sole microtubule structure, the mitotic spindle, of these naturally synchronous syncytia (Paul et al 1987).

One of the functions of the plasmodium specific tubulin genes must be to accommodate the very tight cell cycle regulation of tubulin synthesis in the plasmodium, which is restricted to the last three hours of G2 phase (Schedl et al 1984b). Transcription of tubulin genes is initiated in G2 by the inactivation of a negative regulator of transcription, is rapidly repressed after mitosis, and is followed by the rapid, exponential decay of tubulin mRNA (Carrino & Laffler 1986; Laffler 1987). Since the divergence of the β2 and α2 isotypes is coupled to the restricted expression of these genes, Burland et al (1988) proposed that sequence divergence occurs as a result of neutral drift following the regulatory isolation of a gene to a biological context requiring fewer microtubule related functions. While such a scenario does not fully explain the conservation observed among vertebrate β tubulins, it suggests that regulatory isolation is likely to be an early step in establishing structurally divergent isotypes.

Aspergillus

The filamentous fungus *Aspergillus nidulans* possesses two α tubulin and two β tubulin genes (Morris et al 1984, 1986). Two-dimensional electrophoresis demonstrated that the major genes for α and β tubulin, *tubA* and *benA*, respectively, each give rise to two polypeptides, presumably through posttranslational modification (Weatherbee & Morris 1984). Both *benA* and *tubA* participate in the assembly of mitotic and cytoplasmic microtubules. The second β tubulin gene, *tubC*, encodes a highly divergent polypeptide that appears to function during conidiation, a developmental process associated with asexual sporulation (Weatherbee et al 1985; May et al 1985, 1987). Using integrative transformation to disrupt the *tubC* gene, May et al (1985) were able to test directly whether the differentiation specific *tubC* gene was required for the differentiation process, with the surprising result that conidiation proceeded normally in the absence of a functional *tubC* gene. Thus, in the only example to date where it has been possible to test experimentally for the requirement of a differentiation specific tubulin gene product, the gene has proven to be entirely dispensible.

Yeast

The yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* possess one β and two α tubulin genes (Neff et al 1983; Hiraoka et al 1984; Toda et al

1984; Adachi et al 1986; Schatz et al 1986a,b). The α tubulin gene pairs of the two species, designated *TUB1* and *TUB3* in *S. cerevisiae* and *alpha-1* and *alpha-2* in *S. pombe*, share a number of properties. One of the α tubulin genes, *alpha-1* or *TUB1*, is essential for growth while one is unessential. However, despite 10% divergence in the encoded α tubulin protein sequences, null mutations in the *alpha-1* or *TUB1* can be complemented by increased expression of *alpha-2* or *TUB3*, respectively. The essential features of *alpha-1* and *TUB1* are their expression properties and not, apparently, the unique sequences of the encoded polypeptides. For yeast a single gene for each tubulin chain is sufficient to support a complete life cycle.

Yeasts have proven a fertile substrate for analysis of the structural organization of the tubulin polypeptides. Divergence between the α tubulins of both *S. cerevisiae* and *S. pombe* is clustered in the carboxyl terminus, similar to their vertebrate counterparts, and between residues 35 and 55. Despite apparent functional similarity, the genes of each species share no more homology to each other than to vertebrate α tubulins, which reinforces the conclusion that structural divergence between the essential and nonessential genes is not crucial to tubulin function. Mutagenesis of the amino-terminal variable region has shown that insertions of up to 17 amino acids after residue 43 had no effect on any of the known microtubule processes in yeast (Schatz et al 1987). Presumably, divergence has accumulated because it is accepted in that region and not because it contributes to important aspects of microtubule function.

Taken together, these results clearly demonstrate that eukaryotes utilize multiple tubulin genes to establish independent programs of tubulin gene expression that are linked to other, global patterns of gene expression. Two well defined examples of this are found in the highly differentiated programs of neuronal tubulin expression in vertebrates and in gametogenesis in a large number of species. However, the complex patterns of coexpression of isotypes suggests that a large number of cell types that lack obviously differentiated microtubule structures or functions possess different, genetically specified tubulin isotype pools. While the total range of divergence and functional promiscuity of tubulin isotypes suggests that a great deal of structural variation can be accommodated within the microtubule lattice, it remains possible that tubulin isotypes contribute specialized properties to microtubules. Indeed, the conservation of vertebrate β isotypes is difficult to explain without positive selection since one of the isotypes, class VI, has diverged rapidly throughout the evolution of vertebrates, which demonstrates that amino acid substitution can be accepted on a relatively short time scale. However, the expectation that multiple tubulins would be used for the different microtubule systems

within cells has not been substantiated. A role, if any, for the tubulin isotypes must lie in a more subtle aspect of microtubule function, such as modulating MAP binding or assembly dynamics of microtubules.

POSTTRANSLATIONAL MODIFICATIONS OF TUBULIN: Dynamic Modification of Microtubules

The significance of posttranslational modification as a mechanism for the introduction of biochemical specificity into the backbone of the microtubule polymer has become widely appreciated, largely as the result of the preparation of antibody reagents specific for different modified forms of tubulin. For α tubulin, two types of modification are known: the unique cyclic tyrosination/detyrosination of the carboxyl terminus and lysine-ε-amino acetylation. The new immunological reagents have sparked a rapid and exciting elucidation of the cell biology of these modifications, converging on the description of a novel subset of microtubules present in a variety of cells that differ from the bulk of cytoplasmic microtubules in their assembly dynamics in vivo and their biochemical composition. Analysis of α tubulin modifications in a number of biological systems strongly suggests a link between α tubulin modification, microtubule assembly dynamics, and morphogenesis.

Tyrosination of α Tubulin

An unusual posttranslational modification of α tubulin is the enzymatic removal and addition of the carboxyl-terminal tyrosine residue (Barra et al 1973). Early biochemical work, reviewed by Thompson (1982), identified two specific enzymes, tubulin : tyrosine ligase (TTLase) and tyrosyltubulin carboxypeptidase, that catalyze the two reactions of this unique modification cycle. The demonstration of encoded C-terminal tyrosine on α tubulin initially suggested that detyrosination was the first reaction of the cycle (Valenzuela et al 1981). However, the more recent discovery of α tubulin isotypes that lack encoded carboxyl-terminal tyrosine indicates that primary translation products of α tubulin can begin life on either side of the cycle and that some may not be substrates at all (see above). Nevertheless, the incorporation and turnover of carboxyl-terminal tyrosine in vivo is rapid (Thompson et al 1979) and occurs on the majority of available α tubulin (Gundersen et al 1987). Further, the demonstration that turnover of carboxyl-terminal tyrosine is dependent on microtubules suggested a correlation between the dynamic properties of microtubules and the α tubulin tyrosination cycle (Thompson 1982).

The development of antibodies specific for tyrosinated (Tyr) and untyrosinated (Glu) α tubulin has now allowed biochemical and structural inves-

tigation of the modification cycle at the level of individual cells and microtubules. The first antibody shown to recognize a linear epitope spanning the carboxyl terminus of Tyr α tubulin was a murine monoclonal antibody, YL-1/2, that was originally raised against yeast tubulin (Kilmartin et al 1982; Wehland et al 1984). Application of this antibody in immunofluorescence demonstrated that the microtubule arrays of cultured mammalian cells appear to be uniformly tyrosinated (Wehland et al 1983). However, by preparation of antipeptide antisera specific for Glu or Tyr tubulin, respectively, Gundersen et al (1984) demonstrated that cells, in fact, possess an additional subset of microtubules that are uniquely stained by anti-Glu tubulin. These microtubules have a different morphology and distribution than the bulk of anti-Tyr stained microtubules; they are more sinuous in appearance and usually fail to extend to the cell periphery. Immunogold electron microscopy in CV-1 and PtK2 cells revealed that individual microtubules in vivo are copolymers of Tyr and Glu tubulin that possess detectable amounts of both subunits, but differ in the ratio of reactivity with Glu- or Tyr-antisera (Geuens et al 1986). The tyrosination cycle of α tubulin was thus shown to be correlated with the formation of distinct populations of microtubules in individual cells, a major population rich in Tyr tubulin (Tyr-MT) and a minor, differentiated population enriched for Glu tubulin (Glu-MT).

The differentiation of these two microtubule classes occurs in the polymer phase of cellular tubulin as a postpolymerization reaction and does not involve the selective assembly of Glu-MT versus Tyr-MT (Gundersen et al 1987; see Thompson 1982). Biochemical analysis of TTLase and tyrosyltubulin carboxypeptidase activities initially showed that they are preferentially active on soluble and polymeric tubulin, respectively (Arce et al 1978; Kumar & Flavin 1981). TTLase has now been purified to homogeneity and has been shown to bind tightly to soluble tubulin through a binding site on β tubulin, but both binding and tyrosination were significantly reduced on microtubule substrates (Wehland & Weber 1987b). The activity of TTLase results in a highly tyrosinated monomer pool for the assembly of new microtubules; these microtubules can then be converted to Glu-MT by the action of an endogenous carboxypeptidase (Gundersen et al 1987; Wehland & Weber, 1987b).

High Glu-tubulin content was shown to be correlated with a variety of stable microtubules in several cell types, including cilia and flagella, primary cilia and centrioles, and marginal bands (Gundersen & Bulinski 1986; Sherwin et al 1987). In addition to these specialized microtubule systems, the population of Glu-enriched cytoplasmic microtubules in interphase cells in culture also exhibit enhanced stability toward drug-induced, but not cold-induced microtubule depolymerization (Wehland & Weber

1987a; Kreis 1987; Schulze et al 1987). The most plausible explanation for these observations is that microtubules accumulate modifications in a time dependent manner; most microtubules are unstable and depolymerize before accumulating detectable amounts of Glu tubulin. Glu-MTs correspond to microtubules that are physically older than most microtubules by virtue of enhanced stability.

These experiments raise important questions regarding the role of tubulin modification in regulating the functions and properties of cellular microtubule systems. Before we consider these further, however, we review another modification of α tubulin, acetylation, which raises closely related questions.

Acetylation of α Tubulin

L'Hernault & Rosenbaum (1983, 1985a,b) discovered a second modification of α tubulin, lysine-ε-amino acetylation, which is associated with flagellar morphogenesis in *Chlamydomonas*. A reversible conversion of two isoelectric variants of α tubulin in *Chlamydomonas*, α_1 and α_3, was observed during the formation and resorption of flagella, and this modification was shown to involve acetylation of α_1 to yield α_3. Tubulin acetyltransferase (TAT) is highly specific for α tubulin, is preferentially reactive with polymeric tubulin, and is tightly associated with axonemes (Greer et al 1985; Maruta et al 1986). The complementary tubulin deacetylase (TDT) was found in the cytoplasm in *Chlamydomonas*.

A monoclonal antibody specific for the acetylated form of α tubulin was isolated by Piperno & Fuller (1985) following immunization with tubulin isolated from sea urchin sperm axonemal tubulin. The epitope recognized by antibody 6-11-B1 was shown to be present on the major *Chlamydomonas* flagellar α_3 tubulin isotype as well as flagellar α tubulin in numerous species, but not in soluble cytoplasmic tubulins. In addition, reactivity with 6-11-B1 could be generated specifically on sea urchin egg α tubulin by chemical acetylation of lysine residues with acetic anhydride. Analysis of proteolytic peptides of *Chlamydomonas* flagellar α tubulin identified Lys-40 as acetyllysine and demonstrated that peptides spanning residues 37–138 or 25–50 bind to antibody 6-11-B1 (LeDizet & Piperno 1987). Thus, the epitope recognized by this antibody is specified, at least in part, by the sequence of residues 37–50 of α tubulin and requires acetyllysine in position 40.

Application of this antibody to the analysis of acetylated microtubules distribution in cells revealed that in addition to staining axonemal microtubules, a subset of morphologically distinct microtubules could be detected in the cytoplasmic microtubule networks of a number of cell types (LeDizet & Piperno 1986; Piperno et al 1987). A similar pattern of staining

had previously been observed with another monoclonal anti-α tubulin raised against sea urchin flagellar α tubulin (Thompson et al 1983). This antibody has subsequently been shown to be specific for acetylated α tubulin (Ac-α) (Schulze et al 1987). The subset of cytoplasmic microtubules reactive with the anti Ac-α tubulin antibodies has now been examined in several mammalian cells and has been shown to comprise a class of sinuous, stable microtubules resistant to depolymerization induced by drugs, but not by cold. As for the tyrosination cycle, the acetylation and deacetylation reactions appear to be partitioned between microtubules and monomeric tubulin, respectively (Piperno et al 1987).

Modified Tubulin and Microtubule Stability

The striking similarity of the properties and appearance of cytoplasmic microtubules enriched in Glu tubulin and in acetylated α tubulin suggested that they correspond to the same classes of microtubules in cells. This appears to be largely correct. Using double immunofluorescence in TC-7 (monkey) cells, the population of Ac-α tubulin rich microtubules was demonstrated to be virtually coincident with the population of Glu-MT (Bulinski et al 1988). Neither modification is dependent on the other, however, since cells possessing only acetylated microtubules (HeLa) or Glu microtubules (PtK2) have been described. Further, in cells recovering from microtubule disruption, the kinetics and morphology of recovery of the two modifications differed: Ac-α tubulin was detected rapidly (within 5 min) as small spots or segments of microtubules that increased in length and number as cells approached pretreatment morphology; in contrast, the appearance of uniformly staining Glu-MT was gradual (20–30 min). Thus, two modifications act by different mechanisms on the same subset of microtubules.

Measurements of tubulin assembly dynamics in vivo have revealed that most cellular microtubules are highly unstable, with halflives on the order of 2–5 min, but there exists a population of microtubules that fail to incorporate new tubulin, remaining stable for an average of 1 hr or more (Salmon et al 1984; Soltys & Borisy 1985; Schulze & Kirschner 1987). Kreis (1987) demonstrated that Glu-MT correspond to the less dynamic class of microtubules in Vero cells by colocalization of injected rhodamine labeled tubulin. Using an antibody blocking technique after microinjection of biotinylated tubulin, Schulze et al (1987) demonstrated that for SKNSH (human retinoblastoma) cells, a set of acetylated, tyrosinated, and stable microtubules formed a congruent subclass of microtubules distinct from the abundant class of kinetically dynamic, nonmodified microtubules. However, variations of this theme have been observed in several cell types. In PtK2 cells, which lack acetylated tubulin, a stable class of microtubules

was detected. Chick embryo fibroblasts possess abundant acetylated microtubules and a few Glu-MT, but possess almost no stable microtubules. Thus, modification of microtubules usually, but not always, is associated with a mechanism that stabilizes selected subsets of microtubules in cells.

These experiments have established that cells possess multiple mechanisms for modification of microtubules, which result in the time-dependent accumulation of acetylated and/or detyrosinated α tubulin in stable microtubules. How these modifications affect microtubule function and properties, if at all, is not clear. Biochemically, neither modification appears to affect directly microtubule assembly or disassembly in vitro (Thompson 1982; Kumar & Flavin 1982; Maruta et al 1986) or of Glu tubulin in vivo (Wehland & Weber 1987a; Webster et al 1987; Khawaja et al 1988). Indeed, the fact that both acetylation and detyrosination occur by different mechanisms on the same set of microtubules suggests that that set of microtubules had previously been differentiated by some other mechanism(s), although the time course of acetylation is rapid enough to be involved with this initial differentiation step (Bulinski et al 1988).

Although a biochemical role for the posttranslational modification of tubulin has not yet been described, analysis of modification in a variety of differentiated cells and tissues suggests a strong association between α tubulin modification and the morphological differentiation of cells and intracellular structures. In the central nervous system, the differentiation of certain neuronal populations is associated with extensive detyrosination of axonal microtubules (Cumming et al 1983, 1984), a process mirrored in neuroblastoma (N115) cells induced to differentiate with NGF (Wehland & Weber 1987a). Similarly, acetylation occurs preferentially within axons compared to dendrites, coinciding with detyrosinated microtubules in parallel fiber axons (Cambray-Deakin & Burgoyne 1987). Analysis of retinal tissue by immunofluorescence with antibody 6-11-B1 similarly demonstrated a preferential acetylation of microtubules of neurons as well as a segregation of Ac-α microtubules within outer segments of rod photoreceptor cells (Sale et al 1988). Further, analysis of the activities of enzymes modifying α tubulin suggests that they are specifically modulated during morphogenetic processes (Deanin et al 1981). Proliferating Chinese hamster ovary (CHO) cells possess little α tubulin carboxypeptidase activity, but it can be induced by treatment with forskolin, which induces a transformation of CHO cells from an epithelial to a fibroblastlike morphology (Wehland & Weber 1987a). Induction of chemotaxis in human polymorphonuclear leukocytes results in a reorganization of the microtubule network and an increase in Tyr tubulin content (Nath et al 1981). Further, the evolutionary conservation of these modification cycles sug-

gests that they are an important component of cellular microtubule networks (Diggins & Dove 1987; Sasse et al 1987; Sherwin et al 1987). These results clearly establish a strong link between the differentiation and structural reorganization of cells with dynamic modifications of tubulin and microtubule systems. The challenge now is to define the functional basis for that link.

Other Modifications of Tubulin

In addition to the modifications of α tubulin, β tubulin is subject to phosphorylation although this reaction has not been extensively studied (Gard & Kirschner 1985; Edde et al 1981). Perhaps the most significant aspect of these studies to date is the demonstration that a specific isoelectric variant of β tubulin is phosphorylated during differentiation of neuroblastoma, which documents a cellular process capable of distinguishing between isotypes of tubulin. Further, significant isoelectric heterogeneity of tubulins, particularly brain tubulins, has been extensively documented by isoelectric focusing. The demonstration of at least 12 isoforms of β tubulin in vertebrate brain, which expresses at most six different genetic isotypes, suggests that posttranslational modification may be an important property of β tubulin as well as of α tubulin (Field et al 1984).

LOOKING AHEAD

Analysis of tubulin isotypes in a number of species has revealed both patterns of differential gene regulation and evidence for structural differentiation of the encoded polypeptides. Examination of tubulin has provided at least a partial explanation for why eukaryotes accumulate and maintain multiple genes encoding functionally similar proteins. The existence of multiple tubulin genes allows the synthesis of this ubiquitous cellular component to be regulated under multiple, independent programs of gene expression during differentiation. For this role, a unique structure of the encoded protein may not be necessary, rather the regulatory elements of the gene perform different functions.

Our more specific question of how multiple tubulins provide functional specificity within microtubule systems has been more difficult to answer. Clearly, monomer diversity is a major aspect of the assembly of microtubule networks in most cells, and microtubules have been shown to possess enormous adaptability in utilization of structurally diverse or even abnormal tubulin subunits. This has been a surprise; thinking about the numerous protein : protein interactions that comprise a microtubule led to the expectation that tubulin sequences would be stringently conserved, or at least that variations in sequence would result in disasterous conse-

quences for microtubule assembly. This has also been a boon: We now have a good start on dissecting the organization of structure and function within tubulin through the combined technologies of in vitro molecular genetics and reintroduction of modified genes, sophisticated immunological reagents, microinjection, and classical biochemistry.

Distinctly lacking from this scenario, however, is detailed knowledge of the three-dimensional structure of tubulin and microtubules. It is obvious that microtubule function depends on the association of microtubules with other cellular proteins and organelles, and it is equally obvious that tubulin could, in principle, be an important modulator of these interactions. In order to evaluate this potential role, we need to develop a working knowledge of how the surface of the microtubule is organized for these interactions, where isotypic variation occurs upon this surface, and how associated proteins interact with the tubulin lattice.

The elucidation of the chemical versatility and adaptability of microtubules has been paralleled by significant advances in our understanding of microtubule dynamics in cells, which has led to the proposal that selection of individual microtubules from a dynamic and unstable array plays a fundamental role in mediating microtubule-related morphogenetic events (Kirschner & Mitchison 1986). In spindle morphogenesis, for example, dynamic instability could allow the spindle asters to rapidly sample the entire space of the cell with microtubules, stabilizing only those that yield productive interactions with chromosomes. In a similar way, dynamic instability, and in particular the rapid length oscillations observed at the ends of individual microtubules (Horio & Hotani 1986) affords the cell an opportunity to search the "conformational space" of the microtubule lattice, possibly resulting in the selective assembly or positioning of tubulin isotypes within individual microtubules. In any event, it is clear that continued elucidation of the role of tubulin isotypes in cellular microtubule systems will play an important role in understanding the mechanisms by which microtubules function in cells.

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