

Genetics of Microtubule Systems

ELIZABETH C. RAFF

*Institute for Molecular and Cellular Biology and Department of Biology, Indiana University,
Bloomington, Indiana 47405*

ABSTRACT In most eucaryotes the tubulin genes comprise small multigene families with approximately equal numbers of genes for α - and β -tubulin, the structural proteins of microtubules. The recent isolation of tubulin mutations in several species is proving to be a powerful tool for examining the structure and function of specific sets of microtubules. In *Drosophila melanogaster*, genetic analysis of a testis-specific β -tubulin gene has shown that a single tubulin gene product may fulfill a number of different microtubule functions. In addition to tubulin mutations, mutations in other genes whose products are involved in the regulation or structure of specific microtubule arrays have also been isolated. The combination of analysis of both classes of mutations is beginning to allow a molecular description of the construction and function of three-dimensional cellular structures. In addition, such studies may also shed light on the evolutionary pressures that gave rise to and serve to maintain small families of genes encoding very similar proteins.

Young biochemists of my generation were cautioned not to waste clean thinking on a dirty enzyme. What was harder for us to remember was that it is in a dirty system (i.e., life) that the enzyme normally worked. The awareness that biological function must be viewed within the context of the physical interactions of the cellular architecture is both a cause of, and a result from, the new and very exciting synthesis between what have heretofore been rather separately pursued disciplines of biological thought—molecular biology and biochemistry, developmental biology and genetics, and cell biology. The merging of different technical approaches and viewpoints offers new insight in approaching the fundamental problem of biology: We now have a real possibility of addressing the question of how linearly encoded information can be expressed to result in formation of functional three-dimensional structures, and of following a process from genotype to phenotype.

Having set such a large stage, I want to focus on a single aspect of the overall problem and consider the control of assembly and function of just one of the components of eucaryotic cellular substructure. Microtubules are used to mediate a number of crucial processes including cell division, cell motility, and, as part of the cytoskeleton, the formation and maintenance of cell shape. This system thus affords a good model for study of the mechanisms by which the morphology of eucaryotic cells is determined. A formidable literature exists both on the morphology and function of various microtubule arrays in vivo and on the biochemistry of tubulin.

Genetic analysis has only recently been utilized for examining the relationship between the structure and function of tubulins; it is this relatively new branch of the microtubule literature that I will summarize here, using our work in *Drosophila* as a specific example.

TUBULIN GENE FAMILIES

Microtubules are composed of equimolar amounts of the two 50,000-dalton subunits, α - and β -tubulin. The stable form of tubulin both in vivo and in vitro is as a dimer, and although the direct experimental evidence is not as tight as the universal acceptance of the proposition would imply, circumstantial evidence from many systems is consistent enough to be compelling that the functional unit in microtubule assembly is the α - β heterodimer (1). Historically, thought about tubulin has passed through two distinct phases. Early on, the dominant feature in views of microtubule function was the striking universality and conservation throughout eucaryotic phylogeny both of the structure of microtubules and the biochemical properties of tubulin. This monolithic personality—tubulin is tubulin—was then schizophrenically shattered when, primarily because of improvements in separation techniques, it was found that microheterogeneity of tubulin populations even within a single cell or tissue type was probably the rule, not the exception. Thus the possibility was raised that instead of a single dimer for all seasons, for every tubule there might be a tubulin. Recent elucidation of the

number and distribution of tubulin genes in a wide variety of species has brought these two disparate views into overlapping focus. As summarized in Table I, the tubulins are encoded in small, multigene families containing approximately equal numbers of genes for α - and β -tubulin. There is thus indeed genetic diversity of tubulins, but of a modest sort. It appears that few organisms have as many different tubulins as they have sets of microtubules. Species representing a wide range of phylogenetic diversity contain one to four genes each for α - and β -tubulin, unlinked in the genome. Some of the apparent exceptions to this pattern may actually not represent very significant deviations. The genomes of mammals, so far represented by studies on humans and rats (a venerable combination in the history of science), contain as many as 20 sequences each for α - and β -tubulins. However, in both these species at least some, if not the majority, of genomic tubulin sequences have been found to represent nonfunctional pseudogenes (2, 4, 6, 7, 10). The only striking difference in number and arrangement of tubulin genes is that in the parasitic protozoan *Leishmania*, the α - and β -tubulin genes are clustered in separate tandem repeats (28), and in the trypanosomes, α - and β -tubulin sequences alternate in a tandem repeat of α, β - gene pairs (27). How many of these sequences represent functional genes has not yet been reported.

The unique arrangement of genes in trypanosomes is particularly provocative since it is believed that α - and β -tubulin are derived from a single ancestral sequence, their amino acid sequences exhibiting 40–50% homology (30). Their divergence, however, is clearly an ancient event that occurred very early or indeed at the onset of the origin of eucaryotes (31–33). The highly conserved nature of the tubulins has been amply demonstrated at the biochemical level both by the cross-reactivity of tubulins with heterologous antisera and by the well-known ability of tubulins from diverse sources to copolymerize into microtubules *in vitro*. The remarkable conservation of α - and β -tubulins in evolution has now been directly confirmed at the primary sequence level (8–10, 29, 34–40). However, as Cleveland has recently pointed out (41), it may be misleading to emphasize the homologies since the variation between different tubulin gene products in a single organism may be as great, or even greater than, differences between subunits from different species. The functional significance of small differences in the primary structure of tubulins is thus an important question that has yet to be answered.

THE DROSOPHILA TUBULIN GENE FAMILY

In *Drosophila*, one has the luxurious advantage of being able to relate the genetic recombination map to the visible road map of the genome in the banding pattern of the giant polytene chromosomes in the salivary glands. The tubulin gene family is diagrammed in Fig. 1. This set of genes exhibits the now classical (in terms of the short history of molecular genetics) picture of a multiple gene family. At least one member of each tubulin class is expressed ubiquitously whereas other members are expressed differentially with respect to developmental timing and/or tissue specificity (see Table II). From studies of transcription patterns it appears likely that all of the tubulin sequences in the *Drosophila* genome represent functional genes (16, 18, 19, 42, 43), although to date we have identified protein products for only four of the genes (14, 15, 44, 45; Fig. 2).

As shown in Fig. 2, we have genetically mapped the α -tubulins synthesized during embryogenesis to the polytene chromosome region 84B3-6 by examining tubulin synthesis in embryos homozygous for a deletion of this region. This result is consistent with the observation that transcripts from this gene constitute the bulk of the α -tubulin mRNA population throughout embryogenesis (18, 19, 42). The major α -tubulins synthesized *in vivo* at other times in development and in adult tissues co-migrate with the embryonic α -tubulin in two-dimensional gel electrophoresis and yield similar products in peptide mapping, but we do not know as yet whether these species are all products of the 84B locus or may also represent similar gene products from other loci.

We have characterized three β -tubulin subunits, one of which, like the 84B α -tubulin, is a prominent and ubiquitously expressed protein, and two of which are limited in expression in time or space. We initially identified and mapped a testis-specific subunit (β_2 -tubulin) to the polytene chromosome region 85D4-7 (14, 15); the genetic analysis of the function of this subunit is described in detail below. In Fig. 1, I have tentatively assigned the remaining two β -tubulin subunits to genetic loci based on comparison of protein synthesis patterns with transcription patterns. We have designated the major β -tubulin synthesized throughout embryogenesis and in adult tissues as β_1 -tubulin (14, 44, 45). Based on the ubiquity of expression and prevalence of transcripts, it seems most likely that at least the embryonic species is encoded by the gene at 56D (19; Kimble, M. and E. C. Raff, unpublished data). In

TABLE I
Tubulin Gene Families

Organism	Number of genes each for α - and β -tubulin per haploid genome	Organization in the genome	References
Vertebrates			
Mammals	10–20 (many are pseudogenes)	dispersed	human: 2–7 rat: 8–10
Chicken	4	dispersed	11–13
<i>Drosophila</i>	4	dispersed	14–19
Sea urchin	9–13	α - and β -genes unlinked, but some clustering within families	11, 20
<i>Chlamydomonas</i>	2	dispersed	21, 22
<i>Physarum</i>	3–5	two α -genes linked, otherwise dispersed	23
<i>Aspergillus</i>	2	dispersed	24–26
Trypanosomes	13–17	most genes in tandem repeat cluster ($\alpha\beta$) _n	27
<i>Leishmania</i>	7–15	separate repeat clusters: (α) _n and (β) _n	28
Yeast (<i>Saccharomyces</i>)	1	probably unlinked	29

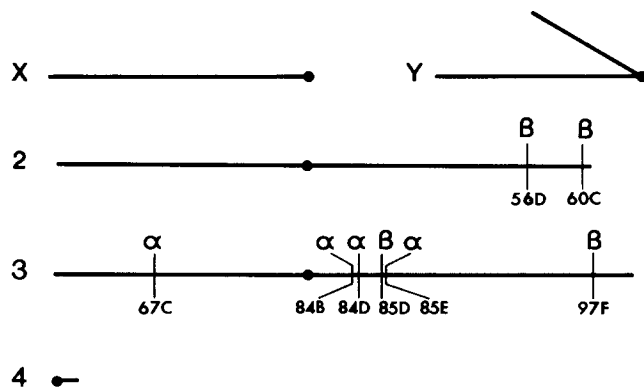


FIGURE 1 The tubulin gene family in *Drosophila melanogaster*. The *Drosophila* chromosomes are shown schematically, approximately to scale. The locations of the tubulin genes in the salivary gland polytene chromosome bands, as determined by in situ hybridization of cloned probes for tubulin sequences are given. (16-19; also, unpublished data of K. Valgiersdottir and M. L. Pardue, and of J. E. Rudolph and E. C. Raff).

addition to β_1 -tubulin, we discovered an embryo-specific subunit, β_3 -tubulin, that is a zygotic gene product transiently expressed only during a tightly restricted time period (15). Our experiments strongly suggested that β_1 - and β_3 -tubulin are different gene products. Since the unique developmental timing observed by Natzle and McCarthy (19) for transcripts from the 60C locus is identical to that we observed for expression of β_3 -tubulin, it seems highly probable that β_3 -tubulin is encoded by this gene.

GENETIC ANALYSIS OF MICROTUBULE FUNCTION

It is clear that neither genetic diversity nor any other single mechanism accounts for cellular control over the enormous versatility of microtubule function. The timing and amount of tubulin synthesis is also regulated not only with respect to which genes are expressed, but as well by a number of complex mechanisms related to the levels of unpolymerized tubulin and other conditions within the cell (21, 41, 53-58). Second, as is discussed in more detail below, additional complexity of tubulin populations may be generated by post-translational modification of specific subunits. Finally, there is the control exerted through the interaction of tubulin with small molecules, in particular, calcium, and, what may well turn out to be the key control for the functional specificity of microtubules, interaction of tubulin with diverse other proteins (the microtubule-associated proteins, or MAPs, of the microtubule literature), which may play both structural and/or regulatory roles. We can, perhaps with some success, assess the relative importance of each of these levels of control for various microtubule organelles; but what we cannot yet do, for even one set of microtubules, is to describe in any detail the sequence of molecular steps involved in its construction and ultimate function within the cell. However, application of the techniques of molecular and classical genetics promises to bridge the gap between the literature on the biology of microtubules and that on the chemistry of tubulin.

Two approaches have been exploited for genetic analysis of microtubule function: (a) isolation of mutations in the structural genes for tubulin subunits themselves, and (b) isolation

TABLE II
Drosophila Tubulin Gene Products

	Transcripts* Kb	Proteins†	Pattern of expression
α-Tubulin genes			
67C4-6	1.7	—	Maternal product: transcripts present in ovaries and very early embryos
84B3-6	1.8	α_1	Ubiquitous
84D4-8	2.0	—	Coordinately with 84B but transcripts much less prevalent
85E6-10	1.65	—	Major 1.65-kb transcript present in pupae, male adults, testis: testis specific?
	(1.85)	—	(1.85-kb transcript present in larvae)
β-Tubulin genes			
56D4-12	1.8	β_1	Ubiquitous
60C6-8	2.3	β_3	Mid-embryogenesis (7-15 h)
85D4-7	2.0	β_2	Testis specific
97F	1.9	—	Coordinately with 56D but transcripts much less prevalent

* The size and patterns of expression of α -tubulin transcripts are from Kalfayan and Wensink (42) for hybridization to mRNA populations by specific probes constructed for each gene from 3'-nontranslated sequences. Similar results have also been reported by Mischke and Pardue (18) and Natzle and McCarthy (19), in which clones that contained tubulin coding sequences were used. The data for the β -tubulin transcripts are from Natzle and McCarthy (19) for hybridization to mRNA by probes for each gene containing primarily tubulin coding sequences.

† The biochemical characterization and patterns of expression of the tubulin proteins were determined in our laboratory (14, 44, 45). The chromosome loci for tubulin gene products were determined by genetic mapping for α_1 - and α_2 -tubulin (84B; see Fig. 2, this paper) and for the testis-specific β_2 -tubulin (85D; 15). Putative genetic loci for the ubiquitous β_1 -tubulin (56D) and the embryo-specific β_3 -tubulin (60C) were assigned on the basis of comparison of the patterns of transcription and protein synthesis, as described in the text. Protein products for the remaining tubulin genes have not yet been identified.

of mutations in other genes that affect the structure, timing, placement, or function of specific sets of microtubules. Classically, genetic analysis has been used to dissect complex biological processes by use of mutants that disrupt different aspects or stages of the process. Tubulin mutations thus constitute specific probes with which to examine the participation of a given tubulin gene product in microtubules in situ in the cell or tissue in which the gene is expressed. Analysis of the phenotypes of such mutations can potentially yield several kinds of information: for example, on the cellular level, the relationship between differential tubulin gene expression and the specificity of microtubule function can be assessed; and on the molecular level, changes in the structure of the tubulin molecule can be directly correlated with specific aspects of function in microtubules in vivo. Finally, the coupling of analysis of tubulin mutations and mutations in other genes involved in microtubule assembly affords a way of following the construction of three-dimensional supramolecular structures within the cell.

Mutations in tubulin genes have now been reported in several species. In this section I will discuss studies in which tubulin mutations have been isolated as a class of mutations that cause altered response to antimitotic drugs. Such mutations were first reported in the fungus *Aspergillus nidulans* by Morris and Oakley and their collaborators, who mapped the

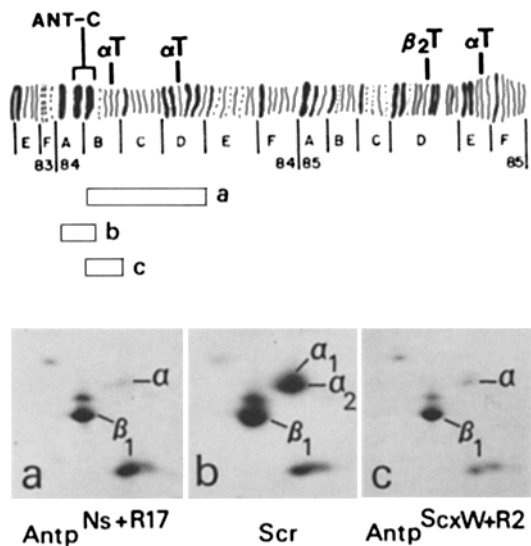


FIGURE 2 Genetic mapping of the major embryonic α -tubulin in *Drosophila*. The gene for embryonic α -tubulin was localized to site 84B by the following experiments performed in collaboration with T. C. Kaufman. The top of the figure shows the locations on the right arm of the third chromosome of three α -tubulin genes, the testis-specific β_2 -tubulin gene, and the *Antennapedia* gene complex. The open bars indicate the extent of three deletions used in this study: (a) *Df(3R)Antp^{Ns+R17}*, which deletes the region 84B1,2-84D13 (46); (b) *Df(3R)Scr*, which deletes the region 84A1,2-84B1,2 (47); (c) *Df(3R)Antp^{ScxW+R2}*, which deletes the region 84A4,5-84B6 (48). Each of these deletions removes a portion of the *Antennapedia* gene complex containing the *ftz* locus (49, 50). Embryos homozygous for the deletions die at the embryo-larval boundary but because of the abnormal segmentation caused by the lesion in the *ftz* locus are readily identifiable by 8 h of development.

The panels at the bottom of the figure are portions of autoradiograms of two-dimensional gels showing the tubulin subunits synthesized by embryos homozygous for each of these deletions. Prelethal stage 22- to 24-h-old embryos were labeled in culture with [³⁵S]methionine. Tubulin was then prepared by vinblastine sulfate precipitation along with carrier tubulin from wild-type eggs and embryos (45). Embryos homozygous for either of the deletions, (a) *Df(3R)Antp^{Ns+R17}* or (c) *Df(3R)Antp^{ScxW+R2}*, failed to synthesize significant amounts of α -tubulin, whereas β_1 -tubulin synthesis was essentially normal. Both of these mutations delete the α -tubulin hybridization site at 84B. The tubulin synthesis pattern in embryos homozygous for the third deletion, (b) *Df(3R)Scr*, which does not delete this site, was the same as that in normal embryos of the same age (45). Thus the gene encoding the major embryonic *Drosophila* α -tubulins is localized within the small region 84B3-84B6 defined by the distal end of *Df(3R)Scr* and the distal end of *Df(3R)Antp^{ScxW+R2}*.

It should be noted that the large deletion *Df(3R)Antp^{Ns+R17}* deletes the α -tubulin gene at 84D as well as that at 84B. However, we have not been able to detect any differences in the tubulin subunits synthesized by prelethal stage embryos homozygous for this deletion and embryos homozygous for *Df(3R)Antp^{ScxW+R2}*, in which only the 84B gene is deleted.

Although it is difficult to see in the rather overloaded gel shown here, (as indicated in b) the embryonic α -tubulins usually resolve in the SDS electrophoresis dimension into a closely spaced doublet, of which the slower moving component (designated α_1) is the minor component. Both species appear to be encoded at 84B and both are synthesized in *in vitro* translation supported by embryonic mRNA preparations (45). The possibility originally raised by Mischke and Pardue (18) that the 84B site may contain two α -tubulin genes seems to be eliminated, however, since overlapping clones for this

gene for β -tubulin to the locus *benA*, and found many mutations that result in resistance or super-sensitivity to the benzimidazole drugs (24). A gene for α -tubulin was subsequently identified among mutations that cause reversion of the drug-sensitive phenotypes of the β -tubulin mutants (25). An elegant aspect of this work is the use of temperature-sensitive mutations, which allows introduction of the mutant block at specific points in the life cycle of the fungus (59-61). For example, these studies showed that the *benA* gene product is required for the function of at least two classes of microtubules (59). Furthermore, a β -tubulin mutation in which hyperstable microtubules are formed at restrictive temperatures was used to demonstrate that chromosome movement requires disassembly of spindle microtubules (60), an aspect of mitotic mechanism long ago proposed by Inoué but for which little direct experimental evidence exists (reviewed in reference 62).

Tubulin mutations resulting in changes in response to antimetabolic drugs have also been reported in yeast (29), in the slime mold *Physarum* (23), and in tissue culture cells (63-68). In the latter two systems, tubulin mutations resulting in altered resistance to antimetabolic drugs were found to have differential effects on microtubule function. For example, Cabral and co-workers (68) found that in Chinese hamster ovary cells expressing a variant β -tubulin, mitotic function was abnormal but the interphase arrangement of cytoplasmic microtubules was normal. Most recently, this group has reported a Chinese hamster ovary cell mutation in which cytoplasmic microtubules appear normal but the mitotic spindle can be formed only in the presence of the microtubule-stabilizing drug taxol (69, 70). Analysis of this mutation has demonstrated that a morphologically normal spindle is required for both chromosome movement and cytokinesis. It has not been determined as yet whether this is a tubulin mutation or whether it represents a microtubule-related mutation.

As mentioned above, the second type of mutations that affect microtubule function are those in genes that encode components, either structural or regulatory, which interact with tubulin or microtubules. Such mutations have been reported in two systems: the first example is the study of the touch receptor neurons in the nematode *Caenorhabditis elegans* by Chalfie and his associates (71-73). This work is not only a nice functional analysis of a specific set of microtubules, but also serves as a reminder of the dangers of generalizations about biological systems. When tubulin is assembled *in vitro*,

region of the *Drosophila* genome give no evidence for duplicate tubulin genes (51).

In each panel the tubulin subunits are designated. The protein above β_1 -tubulin is an as yet unidentified nontubulin component. Actin is also present (lower right-hand corner). As can be seen in a and c, embryos in which the 84B α -tubulin gene is deleted synthesize a trace amount of protein that migrates in the α -tubulin region. This may be a minor tubulin or other protein whose presence is uncovered in the absence of α_1 - and α_2 -tubulin synthesis. (If so, it cannot be a tubulin encoded at site 84D since this protein is present in embryos homozygous for *Df(3R)Antp^{Ns+R17}*.) Perhaps more likely, this may represent residual synthesis of α_2 -tubulin on remaining maternal message (18, 52).

the structures formed (whether bona fide microtubules of various protofilament number or sheets, rings, etc.) depend on the conditions under which assembly is carried out (for example, 74–77; reviewed in references 78 and 79). The wide variety of structures that tubulin can form in vitro reflects the intrinsic functional potential of tubulin subunits to form numerous self-associations. Observations of the morphology of microtubules in vivo, however, have revealed that most cytoplasmic microtubules, as well as the A tubule of the doublet tubules in the axoneme of cilia or flagella, are composed of 13 protofilaments (79). Certainly, examples of microtubules with other protofilament numbers are known (80–82), but the few exceptions have been more or less taken as representing special cases. Because of its universality, the 13-protofilament number has often been implicitly assumed to reflect a general intrinsic structural, and perhaps functional, constraint for cytoplasmic tubules. However, Chalfie and Thomson (73) found that in *C. elegans* and other nematodes there are no 13-protofilament microtubules. Rather, most cells in the worm have 11-protofilament microtubules, whereas the microtubules in the six touch-receptor neurons have 15 protofilaments.

The unique protofilament substructure of the touch cell microtubules appears to be required both for their arrangement in the cell and for their function. Unlike the 11-protofilament microtubules in other cells, the 15-protofilament microtubules, which apparently mediate touch sensitivity by serving as a rigid structure against which physical deformation occurs, are cross-linked in rigid, hexagonally packed bundles; mutations in the gene *mec-7* result in loss of touch sensitivity concomitant with loss of this set of microtubules (71–73). In the mutants they are partially replaced by less regular arrays of uncross-linked 11-protofilament microtubules. Although these microtubules are sufficient for outgrowth of the touch neuron cell processes, they do not mediate touch sensitivity. Chalfie and Thomson (73) concluded that the *mec-7* gene is unlikely to be a tubulin structural gene but rather may encode a product responsible for specifying the protofilament number of the specialized touch cell microtubules. This mutation may thus constitute a first step in identifying the heretofore elusive mechanisms of microtubule nucleation in vivo.

An example of a study of mutations that affect the structural components of a microtubule organelle is the elegant genetic analysis of the *Chlamydomonas* flagellum by Luck and Perno and their colleagues. Using mutations that affect flagellar structure and/or motility, this group has identified specific proteins that comprise various substructures within the flagellum and the order in which these proteins are assembled (reviewed in reference 83). The structural studies have also yielded information about the mechanism of flagellar motility. For example, by analysis of paralyzed mutants lacking radial spokes, Huang et al. (84) identified the proteins from which they are constructed. Subsequent isolation of suppressor mutations yielded unexpected “by-pass” mutants that, although motile, still lacked the radial spoke system previously believed to be required for motility. Analysis of the suppressor mutations revealed a control mechanism involved in the initiation of flagellar beat and also showed that, although not required for axoneme movement per se, the radial spoke system is necessary for the efficient asymmetric flagellar beat in wild type (85, 86).

Mutations in the tubulin genes have not yet been reported in *Chlamydomonas*. However, assembly of the flagellum in

this organism and in the related alga *Polytomella* involves the only post-translational modification of tubulin for which a functional significance has been demonstrated. Studies in the laboratories of Rosenbaum and of Weeks (87–89) have demonstrated that the flagellar isoform of α -tubulin is encoded by the same gene as that which encodes the cell body α -tubulin and is derived by a post-translational modification of the cell body form. Recent work by L'Hernault and Rosenbaum (90) has shown that acetylation of α -tubulin occurs within the flagellar matrix during assembly of the axoneme and is reversed upon disassembly. It is tempting to speculate that the alteration in α -tubulin results in a change in the functional capacity of the molecule.

GENETIC ANALYSIS OF THE *DROSOPHILA* TESTIS-SPECIFIC β_2 -TUBULIN

Spermatogenesis in *Drosophila* has been well characterized at the cytological and ultrastructural level (reviewed in reference 91) and affords an excellent model system for examining microtubule function in construction of a complex organelle. The testis of the adult male may be thought of as representing a steady-state system in both time and space: developing cysts in all stages of spermatogenesis are present and their location along the length of the testis approximately corresponds to their chronological stage of development. There are four crucial sets of microtubules involved in spermatogenesis. At the tip of the testis, gonial cells undergo four mitotic divisions, which form syncytial cysts of 16 primary spermatocytes. These undergo meiosis to form cysts of 64 spermatids. Then, during spermatid differentiation, the sperm flagella axoneme is assembled from the basal body derived from the single centriole remaining after the last meiotic division. Meanwhile, as assembly of the axoneme proceeds, a specialized bundle of cytoplasmic microtubules mediates the shaping of the nucleus into the needle-like head of the mature sperm.

In our initial work in this system, we identified β_2 -tubulin, a subunit that is expressed only in the testis and is the sole β -tubulin component of mature motile sperm (14). Our original hypothesis was that expression of this subunit might be a direct signal for construction of the sperm flagella axoneme, a doublet microtubule-containing structure that does not occur elsewhere in *Drosophila* (there are no ciliated cells in either embryos or adult tissues). Analysis of a number of mutations in the gene for β_2 -tubulin, however, has revealed that the situation is considerably more complicated than this simple model. In fact, β_2 -tubulin serves a number of functions in the tissue in which it is expressed and is required for the function of all classes of microtubules assembled subsequent to its expression, which occurs shortly before the onset of meiosis. Thus, this subunit functions in the meiotic spindle (but not in the mitotic spindles in earlier stages of spermatogenesis), in cytoplasmic microtubules, and in the axoneme.

Table III summarizes the β_2 -tubulin mutations we have isolated. Because this subunit is not expressed at all in females, nor elsewhere in males, these mutations do not affect viability of the flies but exhibit male sterility as their sole phenotype. We first looked for mutations in the gene for the testis-specific subunit by the “brute force” method of screening for male sterile mutations in which the electrophoretic mobility of the subunit was altered. This approach, although perhaps inelegant, was effective. The first such mutation we isolated was the dominant male sterile *B21^P*, which we used to map the

TABLE III
Male Sterile Mutations That Affect Microtubule Function in
Spermatogenesis in *Drosophila*

β_2 -Tubulin mutations		Second-site mutations
Dominant	Recessive	
Stable β_2 -tubulin variant	Class I: Unstable β_2 -tubulin variant	Recessive Fail to complement
Defective meiosis	Testis tubulin pool diminished	Class I β_2 -tubulin mutations
Defective axoneme assembly	No meiosis No nuclear shaping No axoneme assembled	Map to other genes
	Class II: Stable β_2 -tubulin variant Specific defects in microtubule function	

β_2 -tubulin tubulin gene (14, 15). The phenotype of this mutation is illustrated in Fig. 3. Spermatid differentiation in males that express $Bt2^D$ is defective such that both the overall organization of the axonemes in developing sperm tails is disrupted and the morphology of the doublet tubules is aberrant (Fig. 3, *a-f*). However, in addition to the defects in axoneme assembly there are also severe meiotic abnormalities, including formation of multipolar spindles at the second meiotic division in heterozygous males and complete failure to form a spindle in homozygotes (Fig. 3, *g-i*). This observation first raised the possibility that β_2 -tubulin has other functions in addition to assembly of the axoneme. The meiotic defects are consistent with the developmental timing of β_2 -tubulin expression, but because the mutation is dominant, we

could not eliminate the possibility that the variant subunit interferes in processes where the wild-type subunit does not normally function.

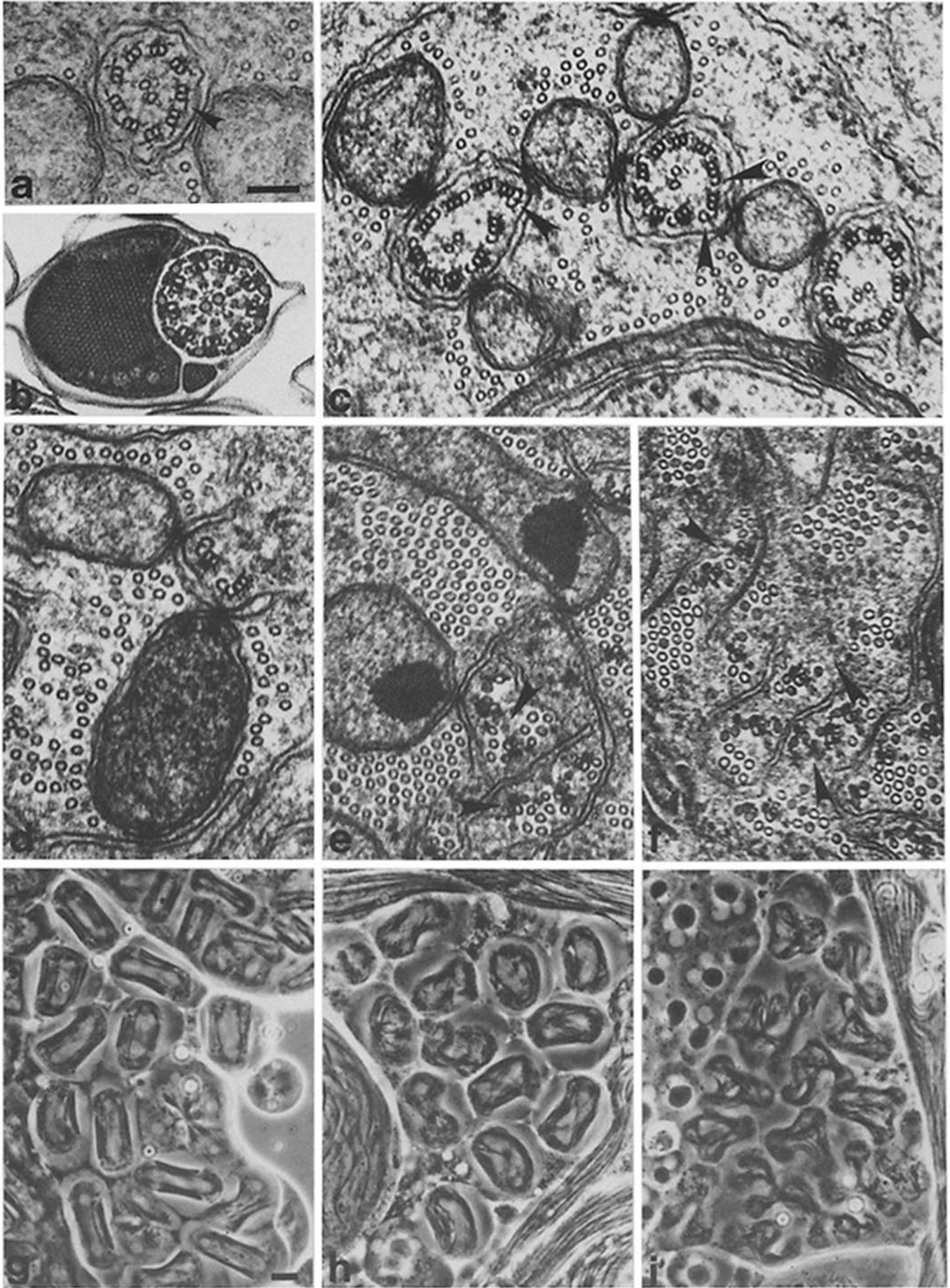
We have answered this question as well as extended our analysis of β_2 -tubulin function by isolating a number of recessive mutations in the gene. These mutations fall into two distinct classes. Class I mutations cause complete failure of β_2 -tubulin function, and it was from these mutations that we deduced the multifunctionality of this tubulin subunit (92–94). In homozygous males, spermatogenesis fails in all aspects of microtubule-mediated function subsequent to expression of the variant subunit. Thus, although early steps in spermatogenesis proceed normally, including the mitotic divisions of the gonial cells to form the cysts of 16 primary spermatocytes, thereafter no microtubules are assembled: meiosis fails to occur (no spindle is formed, there are no chromosome movements, and cytokinesis does not occur); no cytoplasmic microtubules form, the sperm nuclei are not shaped, and no axoneme is formed.

The original group of class I mutations that we reported encode different variant forms of β_2 -tubulin, but all share a common biochemical phenotype (92–94). Whereas the wild-type β_2 -tubulin is extremely stable in the testis protein pool, these mutations encode variant β_2 -tubulin subunits that are synthesized at approximately normal levels but are thereafter unstable and rapidly disappear from the testis tubulin pool. At the same time, the normally stable wild-type α -tubulin also disappears. Thus, in homozygous males the testis tubulin pool is vanishingly small, containing only the normally small amount of the ubiquitous β_1 -tubulin that functions in early spermatogenic stages and a correspondingly small amount of α -tubulin. We concluded that the β_2 -tubulin variants encoded by these mutations are unable to form normal α - β dimers, and that consequently, both the mutant β_2 -tubulin and wild-type α -tubulin are degraded by the proteolytic machinery within the cell. It should be noted that this line of reasoning from the genetic evidence can be turned around, and the co-

FIGURE 3 Phenotype of the dominant β_2 -tubulin mutation ($Bt2^D$) in *Drosophila*. *a* and *b* show spermatid development in wild-type males: (*a*) Early stage spermatid showing an early axoneme and the two associated mitochondrial derivatives. The projection from the B tubule, which constitutes the first stage in assembly of the accessory tubules, is indicated by an arrowhead. (*b*) Mature spermatozoon in the seminal vesicle of a wild-type male, showing the 9 + 2 axoneme and the 9 associated accessory tubules. Note that the lumen of the accessory tubules and the central pair is filled with electron-dense material. The major and minor mitochondrial derivatives, which extend the length of the axoneme, exhibit the typical electron-dense paracrystalline material. *c* shows developing spermatids in a heterozygous $Bt2^D/Bt2^+$ male. The spermatids, at a stage of development comparable with that in *a*, are in abnormal relationships both with respect to each other and to the associated mitochondrial derivatives. Arrowheads indicate aberrant doublet tubules. The defect in microtubule morphology appears to be in construction of the accessory tubules which, in many cases, appear to be assembled in multiple "false starts" and/or at an improper angle to the B tubule, producing structures that resemble triplet tubules.

d-f show spermatid development in a homozygous $Bt2^D/Bt2^D$ male. In this genotype, no complete axonemes are assembled: (*d*) Early stage spermatid comparable in development to that in *a*, showing an incomplete axoneme containing two of the typical aberrant doublet and accessory tubules. In this spermatid the two associated mitochondrial derivatives are in relatively normal relationship to the axoneme. (*e*) and (*f*) Later stages in spermatid development, as indicated by the accumulation of paracrystalline material in the mitochondrial derivatives. Regions that appear to represent abortive axoneme assembly are indicated by arrows (compare the doublet tubules with associated accessory tubules and the surrounding "eyebrow" of electron-dense material in the wild-type axoneme in *b*). Note the remarkable arrays of microtubules in much of the cytoplasm; some of these are filled in with electron-dense material similar to the central pair and accessory tubules and may thus represent ectopic axoneme tubule assembly. Electron micrographs were taken by J. H. Caulton. Magnifications for all of the electron micrographs are given by a bar representing 0.1 μm in *a*.

g-i are phase contrast micrographs of live testis preparations showing meiotic cysts. (*g*) Meiosis I cyst in a wild-type male. Compare the size of the spindles with those in an adjacent meiosis II cyst in the upper right-hand corner of the panel. (*h*) Meiosis I cyst in a heterozygous $Bt2^D/Bt2^+$ male. The spindles are similar in size to those in wild-type but are somewhat abnormal in appearance. (*i*) Meiosis II cyst in the $Bt2^D/Bt2^+$ male, illustrating the large-size multipolar spindles that result following the failure of cytokinesis at meiosis I. Our previous studies have shown that considerable chromosome nondisjunction occurs at both meiotic divisions in males heterozygous for $Bt2^D$ (15). In homozygous $Bt2^D/Bt2^D$ males, no meiotic spindles form. Bar, 10 μm .



degradation within the cell of normal α -tubulin in the presence of the variant β_2 -tubulin can be construed as another strong circumstantial argument that it is the heterodimer that is the normal stable subunit within the cell. Recently, K. A. Matthews has isolated a null mutation for this locus in which no β_2 -tubulin is synthesized (unpublished data). The cytological and biochemical phenotype of this mutation, including loss of normal α -tubulin from the tubulin pool, is identical with that of the other class I mutations.

The second class of recessive mutations in the β_2 -tubulin gene are those that encode variant forms that are stable. Except for the stability of the tubulin subunits they encode, however, unlike the first class mutations, the class II mutations do not share a common phenotype. In the class II mutations the stable β_2 -tubulin variants retain some degree of function so that microtubule-mediated events do not fail to occur, as in class I, but rather proceed abnormally. Each mutation has a unique phenotype and exhibits a different set of defects in microtubule function during spermatogenesis. The phenotypes of these mutations will be described in detail elsewhere (Fuller, M. T., J. H. Caulton, J. A. Hutchens, P. C. Kaufman, E. C. Raff, manuscript in preparation).

Within class I, all interallelic combinations are male sterile and exhibit the same phenotype as homozygotes for a single allele (that is, complete loss of microtubule function in spermatogenesis, beginning with meiosis). Interactions between class I and II alleles, and alleles within class II, however, are more complex. Males that are double heterozygotes for such combinations of alleles do not necessarily exhibit the spermatogenic phenotype of either parent mutation. While analysis of the class I mutations allowed us to determine the normal function of β_2 -tubulin, analysis of the class II mutations, which differentially affect the various functions of β_2 -tubulin, is beginning to allow us to genetically dissect each of these functions.

Finally, Table III also summarizes another very interesting class of mutations we obtained during screens for new β_2 -tubulin mutations. These are recessive male sterile mutations that fail to complement the class I recessive β_2 -tubulin mutations but that map to other genes (for a preliminary report of these mutations, see reference 95). We interpret these nontubulin noncomplementing mutations to represent lesions in genes that encode products that directly interact with β_2 -tubulin during spermatogenesis. Analysis of these mutations together with the tubulin gene mutations should allow us to identify other structural components of the sperm tail axoneme and also to identify proteins that may specify the functional interactions that govern the participation of a single tubulin subunit in a number of different kinds of microtubules.

THE ROLE OF MULTIPLE TUBULIN GENES

Not only the tubulins, but many other proteins of central importance in eucaryotic cell function, for example, actins, collagens, keratins, and the globins, occur as small families of related but distinct genes. This phenomenon raises the general question of the function of multiple copies of genes for very similar proteins. That is, what are the selective pressures in evolution that gave rise to and serve to maintain small families of a few closely related single copy genes?

In the extremes, two disparate mechanisms can be envisioned: (a) perhaps the gene products of the several members of such families each possess slightly different biochemical

properties intrinsic to differences in the primary amino acid sequence and allowing a slightly different mode of function for each protein; or (b) perhaps the different genes arose through the need for cells to exert alternate regulation of expression of basic structural proteins. For the tubulins, then, the question is whether each form possesses unique functional capacities required for a particular set of microtubules, or whether the various forms are equivalent in functional potential for microtubule assembly but are subject to separate control mechanisms allowing differential timing, place, or amount of tubulin synthesis. As with most alternate hypotheses about biological mechanisms, it appears that both possibilities are probably true to different extents in different systems.

As discussed above, our genetic analysis of β_2 -tubulin has revealed the surprising fact that tissue specificity of expression does not necessarily imply restriction of functional specificity. If β_2 -tubulin has unique functional capacity, it is unlikely to be for those structures that involve singlet microtubules (that is, the meiotic spindle and cytoplasmic microtubules) since similar microtubules in other tissues or at times when β_2 -tubulin is not expressed are carried out by other subunits. Perhaps more likely is that if β_2 -tubulin does have unique functional capacity, it is required for assembly of the doublet tubule-containing sperm tail axoneme, a structure that is assembled only in the testis and in which β_2 -tubulin is the only β -tubulin component. However, centrioles, the triplet tubules of which are morphologically extremely similar to the axoneme doublets for which they serve as templates, are normal in all the β_2 -tubulin mutations (92; Caulton, J. and E. Raff, unpublished data). Thus, it appears that other β -tubulins may also participate in construction of this molecular arrangement of tubulin subunits.

Finally, there is the second alternative that multiple tubulin genes may serve regulatory requirements. In addition to β_2 -tubulin, other testis-specific proteins have been described: for example, the testis cytochrome *c* in the mouse (96) and the seminal RNase in the ox (97). The sequences of these two proteins are sufficiently divergent from the somatic or pancreatic forms, respectively, to suggest, if extrapolated directly from a phylogenetic tree constructed on molecular data, that the testis diverged from mammals several million years ago. Since this is an unlikely interpretation, perhaps we can speculate that separate genes for certain proteins evolved to allow specific regulatory control over differentiation of the testis, perhaps consequent to the maintenance of the germ line. We may thus arrive at a circumstantial case for arguing that of the two possibilities proposed above, it may be more likely that a testis-specific tubulin arose as a consequence of such regulatory rather than functional pressures. One always gets in trouble with generalizations, however: the fly in this particular ointment is the chicken, in which the major testis β -tubulin gene is also expressed in other tissues (13).

Recent work from Wensink's laboratory on the structure of the *Drosophila* α -tubulin genes has also yielded some unexpected results. Baum et al. (43) found that although the introns as well as the flanking sequences are different, no differences could be detected within the coding regions for the three α -tubulin genes on the right arm of the third chromosome. This raises the possibility that these three α -tubulins may be very similar or even identical proteins but subject to differential regulation of expression. The fourth gene, however, is different. The homology data suggest that

the maternally expressed α -tubulin encoded on the left arm of the third chromosome is a protein similar to the other α -tubulins in the carboxyl terminal region but only very weakly homologous in the amino terminal half of the molecule. It will be very interesting indeed to find out what the functional significance of this unusual structural divergence may be.

As in *Drosophila*, the expression of different tubulin genes has been shown to be differentially regulated in a number of other organisms, including humans (35, 36), rats (98), chickens (13, 99), sea urchins (100), and *Physarum* (101). This is not necessarily always the case, however. In *Chlamydomonas*, for example, all four tubulin genes are expressed coordinately (21, 22) and appear to have similar control regions (102, 103). The coordinate expression of multiple tubulin genes may be the reason that it has been difficult to isolate tubulin mutations in this organism.

As the functions for specific tubulin genes in different species are defined, we should begin to gain more insight into the precise role the multiple tubulin genes play and thus, implicitly, into the pressures that led to their formation. To extrapolate (always dangerous) from the relatively few examples so far, it appears that in general a given tubulin gene product may be involved in more than one set of microtubules. It may be, however, that we will be unable to arrive at one or even a few generalities. As is made clear by Raff and Kaufman (104) in their discussion of evolutionary mechanisms, diversity of origins, as well as of functions, may well be the only general rule. This indeed is suggested by Frazetta's felicitous analogy of evolution as a process of improving a motor while keeping the engine running (105). Stanislaw Lem, in *His Master's Voice*, remarks that "Evolution is, as an engineer, an opportunist, not a perfectionist" (106). For myself, I like to combine these two images and compare evolutionary processes to that most pragmatic of engineers, a teenager with a junk car (thus, we may expect to find in the molecular record both chewing gum and baling wire).

I wish to thank K. A. Matthews, J. E. Natzle, K. Valgiersdottir and M. L. Pardue, J. V. Ruderman, P. C. Wensink, and W. F. Dove for making available unpublished data while I was preparing this manuscript. The experiments described from my laboratory have been performed over the past few years in collaboration with T. C. Kaufman, R. A. Raff, K. J. Kemphues, M. T. Fuller, J. E. Rudolph, M. Kimble, J. H. Caulton, and J. A. Hutchens, all of whom have contributed not only to the scientific work but also to the accompanying and crucial verbal improvisation usually known as lively discussions.

Our work is presently supported by National Science Foundation grant PCM 83-02149 and United States Public Health Service grant 5R01 HD 16739.

Received for publication 10 January 1984, and in revised form 26 March 1984.

REFERENCES

- Ludueno, R. F. 1979. Biochemistry of tubulin. In *Microtubules*. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., NY. 65-117.
- Cowan, N. J., C. D. Wilde, L. T. Chow, and F. C. Wefald. 1981. Structural variation among human β -tubulin genes. *Proc. Natl. Acad. Sci. USA* 78:4877-4881.
- Wilde, C. D., L. T. Chow, F. C. Wefald, and N. J. Cowan. 1982. Structure of two human α -tubulin genes. *Proc. Natl. Acad. Sci. USA* 79:96-100.
- Wilde, C. D., C. E. Crowther, and N. J. Cowan. 1982. Diverse mechanisms in the generation of human β -tubulin pseudogenes. *Science (Wash. DC)* 217:549-552.
- Wilde, C. D., C. E. Crowther, and N. J. Cowan. 1982. Isolation of a multigene family containing human α -tubulin sequences. *J. Mol. Biol.* 155:533-538.
- Wilde, C. D., C. E. Crowther, T. D. Cripe, M. G.-S. Lee, and N. J. Cowan. 1982. Evidence that a human β -tubulin pseudogene is derived from its corresponding mRNA. *Nature (Lond.)* 297:83-84.
- Lee, M. G.-S., S. A. Lewis, C. D. Wilde, and N. J. Cowan. 1983. Evolutionary history of a multigene family: an expressed human β -tubulin gene and three processed pseudogenes. *Cell* 33:477-487.
- Ginzburg, A., L. Behar, D. Givol, and U. Z. Littauer. 1981. The nucleotide sequence of rat α -tubulin: 3'-end characteristics and evolutionary conservation. *Nucleic Acids Research* 9:2691-2697.
- Lemischke, I. R., S. Farmer, V. R. Racancielo, and P. A. Sharp. 1981. Nucleotide sequence and evolution of a mammalian α -tubulin messenger mRNA. *J. Mol. Biol.* 151:101-120.
- Lemischke, I. R., and P. A. Sharp. 1982. The sequences of an expressed rat α -tubulin gene and a pseudogene with an inserted repetitive element. *Nature (Lond.)* 300:330-335.
- Cleveland, D. W., M. A. Lopata, N. J. Cowan, R. J. MacDonald, W. J. Rutter, and M. W. Kirschner. 1980. Number and evolutionary conservation of α - and β -tubulin and cytoplasmic β - and γ -actin genes using specific cloned cDNA probes. *Cell* 20:95-105.
- Cleveland, D. W., S. H. Hughes, E. Stubblefield, M. W. Kirschner, and H. E. Varmus. 1981. Multiple α - and β -tubulin genes represent unlinked and dispersed gene families. *J. Biol. Chem.* 256:3130-3134.
- Lopata, M. A., J. C. Havercroft, L. T. Chow, and D. W. Cleveland. 1983. Four unique genes required for β -tubulin expression in vertebrates. *Cell* 32:713-724.
- Kemphues, K. J., R. A. Raff, T. C. Kaufman, and E. C. Raff. 1979. Mutation in a structural gene for a β -tubulin specific to testis in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 76:3991-3995.
- Kemphues, K. J., E. C. Raff, R. A. Raff, and T. C. Kaufman. 1980. Mutation in a testis-specific β -tubulin in *Drosophila*: analysis of its effects on meiosis and map location of the gene. *Cell* 21:445-451.
- Sanchez, F., J. E. Natzle, D. W. Cleveland, M. W. Kirschner, and B. J. McCarthy. 1980. A dispersed multigene family encoding tubulin in *Drosophila melanogaster*. *Cell* 22:845-854.
- Kalfayan, L., and P. C. Wensink. 1981. α -tubulin genes of *Drosophila*. *Cell* 24:97-106.
- Mischke, D., and M. L. Pardue. 1982. Organization and expression of α -tubulin genes in *Drosophila melanogaster*. One member of the α -tubulin gene family is transcribed in both oogenesis and later embryonic development. *J. Mol. Biol.* 156:449-466.
- Natzle, J., and B. J. McCarthy. 1984. Regulation of *Drosophila* α - and β -tubulin genes during development. *Dev. Biol.* In press.
- Alexandraki, D., and J. V. Ruderman. 1981. Sequence heterogeneity, multiplicity, and genomic organization of α - and β -tubulin genes in sea urchins. *Mol. Cell. Biol.* 1:1125-1137.
- Siflow, C. D., and J. L. Rosenbaum. 1981. Multiple α - and β -tubulin genes in *Chlamydomonas* and regulation of tubulin mRNA levels after deflagellation. *Cell* 24:81-88.
- Brunke, K. J., E. E. Young, B. U. Buchbinder, and D. P. Weeks. 1982. Coordinate regulation of the four tubulin genes of *Chlamydomonas reinhardtii*. *Nucleic Acids Research* 10:1295-1310.
- Burland, T. G., T. Schedle, and W. F. Dove. 1983. Genetic analysis of tubulin genes in *Physarum*. *J. Cell Biol.* 97:216a. Abstr.
- Sherr-Neiss, G., M. H. Lai, and N. R. Morris. 1978. Identification of a gene for β -tubulin in *Aspergillus nidulans*. *Cell* 15:639-648.
- Morris, N. R., M. H. Lai, and C. E. Oakley. 1979. Identification of a gene for α -tubulin in *Aspergillus nidulans*. *Cell* 16:437-442.
- Weatherbee, J. A., and N. R. Morris. 1983. *Aspergillus nidulans* contains multiple tubulin genes. *J. Cell Biol.* 97:217a. Abstr.
- Thomashow, L. S., M. Milhausen, W. J. Rutter, and N. Agabian. 1983. Tubulin genes are tandemly linked and clustered in the genome of *Trypanosoma brucei*. *Cell* 32:35-43.
- Landfear, S. M., D. McMahon-Pratt, and D. F. Wirth. 1983. Tandem arrangement of tubulin genes in the protozoan parasite *Leishmania enrietti*. *Mol. Cell Biol.* 3:1070-1076.
- Neff, N. F., J. H. Thomas, P. Grisati, and D. Botstein. 1983. Isolation of the β -tubulin gene from yeast and demonstration of its essential function *in vivo*. *Cell* 23:211-219.
- Ponstingl, H., E. Krauhs, and M. Little. 1983. Tubulin amino acid sequence and consequences. *J. Submicrosc. Cytol.* 15:359-362.
- Little, M., R. F. Ludueno, G. M. Langford, C. F. Asnes, and K. Farrell. 1981. Comparison of proteolytic cleavage patterns of α -tubulins and β -tubulins from taxonomically distant species. *J. Mol. Biol.* 149:95-107.
- Ludueno, R. F., and M. Little. 1981. Comparative structure and chemistry of tubulins from different eucaryotes. *Biosystems* 14:231-238.
- Little, M., E. Krauhs, and H. Ponstingl. 1981. Tubulin sequence conservation. *Biosystems* 14:239-246.
- Ponstingl, H., E. Krauhs, M. Little, and T. Kempf. 1981. Complete amino acid sequence of α -tubulin from porcine brain. *Proc. Natl. Acad. Sci. USA* 78:2757-2761.
- Krauhs, E., M. Little, T. Kempf, R. Hofer-Warbinek, W. Ade, and H. Ponstingl. 1981. Complete amino acid sequence of β -tubulin from porcine brain. *Proc. Natl. Acad. Sci. USA* 78:4156-4160.
- Valenzuela, P., M. Quiroya, J. Zaldivar, W. J. Rutter, M. W. Kirschner, and D. W. Cleveland. 1981. Nucleotide and corresponding amino acid sequence encoded by α - and β -tubulin mRNAs. *Nature (Lond.)* 289:650-655.
- Cowan, N. J., P. R. Dobner, E. V. Fuchs, and D. W. Cleveland. 1983. Expression of human α -tubulin genes: interspecies conservation of 3' untranslated regions. *Mol. Cell Biol.* 3:1738-1745.
- Hale, J. L., L. Dudley, P. R. Dobner, S. A. Lewis, and N. J. Cowan. 1983. Identification of two human β -tubulin isotypes. *Mol. Cell Biol.* 3:859-862.
- Sullivan, K. F., H. Sternlicht, and D. W. Cleveland. 1983. Nucleotide and corresponding amino acid sequence of a divergent β -tubulin gene in the chicken. *J. Cell Biol.* 97:216a. (Abstr.)
- Alexandraki, D., and J. V. Ruderman. 1983. Evolution of α - and β -tubulin genes as inferred by the nucleotide sequences of sea urchin cDNA clones. *J. Mol. Evol.* 19:397-410.
- Cleveland, D. W. 1983. The tubulins: from DNA to RNA to protein and back again. *Cell* 34:330-332.
- Kalfayan, L., and P. C. Wensink. 1982. Developmental regulation of *Drosophila* α -tubulin genes. *Cell* 29:91-98.
- Baum, H. J., Y. Livneh, and P. C. Wensink. 1983. Homology maps of the *Drosophila* α -tubulin gene family: one of the four genes is different. *Nucleic Acids Research* 11:5569-5587.
- Loyd, J. E., E. C. Raff, and R. A. Raff. 1981. Site and timing of synthesis of tubulin and other proteins during oogenesis in *Drosophila melanogaster*. *Dev. Biol.* 86:272-284.
- Raff, E. C., M. T. Fuller, T. C. Kaufman, K. J. Kemphues, and R. A. Raff. 1982. Regulation of tubulin gene expression during embryogenesis in *Drosophila melano-*

- gaster*. *Cell*. 28:33-40.
46. Duncan, I. W., and T. C. Kaufman. 1975. Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*. Mapping of the proximal right arm. *Genetics*. 80:733-752.
 47. Kaufman, T. C., R. Lewis, and B. Wakimoto. 1980. Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*. The homeotic gene complex in polytene chromosome interval 84B-D. *Genetics*. 94:115-133.
 48. Hazelrigg, T., and T. C. Kaufman. 1983. Revertants of dominant mutations associated with the antennapedia gene complex of *Drosophila melanogaster*: cytology and genetics. *Genetics*. 105:581-600.
 49. Wakimoto, B. T., and T. C. Kaufman. 1981. Analysis of larval segmentation in lethal genotypes associated with the antennapedia gene complex in *Drosophila melanogaster*. *Dev. Biol.* 81:51-64.
 50. Dennell, R. E., K. R. Hummels, B. T. Wakimoto, and T. C. Kaufman. 1981. Developmental studies of lethality associated with the Antennapedia gene complex in *Drosophila*. *Dev. Biol.* 81:43-50.
 51. Scott, M. P., A. J. Weiner, T. I. Hazelrigg, B. A. Poisky, V. Pirrotta, F. Scalenghe, and T. C. Kaufman. 1983. The molecular organization of the *Antennapedia* locus of *Drosophila*. *Cell*. 35:763-776.
 52. Anderson, K. V., and J. A. Lengyel. 1981. Changing rates of DNA and RNA synthesis in *Drosophila* embryos. *Dev. Biol.* 82:127-138.
 53. Ben-Ze'ev, A., S. Farmer, and S. Penman. 1979. Mechanisms of regulating tubulin synthesis in cultured mammalian cells. *Cell*. 17:319-325.
 54. Lefebvre, P. A., C. Silflow, E. Wiehen, and J. Rosenbaum. 1980. Increased levels of mRNAs for tubulin and other flagellar proteins after amputation or shortening of *Chlamydomonas* flagella. *Cell*. 20:469-477.
 55. Minami, S. A., P. S. Collis, E. E. Young, and D. P. Weeks. 1981. Tubulin induction in *Chlamydomonas reinhardtii*: requirement for tubulin mRNA synthesis. *Cell*. 24:89-95.
 56. Cleveland, D. W., M. A. Lopata, P. Sherline, and M. W. Kirschner. 1981. Unpolymerized tubulin modulates the level of tubulin mRNAs. *Cell*. 25:537-546.
 57. Cleveland, D. W., M. F. Pittenger, and J. R. Feramisco. 1983. Elevation of tubulin levels by microinjection suppresses new tubulin synthesis. *Nature (Lond.)*. 305:738-740.
 58. Cleveland, D. W., and J. C. Havercroft. 1983. Is apparent regulatory control of tubulin synthesis nontranscriptionally regulated? *J. Cell Biol.* 97:919-924.
 59. Oakley, B. R., and N. R. Morris. 1980. Nuclear movement is β -tubulin dependent in *Aspergillus nidulans*. *Cell*. 19:255-262.
 60. Oakley, B. R., and N. R. Morris. 1981. A β -tubulin mutation in *Aspergillus nidulans* that blocks microtubule function without blocking assembly. *Cell*. 24:837-845.
 61. Oakley, B. R. 1983. Conditionally lethal tubulin mutants of *Aspergillus nidulans*. *J. Submicrosc. Cytol.* 15:363-366.
 62. Inoué, S. 1981. Cell division and the mitotic spindle. *J. Cell Biol.* 91:131S-147S.
 63. Ling, V., J. E. Aubin, A. Chase, and F. Sarangi. 1979. Mutants of Chinese hamster ovary (CHO) cells with altered colcemid-binding capacity. *Cell*. 18:423-430.
 64. Cabral, F., M. E. Sobal, and M. M. Gottesman. 1980. CHO mutants resistant to colchicine, colcemid, or griseofulvin have an altered β -tubulin. *Cell*. 20:29-36.
 65. Cabral, F., I. Abraham, and M. M. Gottesman. 1981. Isolation of a taxol-resistant Chinese hamster ovary cell mutant that has an alteration in α -tubulin. *Proc. Natl. Acad. Sci. USA*. 78:4388-4391.
 66. Connolly, J. A., V. I. Kalnins, and V. Ling. 1981. Microtubules in colcemid-resistant mutants of CHO cells. *Exp. Cell Res.* 132:147-155.
 67. Keates, R. A. B., F. Sarangi, and V. Ling. 1981. Structure and functional alterations in microtubule proteins from Chinese hamster ovary cell mutants. *Proc. Natl. Acad. Sci. USA*. 78:5638-5642.
 68. Cabral, F., I. Abraham, and M. M. Gottesman. 1982. Revertants of a Chinese hamster ovary cell mutant with an altered β -tubulin: evidence that the altered tubulin confers both colcemid resistance and temperature sensitivity on the cell. *Mol. Cell Biol.* 2:720-729.
 69. Cabral, F. R. 1983. Isolation of Chinese hamster ovary cell mutants requiring the continuous presence of taxol for cell division. *J. Cell Biol.* 97:22-29.
 70. Cabral, F., L. Wible, S. Brenner, and B. R. Brinkley. 1983. Taxol-requiring mutant of Chinese hamster ovary cells with impaired mitotic spindle assembly. *J. Cell Biol.* 97:30-39.
 71. Chalfie, M., and J. N. Thomson. 1979. Organization of the neuronal microtubules in the nematode *Caenorhabditis elegans*. *J. Cell Biol.* 82:278-289.
 72. Chalfie, M., and J. Sulston. 1981. Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* 82:358-370.
 73. Chalfie, M., and J. N. Thomson. 1982. Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. *J. Cell Biol.* 93:15-23.
 74. Larsson, H., M. Wallin, and A. Edstrom. 1976. Induction of a sheet polymer of tubulin by Zn^{2+} . *Exp. Cell Res.* 100:104-110.
 75. Mandelkow, E.-M., P. N. T. Unwin, E. Mandelkow, and C. Cohen. 1977. Tubulin hoops. *Nature (Lond.)*. 265:655-657.
 76. Pierson, G. B., P. R. Burton, and R. H. Himes. 1978. Alterations in the number of protofilaments in microtubules assembled *in vitro*. *J. Cell Biol.* 76:223-228.
 77. Green, L. H., E. C. Raff, and R. A. Raff. 1979. Tubulin from a developing insect embryo undergoing rapid mitosis: factors regulating *in vitro* assembly of tubulin from *Drosophila melanogaster*. *Insect. Biochem.* 9:489-495.
 78. Scheele, R. B., and G. G. Borisy. 1979. *In vitro* assembly of microtubules. In *Microtubules*. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., NY. 175-254.
 79. Amos, L. A. 1979. Structure of microtubules. In *Microtubules*. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., NY. 1-64.
 80. Burton, P. R., and R. E. Hinkley. 1974. Further electron microscopic characterization of axoplasmic microtubules of the ventral cord of the crayfish. *J. Submicrosc. Cytol.* 6:311-326.
 81. Burton, P. R., R. E. Hinkley, and G. B. Pierson. 1975. Tannic acid-stained microtubules with 12, 13, and 15 protofilaments. *J. Cell Biol.* 65:227-233.
 82. Nagano, T., and F. Suzuki. 1975. Microtubules with 15 subunits in cockroach epidermal cells. *J. Cell Biol.* 64:242-245.
 83. Luck, D. J. L. 1984. Genetic and biochemical dissection of the eucaryotic flagellum. *J. Cell Biol.* 98:789-794.
 84. Huang, B., G. Piperno, Z. Ramanis, and D. J. L. Luck. 1981. Radial spokes of *Chlamydomonas* flagella: genetic analysis of assembly and function. *J. Cell Biol.* 88:80-88.
 85. Huang, B., Z. Ramanis, and D. J. L. Luck. 1982. Suppressor mutants in *Chlamydomonas* reveal a regulatory mechanism for flagellar function. *Cell*. 28:115-124.
 86. Brokaw, C. J., D. J. L. Luck, and B. Huang. 1982. Analysis of the movement of *Chlamydomonas* flagella: the function of the radial spoke system is revealed by comparison of wild-type and mutant flagella. *J. Cell Biol.* 92:722-732.
 87. McKeithan, T. W., and J. L. Rosenbaum. 1981. Multiple forms of tubulin in the cytoskeletal and flagellar microtubules of *Polytomella*. *J. Cell Biol.* 91:352-360.
 88. Brunke, K. J., P. S. Collis, and D. P. Weeks. 1982. Post-translational modification of tubulin dependent on organelle assembly. *Nature (Lond.)*. 297:516-518.
 89. McKeithan, T. W., P. A. Lefebvre, C. D. Silflow, and J. L. Rosenbaum. 1983. Multiple forms of tubulin in *polytomella* and *Chlamydomonas*: evidence for a precursor of flagellar α -tubulin. *J. Cell Biol.* 96:1056-1063.
 90. L'Hernault, S. W., and J. L. Rosenbaum. 1983. *Chlamydomonas* α -tubulin is post-translationally modified in flagella during flagellar assembly. *J. Cell Biol.* 97:258-263.
 91. Lindsley, D. L., and K. T. Tokuyasu. 1980. Spermatogenesis. In *The Genetics and Biology of Drosophila*, Vol 2. M. Ashburner and T. R. F. Wright, editors. Academic Press, Inc., NY. 226-294.
 92. Kempthues, K. J., T. C. Kaufman, R. A. Raff, and E. C. Raff. 1982. The testis-specific β -tubulin subunit in *Drosophila melanogaster* has multiple functions in spermatogenesis. *Cell*. 31:655-670.
 93. Kempthues, K. J., E. C. Raff, and T. C. Kaufman. 1983. Genetic and cytogenetic analysis of *B2t*, the gene for a testis-specific tubulin subunit in *Drosophila melanogaster*. *Genetics*. 105:345-356.
 94. Raff, E. C., and K. J. Kempthues. 1983. The expression and function in spermatogenesis of a testis-specific tubulin subunit in *Drosophila melanogaster*. *J. Submicrosc. Cytol.* 15:341-347.
 95. Fuller, M. T., T. C. Kaufman, and E. C. Raff. 1982. Genetic analysis of gene products which may interact with β -tubulin. *J. Cell Biol.* 95:352a Abstr.
 96. Hennig, B. 1975. Change of cytochrome *c* structure during development of the mouse. *Eur. J. Biochem.* 55:167-183.
 97. Beintema, J. J., and J. A. Lenstra. 1982. Evolution of mammalian pancreatic ribonucleases. In *Macromolecular Sequences in Systematic and Evolutionary Biology*. M. Goodman, editor. Plenum Press, NY. 43-74.
 98. Bond, J. F., and S. R. Farmer. 1983. Regulation of tubulin and actin mRNA production in rat brain: expression of a new β -tubulin mRNA with development. *Mol. Cell Biol.* 3:1333-1342.
 99. Murphy, D. B., and K. T. Wallis. 1983. Brain and erythrocyte microtubules from chicken contain different β -tubulin polypeptides. *J. Biol. Chem.* 258:8357-8496.
 100. Ruderman, J. V., and D. Alexandraki. 1983. Organization and expression of the tubulin gene families in the sea urchin. *J. Submicrosc. Cytol.* 15:349-352.
 101. Burland, T. G., K. Gull, T. Schedl, R. S. Boston, and W. T. Dove. 1983. Cell type-dependent expression of tubulins in *Physarum*. *J. Cell Biol.* 97:1852-1859.
 102. Brunke, K. J., B. U. Buchbinder, E. J. Steinberg, J. G. Anthony, and D. P. Weeks. 1982. Sequence homology in the 5' flanking regions of the coordinately regulated tubulin genes from *Chlamydomonas reinhardtii*. *J. Cell Biol.* 95:216a Abstr.
 103. Brunke, K. J., J. G. Anthony, E. J. Steinberg, and D. P. Weeks. 1983. All four coordinately regulated tubulin genes of *Chlamydomonas* contain sequence homologies in 5'-noncoding and flanking regions. *J. Cell Biol.* 97:8a Abstr.
 104. Raff, R. A., and T. C. Kaufman. 1983. Embryos, Genes, and Evolution. MacMillan and Co. NY.
 105. Frazetta, T. H. 1975. Complex Adaptations in Evolving Populations. Sinauer Associates, Inc., Sunderland, MA. p. 20.
 106. Lem, S. 1983. *His Master's Voice*. Translated by M. Kandel. Harcourt, Brace, Jovanovich, NY. p. 23.