Mutations That Encode Partially Functional β2 Tubulin Subunits Have Different Effects on Structurally Different Microtubule Arrays

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Abstract. The testis-specific β_2 tubulin of *Drosophila* is required for assembly and function of at least three architecturally different microtubule arrays (Kemphues et al., 1982). Two recessive male-sterile mutations in the *B2t* locus that encode partially functional, stable, variant forms of β_2 tubulin cause defects in only certain microtubule-based processes during spermatogenesis. These mutations could thus identify aspects of β tubulin primary structure critical for function only in specific microtubule arrays. In males carrying the $B2t^6$ mutation, meiotic chromosome segregation and nuclear shaping are normal and flagellar axonemes are formed, but there is a subtle defect in axoneme structure; the outer doublet microtubules fill in with a central core normally seen only in the central pair and accessory microtubules. In homozygous $B2t^7$ males, chromosome movement is usually normal during meiosis but cytokinesis often fails, cytoplasmic microtubules are assembled and nuclear shaping appears to be normal, but the flagellar axoneme lacks structural integrity. In contrast, the $B2t^8$ allele affects a general property of tubulin, the ability to form normal side-to-side association of protofilaments (Fuller et al., 1987), and causes defects in meiosis, axoneme assembly and nuclear shaping. Certain combinations of these β_2 tubulin mutations show interallelic complementation; in $B2t^6/B2t^8$ males functional sperm are produced and both variant subunits are incorporated into mature sperm, in the absence of wild-type β_2 tubulin. Comparison of the phenotypes of the three partially functional β_2 tubulin alleles reveals some aspects of tubulin primary structure more important for function in specific subsets of microtubule arrays, and other aspects required for the construction of microtubules in general.

MICROTUBULES that mediate different events in eukaryotic cells characteristically are organized into architecturally different arrays. For example, the mitotic spindle is structurally distinct from the flagellar axoneme. The major proteins of microtubules, α and β tubulin, are highly conserved (reviewed in Cleveland and Sullivan, 1985). Much of this conservation undoubtedly reflects general requirements for function in all types of microtubule arrays: the ability to dimerize, form protofilaments, and correctly align protofilaments to form closed microtubules. Other aspects of tubulin primary structure may reflect specific requirements needed for function in only certain kinds of microtubule arrays. Such function-specific regions of sequence may or may not be conserved, depending on the versatility of the particular tubulin gene product. Isolation of mutations that encode partially functional tubulin subunits, coupled with analysis of their effects on microtubule function in vivo, should reveal elements of tubulin primary structure required for function in specific kinds of microtubule arrays, as well as elements important for general microtubule function beyond the formation of the initial dimer. Such mutational analysis, whether following in vivo or in vitro mutagenesis, will ultimately be essential for full understanding of how the function of tubulin in the cell is encoded in the structural design of the tubulin protein.

The *B2t* gene of *Drosophila* encodes a testis-specific form of β tubulin (β_2) that is a major structural component of the meiotic spindle, the sperm tail axoneme and the array of cytoplasmic microtubules involved in shaping the sperm nucleus (Kemphues et al., 1982, 1983; Fuller et al., 1987). The multiple functions of this versatile subunit were first deduced by analysis of recessive male sterile mutations that encode afunctional, unstable variants of β_2 tubulin (designated class I *B2t* alleles; Raff and Fuller, 1984; Fuller, 1986). Testis α tubulin was also unstable in these mutants. In the absence of the normal pools of α and β_2 tubulin, chromosome movement and cytokinesis failed during meiosis, no flagellar axonemes were assembled, and nuclear elongation was defective (Kemphues et al., 1982, 1983).

Because β_2 tubulin functions in several microtubule-based processes, and because it is the major form of β tubulin in the adult testis (Kemphues et al., 1982), the *B2t* gene offers an excellent system for genetic analysis of the relationship between β tubulin structure and the assembly and function

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of morphologically different microtubule arrays in vivo. To identify aspects of β tubulin primary structure required only in certain types of microtubule-based organelles, we have isolated mutations at the *B2t* locus that encode partially functional β_2 tubulin subunits (designated class II alleles; Raff and Fuller, 1984; Fuller, 1986). Identification of this class of mutations was facilitated by the observation that α tubulin is degraded in the testis of males homozygous for a null mutation at the *B2t* locus (Fuller, 1986), indicating that undimerized α tubulin is not stable. Thus, *B2t* mutations in which testis α tubulin is stable encode partially functional β_2 tubulin subunits at least able to form the αβ tubulin heterodimer.

We have characterized in detail three class II *B2t* alleles. One of these, $B2t^8$, causes a defect in tubulin structure that results in a general inability to assemble closed microtubules (Fuller et al., 1987; Rudolf et al., 1987). In this paper we describe two *B2t* mutations that cause defects in only a subset of microtubule based events in the testis and thus may identify aspects of β_2 tubulin primary structure more important for function of some kinds of microtubule arrays than for others. We also present more detailed analysis of the phenotype of the $B2t^8$ mutation and compare the effects of all three partially functional alleles on microtubule based events during spermatogenesis.

Materials and Methods

Fly Stocks, Screen for Mutants, and Recombination Mapping

Flies were raised at 25°C on standard medium. Visible mutations and balancers are described in Lindsley and Grell (1968). Class I *B2t* alleles, including $B2t^3$, and the deletion *Df(3R)B2t*, are described in Kemphues et al. (1982, 1983). The null mutation $B2t^{nu11}$ is described in Fuller (1986). $B2t^6$ was originally named *NNI57* and came from a collection of recessive male sterile mutations on chromosome III kindly provided by Dr. D. Lindsley. $B2t^7$ and $B2t^8$ were isolated in the following screen for new alleles of *B2t*. Homozygous *red e* males treated with 25 mM ethyl methanesulfonate (Lewis and Bacher, 1968) were mated to females heterozygous for the balancer chromosomes. *In(3LR)TM3,* p^p *Sb Ser bx* e^s and *In(3LR) CXD, D.* Female progeny were individually mated to $B2t^3$/*TM3* males, and their $B2t^3$/*red e* sons tested for fertility by mating. If the $B2t^3$/*red e* males were sterile, the new mutation was recovered through their *red e/TM3* siblings. All three mutations were mapped by recombination using the visible markers, *Ki*, p^p, *by* and *cu*. Recombinants were scored for the *β2t* mutation by testing for failure to complement class I *B2t* alleles. Map positions are given with 95% confidence intervals calculated as described in O'Brien and MacIntyre (1978). Data from flies carrying the original $B2t^8$ *red e* or $B2t^7$ *red e* chromosomes are shown in all figures except Tables I and II, and Fig. 7 *d*, where the recombinant line, $B2t^7$ *cu* was used. The $B2t^6$ line used for the electrophoretic mobility analysis shown in Figs. 1 and 8 was marked with *Ki* and carried a second recessive male sterile mutation, which was later removed by recombination between *Ki* $B2t^6$ and a multiply marked third chromosome (*ru h th st e ca*). The resulting lines, *ru h th st* $B2t^6$ *ca* (Figs. 2, 3, and 7) and *ru h th st Ki* $B2t^6$*ca* (Fig. 5), were used in all subsequent analysis. These lines encoded β_2 tubulin with the characteristic altered electrophoretic mobility, which segregated with the *B2t* locus in recombination analysis and was thus not due to the secondary male sterile mutation in the original line. *red e*, *Ore-R*, and *CXD/TM3* behaved genetically as $B2t^+$, encoded a β_2 tubulin subunit that migrated at the same position in 2D gels, and were used for wild type as indicated in the figure legends.

Sample Preparation and PAGE

Testis proteins were isotopically labeled in organ culture as described by Kemphues et al. (1979). Mature sperm were isolated by dissection from the seminal vesicles of male flies held apart from females for 2 to 4 w. Two-dimensional gel electrophoresis was performed as described in Fuller et al. (1987). Gels are presented with the acidic end to the left, and with only the tubulin region shown.

Cytology and Ultrastructure

Squashes of unfixed testes were prepared for phase contrast microscopy as described in Kemphues et al. (1982), except that TB-1 buffer (7 mM K_2HPO_4, 7 mM KH_2PO_4, 80 mM KCl, 16 mM NaCl, 5 mM $MgCl_2$, 1% PEG 6,000, pH 6.7) was used instead of insect Ringers. For observation of meiotic chromosomes and spermatid nuclei, testes were fixed, stained and squashed in 45% acetic acid and lacto-aceto orcein as described by Lifschytz and Hareven (1977). Testes were prepared for electron microscopy as described in Fuller et al. (1987).

Results

Three Mutations in the B2t Structural Gene Encode Stable, Electrophoretic Variant, Partially Functional Subunits of the Testis-specific β tubulin

Three male sterile mutations that fail to complement previously isolated class I β_2 tubulin alleles were mapped to the region containing the *B2t* locus by recombination between the visible markers p^p (48.0) and *by* (48.7). $B2t^6$ mapped to 48.45 ± 0.2, $B2t^7$ mapped to 48.57 ± 0.16, and $B2t^8$ mapped to 48.54 ± 0.16 map units. These map positions agree with the position of 48.49 ± 0.06 for the dominant male sterile allele, $B2t^D$, determined by Kemphues et al. (1980) using the same markers. As shown in Table I, each

Table I. Complementation Tests: Male Fertility in B2t Homozygotes and Heterozygotes

	$B2t^6$	$B2t^7$	$B2t^8$	*ORE R*
$B2t^{nu11}$	S	S	S	F (791 ± 320)
$B2t^6$	S	S*	F (197 ± 34)	F (1,128 ± 74)
$B2t^7$		S	S	F (949 ± 219)
$B2t^8$			S‡	F (310 ± 215)
ORE-R				F (1,084 ± 150)

(S) Male sterile: no progeny from 100 males mated in batches of up to 10 males with an equal number of females per vial.
‡ 96 $B2t^8$*red e* homozygotes, 100 $B2t^8$*cu* homozygotes and 87 $B2t^8$*by cu* homozygous males were tested. All were sterile.
(S*) A few progeny were produced when 100 $B2t^6/B2t^7$ males were tested for fertility in vial tests. No progeny were produced by 30 $B2t^6/B2t^7$ males tested in bottle tests as described below.
(F) Males fertile: (average number of progeny per bottle from 5 males and 15 females.)
All crosses were done at 25°C. All test males were 1-d old or less when mated to 1 to 7-d old ry^{506}/ry^{506} virgin females. Marked females were used to allow easy detection of progeny from accidental nonvirgins.

Although fully fertile males continued to produce large numbers of progeny when given fresh females, poorly fertile males tended to show loss of fertility with age in brooding experiments. Some of this decrease in fecundity was probably caused by obstruction due to the accumulation of debris from degenerating spermatids, and probably did not truly reflect the capacity to produce functional sperm. Therefore mating tests were set up to assay initial fertility, rather than fecundity over the total lifespan. For fertile genotypes, the level of initial fertility was scored by mating in bottles as follows: Five males were mated with fifteen females per bottle on yeasted food. Parents were discarded on the seventh day and progeny were counted up to and including the eighteenth or nineteenth day after mating. Numbers were averaged from five to six bottles. For fully fertile genotypes, the level of progeny produced under these conditions was probably limited by availability of females and of food for larval growth, not by male fertility.

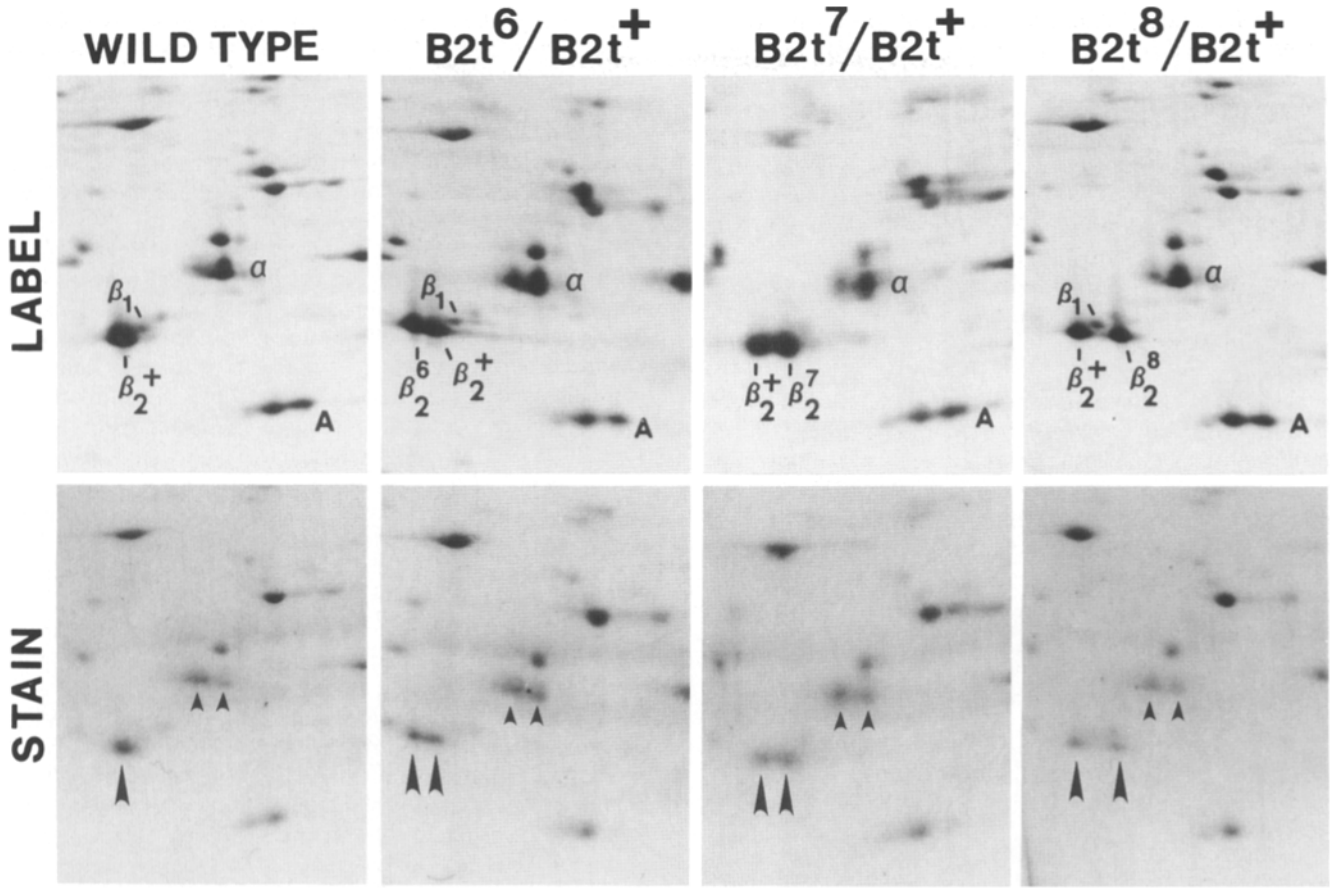

Figure 1. Class II *B2t* alleles encode stable, electrophoretic variant subunits of the testis-specific β tubulin. Portions of two-dimensional gels showing tubulin synthesis patterns and total tubulin pools in testes from class II *B2t*/+ heterozygotes. *Top row*: (*Label*) Autoradiograms showing newly synthesized proteins labeled during incubation for 1 h in [^{35}S]methionine. (α) α tubulins. (β_1) ubiquitous somatic β tubulin. (β_2) Wild-type, testis specific, β_2 tubulin. ($B2^{6,7,8}$) electrophoretic variant β_2 tubulins. (*A*) Actin isoforms. Wild-type β_2 tubulin was distinguished from the variant forms by its position in the gels with respect to β_1 tubulin, α tubulin, and actin. *Bottom row*: (*Stain*) Coomassie Blue–stained pattern of same gels, showing stable testis protein pools. (*Small arrows*) α tubulins; (*large arrows*) β_2 tubulins. Each sample contained four labeled and 10 unlabeled adult testes. Genotypes were *CXD/TM3* for wild type; *Ki B2t^6/CXD*; *B2t^7 red e/TM3*; and *B2t^8 red e/TM3*.

of the three mutations was male sterile in heterozygous combination with *B2t^n*, a null allele of the *B2t* locus. The male sterility associated with *B2t^6* and *B2t^7* was recessive. *B2t^8* showed some semi-dominant effects; although *B2t^8*/+ males produced progeny, their fertility was significantly reduced (Table I).

Each mutation encodes a different electrophoretic variant of the β_2 tubulin subunit (Fig. 1). The *B2t^6* variant is acidic, the *B2t^7* variant is basic, and the *B2t^8* variant is shifted by two charge units toward the basic direction. For all three, roughly equal amounts of variant and wild-type β_2 protein were synthesized in testes from heterozygous males (Fig. 1, *upper panels*). These observations, in combination with the map positions, indicate that the mutations lie in the β_2 tubulin structural gene.

The mutations have biochemical characteristics different from the class I *B2t* alleles, which encode unstable β_2 tubulin subunits (Kemphues et al., 1982, 1983). The variant subunits encoded by the class II mutations are stable and accumulate to roughly the same level as wild type β_2 tubulin in testes from heterozygotes (Fig. 1, *lower panels*). In mutant homozygotes, both the variant β_2 tubulin and α tubulin are synthesized and accumulate in adult testes. Because the testis α tubulins are stable in homozygous mutant males, the class II alleles must encode partially functional β_2 subunits at least capable of forming the αβ tubulin heterodimer.

Partially Functional β Tubulin Mutations have Different Effects on Meiosis

During normal spermatogenesis in *Drosophila melanogaster*, a cyst of 16 primary spermatocytes proceeds through meiosis and differentiation in synchrony. During meiosis, mitochondria line up along the perimeter of the spindle region and are distributed equally to the daughter cells upon cytokinesis (Tates, 1971). Following meiosis II, the mitochondria in each cell coalesce into a single, spherical mitochondrial derivative known as the nebenkern (Tokuyasu, 1975). Thus, the product of normal meiosis is a cyst of 64 early spermatids, each with a single nucleus paired with a mitochondrial derivative, where both the nuclei and the mitochondrial derivatives are uniform in size (reviewed by

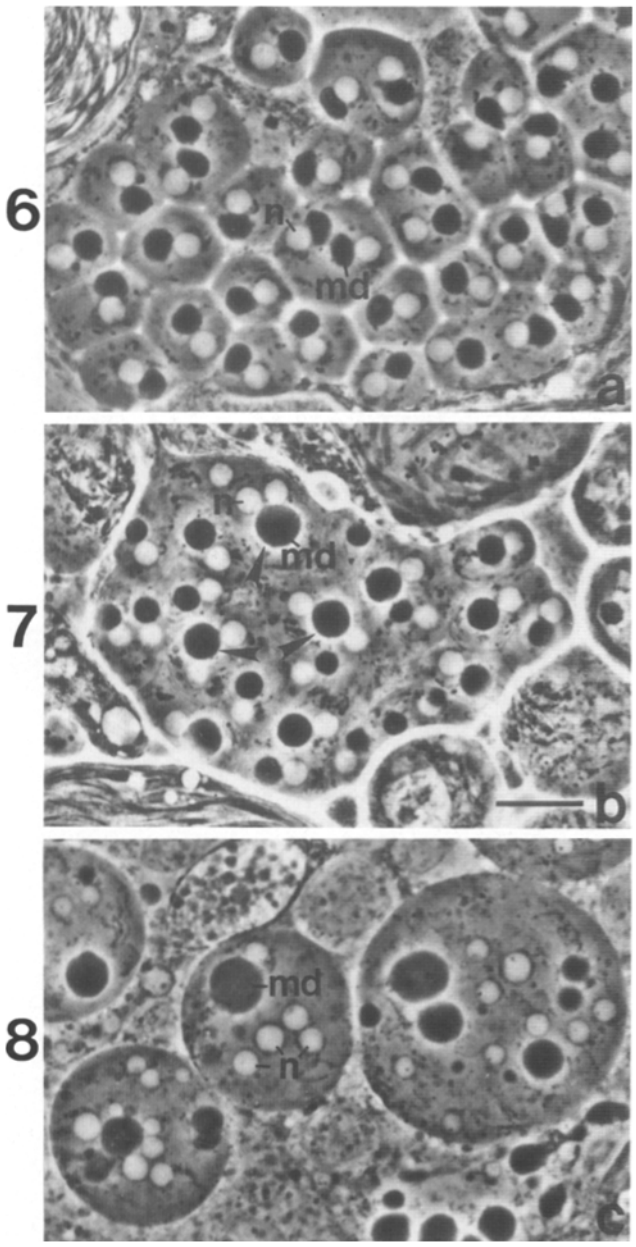

Figure 2. Different effects of *B2t* mutations on meiosis are reflected in the morphology of early spermatids. Onion stage early spermatids in live squashes viewed by phase contrast light microscopy. (*a*) $B2t^6/B2t^6$: Spermatid nuclei uniform in size. Mitochondrial derivatives usually uniform in size and paired with a single nucleus (see text for exceptions). (*b*) $B2t^7/B2t^7$: (*arrows*) Large mitochondrial derivatives associated with two normal-sized nuclei indicate failure of cytokinesis during one of the preceding meiotic divisions. (*c*) $B2t^8/B2t^8$: Abnormal size and distribution of nuceli (*n*) and mitochondrial derivatives (*md*) indicate failure of normal chromosome segregation and cytokinesis during meiosis. Bar, 20 μm.

Lindsley and Tokuyasu, 1980; see Kemphues et al., 1982 and Fuller, 1986 for phase-contrast light micrographs). This is known as the onion stage of spermatogenesis, because of the concentric layers of membrane in the nebenkern in early spermatids.

Abnormalities is meiosis often result in deviations from the regular array of nuclei and mitochondrial derivatives at the early spermatid stage. To assay effects of the partially functional β_2 tubulin mutations on meiosis we examined onion stage early spermatid cysts (Fig. 2) and cells in meiosis in squashed preparations of unfixed testis from homozygous mutant males. In addition, fixed preparations were stained with orcein to examine chromosome behavior during meiosis (Fig. 3).

Chromosome movement during meiosis appeared to be normal in males homozygous for $\beta 2t^6$. Nuclei were consistently equal in size in onion stage spermatid cysts. Usually each nucleus was paired with a single, normal-sized mitochondrial derivative (Fig. 2 *a*). However, in $B2t^6$ homozygotes almost every testis had a few examples of cells containing a large mitochondrial derivative associated with four or sometimes two normal-sized nuclei. As discussed below for $B2t^7$, this defect probably arises from occasional failure of cytokinesis durng meiosis. The noticeable occurrence of large mitochondrial derivatives associated with more than one nucleus in $B2t^6$ homozygotes is probably due to a secondary mutation on the *ru h th st* $B2t^6$ca chromosome, because nuclei and mitochondrial derivatives were almost always normal in males heterozygous for $B2t^6$ and the null mutation, $B2t^n$. However, mitochondrial derivatives associated with two nuclei were occasionally observed in $B2t^6/B2t^n$ males, and seemed more prevalent than in wild type. Alternatively, the large mitochondrial derivatives associated with four or two nuclei observed in homozygotes could result from two copies of the $B2t^6$ mutation. Meiotic chromosomes in homozygous $B2t^6$ males appeared to separate and segregate to the spindle poles normally in both meiosis I and II (Fig. 3 *a–c*). In most cases, cells in meiosis I and II had normal spindle morphology when viewed with phase contrast optics in squashed preparations.

In males homozygous for $B2t^7$, chromosome movement appeared to be normal most of the time, but the results of failure of cytokinesis after meiosis I or II were frequent. Cysts of onion stage early spermatids from $B2t^7$ homozygotes usually contained equal sized nuclei, but in many cases two or sometimes four nuclei were found associated with a single, abnormally large mitochondrial derivative (arrows in Fig. 2 *b*). Mitochondrial derivatives smaller than normal were common. The frequency of these abnormalities varied, but almost every cyst showed at least one defective spermatid, and in some cases as many as fifty percent of the cells in a cyst showed defects. Early spermatid cysts from males heterozygous for $B2t^7$ and the null mutation $B2t^n$ had a similar range of defects in distribution of mitochondrial derivatives, but in addition also occasionally showed nuclei that were slightly smaller or slightly larger than normal. Thus, with respect to its effects on meiosis as deduced from the appearance of onion stage cysts, $B2t^7$ seemed to act as a hypomorph; $B2t^7/B2t^n$ sometimes appeared to affect chromosome segregation, while chromosome segregation appeared to be normal in $B2t^7/B2t^7$. Defects in chromosome segregation were never observed in orcein stained preparations of cells in meiosis from $B2t^7$ homozygotes. However, anaphase II cells similar to those shown in Fig. 3 *d* were common. Although the chromosomes separated and segregated correctly to the poles, in two of the cells shown in Fig. 3 *d* failure of cytokinesis in the previous division resulted in two sets of chromosomes segregating as if on independent spindles in a

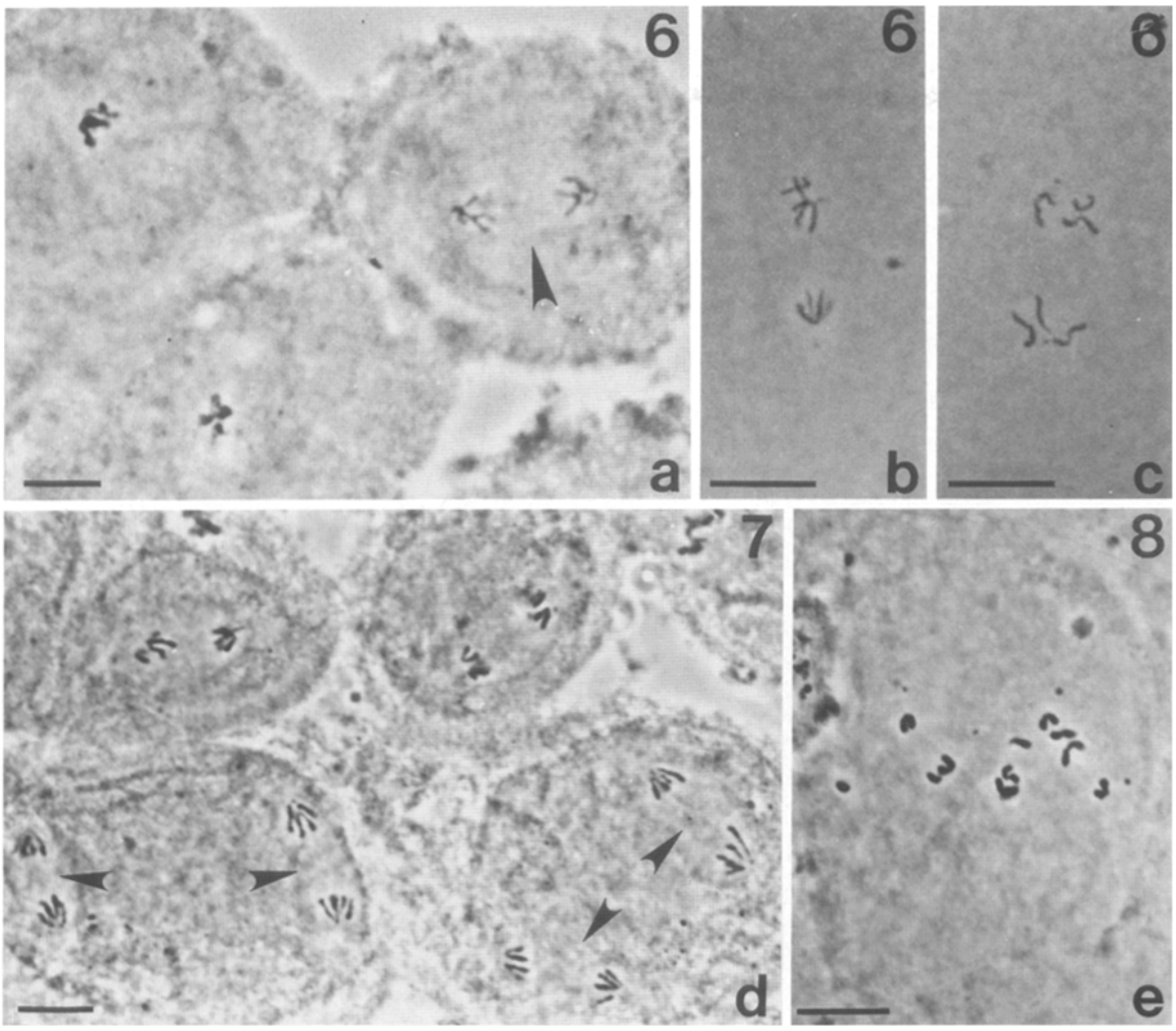

Figure 3. Partially functional *B2t* alleles have different effects on meiotic chromosome behavior. Squashes of fixed, orcein stained testes. (*a–c*) Meiosis II in $B2t^6/B2t^6$ resembles wild-type. (*a*) Two cells in metaphase II at left. (*arrow*) Cell in anaphase II. (*b*) Anaphase II. (*c*) Telophase II. (*d*) Anaphase II in $B2t^7/B2t^7$: Lower two cells each contain two sets of meiotic figures (*arrows*), probably due to failure of cytokinesis at the previous meiotic division. (*d*) $B2t^8/B2t^8$: Single cell in meiosis II, showing almost a complete 4N complement of scattered chromatids. Bars, 10 μm.

single anaphase II cell (*arrows*). Although cytokinesis is normally incomplete during meiosis in *Drosophila melanogaster* males, it usually results in clearly distinguishable daughter cells, as shown in the upper two cells in Fig. 3 *d*. Cells in meiosis from $B2t^7/B2t^7$ males usually had normal morphology, although abnormally shaped spindles were occasionally observed by phase contrast optics in squashed preparations.

Both chromosome segregation and cytokinesis were abnormal during meiosis in $B2t^8$ homozygous males. Early spermatids from $B2t^8$ homozygotes characteristically had multiple nuclei of a variety of sizes, often associated with large mitochondrial derivatives (Fig. 2 *c*). The variation in nuclear size indicates that defects in chromosome segregation during meiosis caused onion-stage nuclei to receive unequal amounts of chromatin (Hardy, 1975; Gonzales et al., 1988). $B2t^8$ in combination with a deletion of the *B2t* locus, a class I *B2t* mutation, or the null allele $B2t^n$ all showed early spermatid defects similar to those observed in $B2t^8$ homozygotes. In unfixed preparations from $B2t^8/B2t^8$ testes, meiotic spindles were rarely observed, either because they were so disorganized as to be unrecognizable, or because they were abnormally fragile and consequently were easily disrupted during preparation. However, meiotic spindles were observed in $B2t^8$ homozygotes by light microscopy of thick sections from fixed and embedded testis, as well as by electron microscopy (Fuller et al., 1987). In orcein-stained preparations of testes from $B2t^8$ homozygotes, prometaphase tetrads looked normal at meiosis I but a normal metaphase I plate was never observed. Some separation of chromosomes during meiosis does occur in $B2t^8$. Separation of homologues in meiosis I and of sister chromatids during meiosis II generally took place, but during both meiosis I and II the chromosomes failed to move to the poles and remained scattered. Almost the full 4N complement of separated sister chromatids can be seen in the cell in meiosis II shown in Fig. 3 *e*. These scattered chromatids appear to be incorporated into nuclei as aneuploid sets at the completion of meiosis,

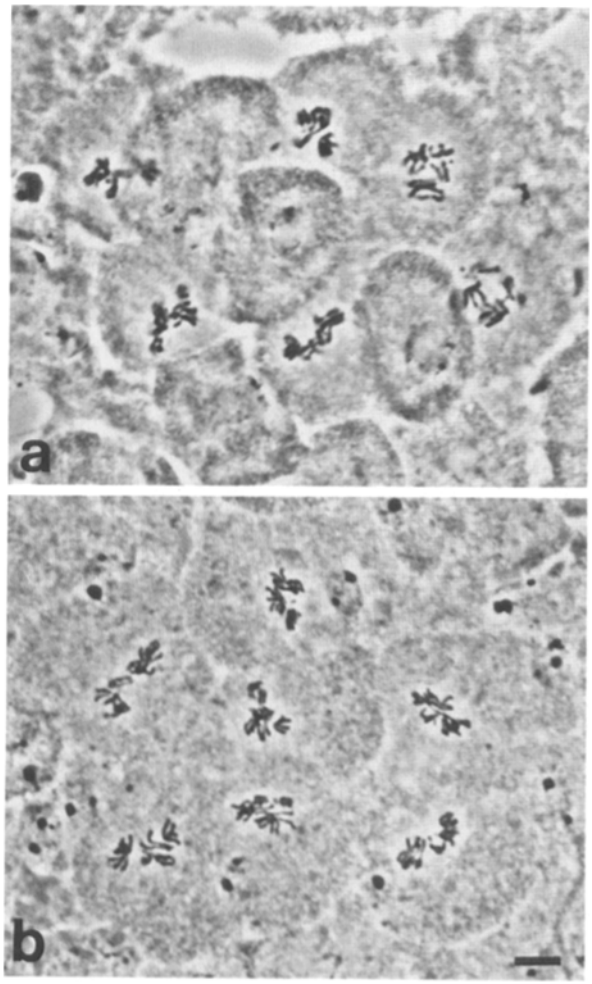

Figure 4. Normal mitosis in $B2t^8$ homozygotes. Orcein-stained preparations of spermatocyte cysts in the mitotic division before meiosis. (*a*) Wild type (*red e/red e*): only six of the eight cells are in focus. (*b*) $B2t^8$ homozygote: The eighth cell was present in the preparation, but not in this photographic field. Bar, 10 μm.

giving rise to the unequal sized nuclei characteristic of onion stage spermatids in this mutant (Fig. 2 *c*). We had previously shown in fixed, sectioned material that onion stage spermatid cells from $B2t^8$ homozygotes were the same size at the completion of meiosis as premeiotic primary spermatocytes (Fuller et al., 1987), suggesting that cytokinesis fails to occur after both meiosis I and II. Failure of cytokinesis, combined with failure of the separated chromosomes to migrate correctly to the poles, can explain the observation that early spermatid cells from $B2t^8$ homozygotes often have more than four nuclei.

Mitotic Spindle Function is Normal in β_2-tubulin Mutants

Despite the defects in chromosome movement and cytokinesis during meiosis in $B2t^8$ males, the preceding mitotic divisions appear to be normal. Fig. 4 shows a cyst of cells in the final mitotic division from a wild-type male (Fig. 4 *a*) and from a $B2t^8$ homozygote (Fig. 4 *b*). Mitotic spindle function appears to be normal for all *B2t* alleles examined. In the class I *B2t* mutations and the three class II alleles described here, pre-meiotic cysts of primary spermatocytes had nuclei regular in size. It is likely that *B2t* mutations have no effect on the mitotic divisions because synthesis of β_2 tubulin probably does not begin until after the last mitotic division prior to the onset of meiosis (Kemphues et al., 1982).

Partially Functional B2t Alleles Cause Different Defects in Axoneme Structure

The newly formed axoneme of the *Drosophila* sperm tail flagellum consists of nine outer doublets surrounding two central pair microtubules. As the axoneme matures, an accessory tubule grows out of the B subfiber of each outer doublet and the resulting complex becomes decorated with associated structures. Finally, a densely staining core appears in the lumen of the central pair and accessory tubules (Kiefer, 1970), but not in the outer doublets (Fig. 5 *a*).

Although growing axonemes from $B2t^6$ homozygotes look morphologically normal, mature axonemes exhibit a characteristic structural defect; the core of densely staining material appears in the A subfibers of the outer doublet microtubules (*arrow* in Fig. 5 *b*) as well as in the central pair and accessory tubules. On rare occasions, filled outer doublet B tubules were also observed. The spermatid shown in Fig. 5 *b* did not undergo individualization and has begun to show signs of degeneration. However, cysts of individualized or partially individualized sperm were commonly observed. Axonemes from males heterozygous for $B2t^6$ and a class I allele or a deletion of the *B2t* locus also showed the filled outer doublets characteristic of $B2t^6$ homozygotes. The cytoplasmic microtubules near the mitochondrial derivatives in developing spermatids appeared to be normal in $B2t^6$ males.

In $B2t^7$ homozygotes, cross sections of spermatid cysts contained many fewer axonemes than normal, and virtually all axonemes lacked one or both central pair microtubules (Fig. 5, *c* and *d* and Table II). Broken axonemes (Fig. 5 *e*) or axoneme fragments were common. In late stage $B2t^7$ axonemes or fragments, accessory tubules filled in with the dense central material, but filled outer doublet microtubules like those in $B2t^6$ were never observed. Cytoplasmic microtubules near the mitochondrial derivatives were present and appeared to be normal in $B2t^7$ (Fig. 5, *c–e*).

As previously described, $B2t^8$ homozygotes had clusters of abnormal microtubules in place of axonemes (Fig. 5 *f*). In cross-section, the most typical aberrant structures were S-shaped microtubules. Other structures included closed single microtubules (*arrows* in Fig. 5 *f*) and S-shaped microtubules with hooked projections. In late spermatids many of the aberrant structures appeared to become filled with the dense core material characteristic of central pair and accessory microtubules in wild type.

Some elongation of spermatid cysts occurred in $B2t^8$ homozygotes, although the extent of elongation was very poor. Flagellar elongation was better in $B2t^7$ homozygotes, although it was never as good as in wild type. As discussed below, intact axonemes were on average longer in $B2t^7$ males than in $B2t^8$. The greater extent of flagellar elongation in $B2t^7$ than in $B2t^8$ could be due to longer flagellar axonemes, the presence of normal cytoplasmic microtubules running lengthwise along the flagella in $B2t^7$ males (Fig. 5, *c–e*) or both.

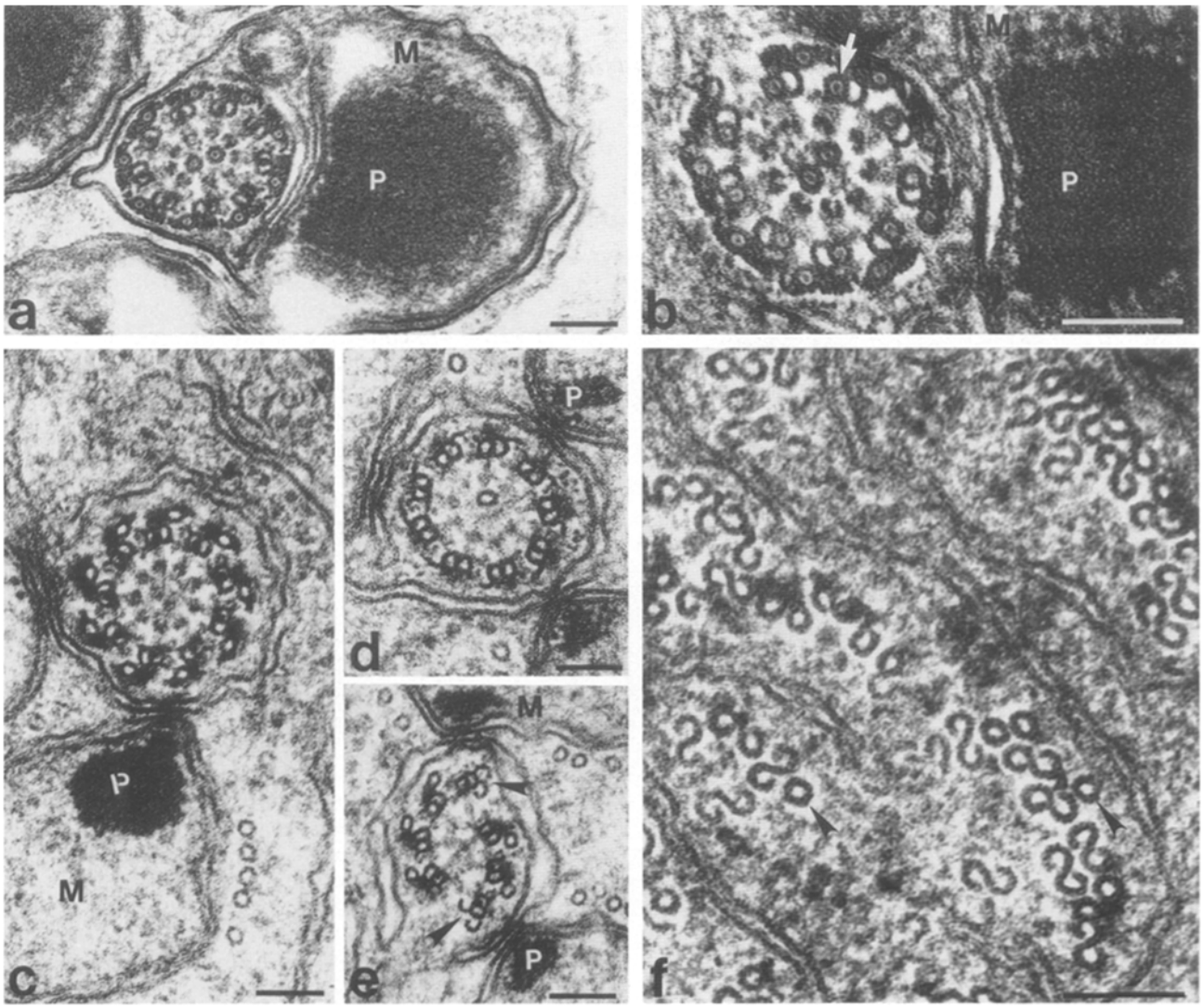

Figure 5. Partially functional β_2 tubulin mutations cause different defects in axoneme morphology. (*a*) Wild type: cross section of mature, individualized sperm. The lumen of the central pair and accessory tubules is filled with darkly staining material, but the outer doublets are not filled. (*b*) *B2t*^{6} homozygote: Late stage axoneme showing outer doublet microtubules with lumen of A subfiber filled with darkly staining material (*arrow*). Separation of adjacent accessory microtubule complexes and loss of dynein arms are typical of the beginning of degeneration (Kiefer, 1970). (*c*) *B2t*^{7} homozygote: developing axoneme lacking central pair microtubules. (*d*) *B2t*^{7} homozygote: Young axoneme containing one central tubule. (*e*) *B2t*^{7} homozygote: Fragmented young axoneme containing seven doublets. (*arrows*) Doublet microtubules with abnormal accessory tubule projections. (*f*) *B2t*^{8} homozygote: abnormal microtubules in axoneme region. (*arrows*) Closed singlet microtubules. (*M*) Mitochondrial derivative; (*P*) paracrystaline material. Bars, 0.1 μm.

Organized Rings of Outer Doublet Microtubules are Assembled Adjacent to the Basal Body, Even in Males Homozygous for Axoneme-defective B2t Alleles

The low number of intact axonemes in cross sections of spermatid cysts from *B2t*^{7} homozygotes (Table II) could result from at least two scenarios. Only a few basal bodies might successfully initiate axoneme assembly, but once initiated axonemes may attain nearly normal length. Because the spatial relationship between the nebenkern and nucleus in early spermatids probably depends on the association between the mitochondrial derivative and the axoneme that grows from the basal body embedded in each nucleus, the relatively regular nucleus-nebenkern association in *B2t*^{7} onion stage cysts (Fig. 2 *b*) argues against this possibility. An alternative scenario would be that all 64 basal bodies initiate assembly of axonemes, but the axonemes are abnormally short. In this case, if the spermatid nuclei with their associated basal bodies were scattered along the length of the cyst (see below), a given cross section would have only a few intact axonemes. To determine if axonemes are abnormally short in *B2t*^{7} homozygotes, we followed axonemes from four different *B2t*^{7} spermatid cysts in the thin sections from two series consisting of alternating 10-μm thick and 1-μm thin cross sections covering 70–80 μm of length per series. Axoneme starts were scattered in the longitudinal dimension of the spermatid cysts. The axonemes were short and tended to fray apart into fragments at their distal ends. Thus the broken axonemes and fragments common in *B2t*^{7} homozygotes (Fig. 5 *e*) probably arise from more proximal intact axonemes. In no case were central pair microtubules observed, even close to the basal

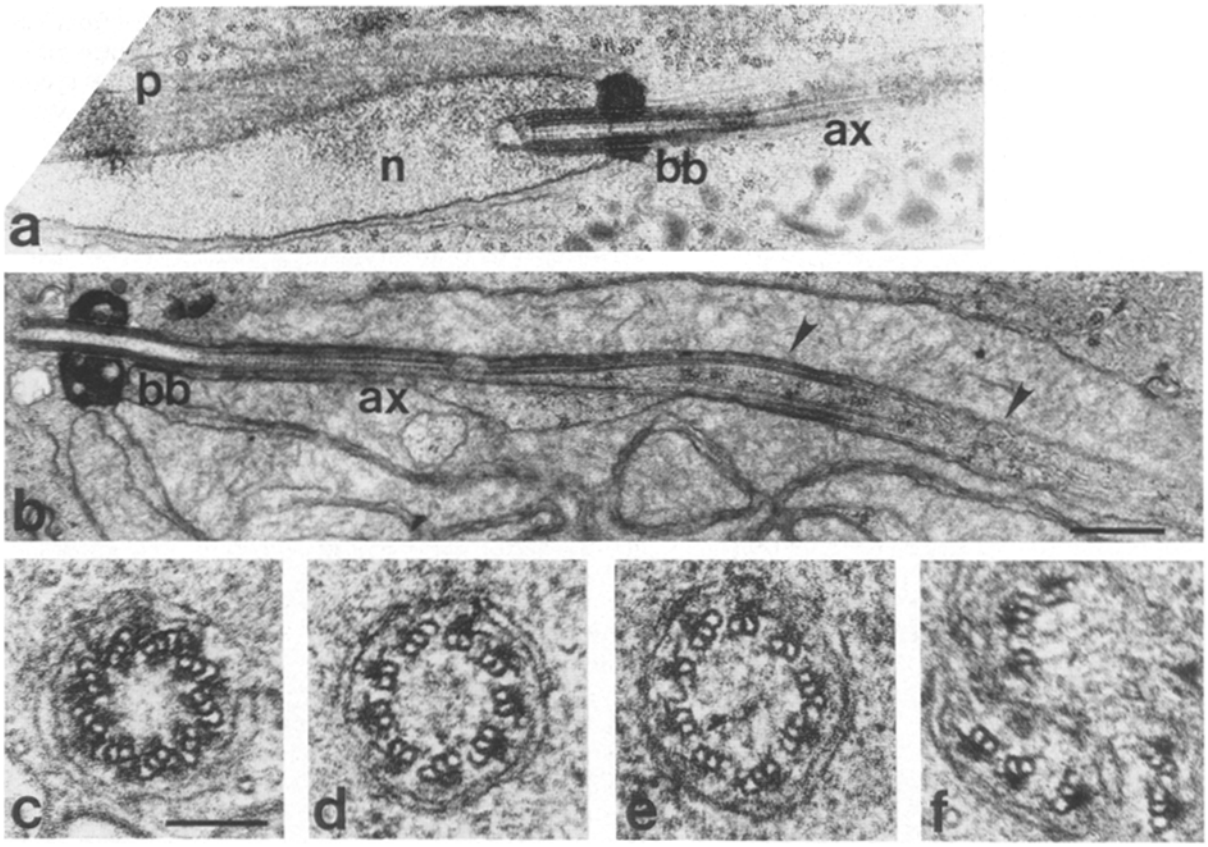

Figure 6. Short axonemes adjacent to the basal body in $B2t^8$ homozygotes. Basal bodies with adjacent axonemes from developing spermatids. (*a*) Longitudinal section of wild-type (*red e/red e*). (*b*) Longitudinal section of $B2t^8/B2t^8$: (*arrows*) Axoneme disintegration by 3–4 μm from the basal body. (*c–f*) Representative serial cross-sections from a $B2t^8$ homozygote. Sequential sets of 20 serial sections were cut, covering 2 μm per set. A single section from each 2 μm set was examined. (*c*) Basal body; (*d*) axoneme 0–2 μm from *c*; (*e*) axoneme 4–6 μm from *c*; (*f*) fragmented axoneme 6–8 μm from *c*. (*ax*) axoneme; (*bb*) basal body; (*n*) nucleus; (*p*) perinuclear cytoplasmic microtubules. Bars: (*a* and *b*) 0.5 μm; (*c–f*) 0.1 μm.

*Table II. Average Number of Axoneme-like Structures per Spermatid Cyst Cross section in $B2t^7$**

Genotype‡	Whole axonemes§	Partial axonemes‖	Number of axonemes per cyst separated according to number of central pair microtubules¶			Abnormal**	No. of cysts	No. of males
			0	1	2			
7/7:								
$B2t^7$ red e/$B2t^7$ red e	18 ± 14	19 ± 12	15 ± 11 (83%)	0.5 ± 0.5 (3%)	0	3	18	4
$B2t^7$cu/$B2t^7$ cu	4 ± 5	14 ± 8	2 ± 3 (50%)	0	0	2	5	4
7/8:								
$B2t^7$ red e/$B2t^8$	46 ± 9	5 ± 7	12 ± 6 (26%)	8 ± 5 (17%)	15 ± 8 (32%)	7	9	6
$B2t^7$cu/$B2t^8$	49 ± 10	6 ± 10	22 ± 4 (44%)	14 ± 5 (28%)	7 ± 2 (14%)	10	6	3
7/+:								
$B2t^7$ red e/red e	63 ± 1	1 ± 2	3 ± 2 (4%)	6 ± 5 (10%)	54 ± 6 (86%)	2	6	3
$B2t^7$cu/red e	61 ± 3	0	5 ± 3 (8%)	6 ± 5 (10%)	48 ± 8 (78%)	1	6	2
Wildtype:‡‡								
Ore-R	62.6 ± 0.4		0	0	(100%)	0	7	3
red e	63.5 ± 0.2		0	0	(100%)	0	6	3
CxD/TM3	64.0 ± 0.0		0	0	(100%)	0	2	1

* Cross sections including an entire spermatid cyst were scored for number of axoneme-like structures per cyst. Results expressed as average numbers per cyst, with SDs.
‡ Data are given for both the original *$B2t^7$red e* isolate and a recombinant line marked with *cu*.
§ Whole axonemes were defined as any structure with a complete ring of outer doublets.
‖ Partial axonemes consisted of broken rings of outer doublets or clusters of axoneme fragments.
¶ Percentages given in parentheses express the numbers as percent of whole axonemes in *.
** Abnormally shaped microtubules or structures with indistinguishable morphology in the central pair position.
‡‡ Defects in spermatid organization, such as missing or morphologically abnormal mitochondrial derivatives were occasionally seen in wild type. However, defects in axoneme morphology were only rarely noticed. Lack of central pair microtubules was not observed in our wild-type samples.

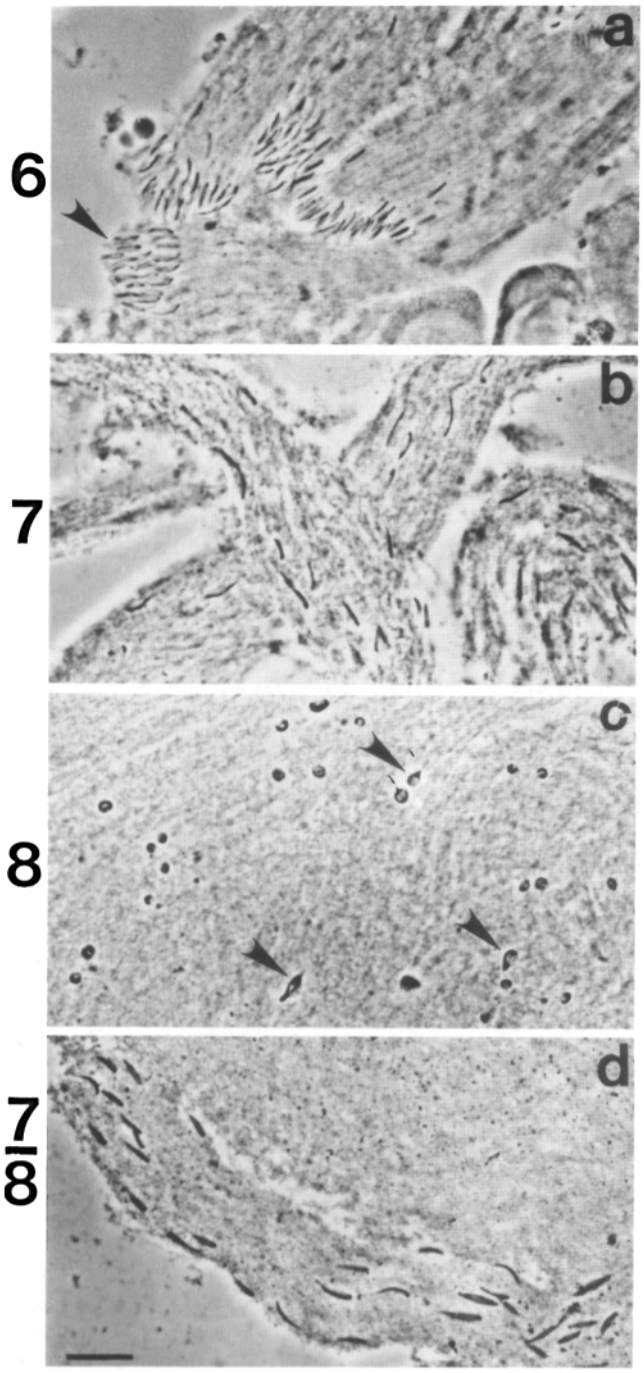

Figure 7. Partially functional *B2t* alleles have different effects on nuclear shape and alignment. Condensed chromatin in mature nuclei in elongated spermatid bundles stained with aceto-orcein. (*a*) Elongated spermatid bundles in $B2t^6$ homozygote: nuclear shaping and head alignment resemble wild type. (*arrow*) Less mature bundle containing nuclei not yet fully elongated. (*b*) $B2t^7$ homozygote: nuclei shaped but heads scattered along cyst. (*c*) $B2t^8$ homozygote: nuclear shaping and head alignment both fail to occur, although a few nuclei undergo aberrant shaping (*arrows*). (*d*) $B2t^7/B2t^8$: Nuclear shaping normal but head alignment poor. Bar, 20 μm.

body. Seventeen intact 9 + 0 axonemes and one 8 + 0 axoneme from two of the cysts initiated within the set of serial cross sections. Except for the lack of central pair microtubules, the axonemes appeared to be morphologically normal near the site of initiation. These axonemes ranged from less than 10 to more than 70-μm long, with an average intact length of only 25 μm. In contrast, mature wild-type sperm tail axonemes exceed 1.5 mm in length (Lindsley and Tokuyasu, 1980).

Serial cross-section analysis also revealed that even in males homozygous for $B2t^8$, 9 + 0 axonemes with an intact ring of outer doublet microtubules are initiated at the basal body. However, as in $B2t^7$ males, the axonemes fray apart, this time within a few microns of the basal body (Fig. 6). In longitudinal sections through five different basal bodies from homozygous $B2t^8$ males, the basal body gave rise to an axoneme in each case. In the example shown in Fig. 6 *b*, the axoneme had relatively normal morphology, including cross-striations, for the first 2 μm of its length, but began to splay apart by 3–4 μm away from the basal body (*arrows* in Fig. 6 *b*). Fig. 5, *c–f* shows representatives from a set of serial 1 μm cross-sections beginning in a basal body and extending into its attached axoneme. The normal morphology of the basal body is not surprising, since basal bodies are assembled prior to meiosis (Tates, 1971) and thus might be composed of the β_1 form of tubulin. The ring of axonemal outer doublet microtubules is well organized near the basal body (Fig. 6 *d*), but becomes increasingly abnormal as it extends away and is clearly fragmented at 6–8 μm from the basal body (Fig. 6 *f*). In each of the six series of cross-sections of different axonemes from $B2t^8$ homozygotes examined, axonemes were well organized near the basal body but had frayed apart within 2–8 μm. In all cases, the axonemes from $B2t^8$ homozygotes lacked central pair microtubules. It is possible that the rapid disintegration of $Bt2^8$ axonemes with distance from the basal body may interfere with the association of the axoneme and the mitochondrial derivatives, thus contributing to the disruption of nucleus-nebenkern association sometimes observed in onion stage cysts from $B2t^8$ (Fig. 2 *c*). However, since $B2t^8$ onion stage spermatids cells and nebenkerns are abnormally large (Fuller et al., 1987). The disrupted spatial arrangement of cellular components in unfixed squashed preparations must be interpreted with caution.

The better organization of rings of axonemal outer doublet microtubules near the basal body in both $B2t^7$ and $B2t^8$ homozygotes could be due to physical constraints imposed by the basal body, which might exert a template effect on the organization of the ring of axonemal outer doublet microtubules that grows from it. Alternatively, the first few microns of axonemes in each sperm tail could be assembled from β_1 rather than β_2 tubulin. A small amount of β_1 tubulin was detected in mature sperm by staining gel transfers with a β_1 specific monoclonal antibody (Raff, E. C., unpublished experiments).

The Partially Functional β2 Tubulin Mutations Have Different Effects On Nuclear Shaping and Alignment

During spermatogenesis in wild type, round spermatid nuclei undergo a dramatic shape change to form the needle-like, condensed nuclei of mature sperm. A concave groove containing a region of dense cytoplasm and a longitudinal bundle of microtubules (Fig. 6 *a*) forms along one side of the nucleus, which then elongates along the microtubules (Tokuyasu, 1974). Nuclear shaping occurs in $B2t^6$ (Fig. 7 *a*) and $B2t^7$ (Fig. 7 *b*) homozygotes. However, in $B2t^8$ homozygotes,

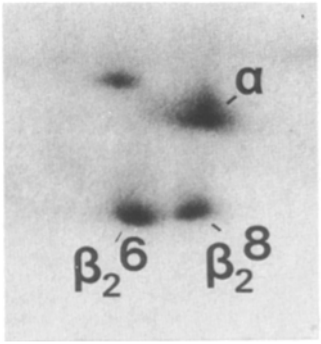

Figure 8. Variant β_2-tubulin subunits are incorporated into sperm in *B2t*^{6}/*B2t*^{8} heterozygotes. Tubulin region of Coomassie Blue-stained two-dimensional gel showing proteins present in mature sperm dissected from seminal vesicles of 20 fertile *B2t*^{6}/*B2t*^{8} males. (β_2^6) *B2t*^{6} product; (β_2^8) *B2t*^{8} product; (α) α tubulin.

in which the perinuclear microtubules have abnormal structure (Fuller et al., 1987), most nuclei remain round (Fig. 7 *c*) although chromatin condenses at the perimeter. The nuclei vary in size due to the defects in meiosis typical of *B2t*^{8} homozygotes.

Normally, as a cyst of developing spermatids elongates, all the nuclei remain in a cluster at the basal end of the bundle. Nuclear alignment is largely normal in *B2t*^{6} homozygotes (Fig. 7 *a*), with only a few sperm heads out of alignment in each bundle. Head alignment is defective in both *B2t*^{7} (Fig. 7 *b*) and *B2t*^{8} (Fig. 7 *c*) homozygotes. Defects in nuclear alignment may be secondary consequences of aberrant spermatid development in these mutants and do not necessarily indicate that β_2 tubulin has a direct role in head alignment. In fertile *B2t*^{6}/+, *B2t*^{7}/+ or *B2t*^{8}/+ heterozygotes, nuclear shaping appears to proceed normally, and head alignment is very good, with only a few nuclei found outside of the terminal cluster.

Partial Intragenic Complementation Among Class II B2t Alleles

The defects in axoneme assembly characteristic of each of the class II *B2t* mutations are partially corrected in males heterozygous for two different class II alleles. In testes from *B2t*^{6}/*B2t*^{7} flies, the number of whole axonemes per cyst in a cross-section was increased dramatically compared with *B2t*^{7} homozygotes. The morphology of many axonemes in *B2t*^{6}/*B2t*^{7} males was normal, although a significant fraction still lacked central pair microtubules. The filled in outer doublets characteristic of *B2t*^{6} were never observed. *B2t*^{6}/*B2t*^{7} males occasionally produced a few progeny (Table I).

Complementation between *B2t*^{7} and *B2t*^{8} was less complete. Although the number of whole axonemes with complete outer doublet rings per cyst cross-section in *B2t*^{7}/*B2t*^{8} heterozygotes was below the expected 64 (Table II), it was significantly more than in either *B2t*^{7} or *B2t*^{8} homozygotes. As discussed above, the low number of whole axonemes per cyst cross section in *B2t*^{7} is probably due to a combination of abnormally short axonemes and the scattering of nuclei along the spermatid bundle. Late stage nuclei remained scattered along the cyst in *B2t*^{7}/*B2t*^{8} males as well (Fig. 7 *d*). Thus, the increased number of intact axonemes per cyst cross section in *B2t*^{7}/*B2t*^{8} males could indicate that axonemes are longer than in *B2t*^{7} or *B2t*^{8} homozygotes. Although many axonemes lacked one or both central pair microtubules in *B2t*^{7}/*B2t*^{8}, a significant percentage had both central pair microtubules present (Table II). S-shaped microtubules characteristic of *B2t*^{8} homozygotes were often observed mixed with normal singlet microtubules in the spermatid cytoplasm or in the central pair position of *B2t*^{7}/*B2t*^{8} axonemes. Onion stage early spermatid cysts from *B2t*^{7}/*B2t*^{8} males resembled those from *B2t*^{7} homozygotes, indicating that the problems in chromosome separation characteristic of *B2t*^{8} were largely alleviated. The defects in nuclear shaping characteristic of *B2t*^{8} homozygotes were also corrected in *B2t*^{7}/*B2t*^{8}, although head alignment did not occur (Fig. 7 *d*). Progeny from *B2t*^{7}/*B2t*^{8} males were never observed (Table I).

Complementation between *B2t*^{7} and *B2t*^{8} may be limited because both mutations have some semi-dominant effects at the ultrastructural level. Abnormal, S-shaped microtubules were often observed in spermatid cytoplasm in *B2t*^{8}/+ heterozygotes (Fuller et al., 1987), although never in the central pair position as seen in *B2t*^{7}/*B2t*^{8}. In *B2t*^{7}/+ males, some whole axonemes lack one or both central pair tubules (Table II). Both *B2t*^{7}/+ and *B2t*^{8} flies are fertile, but the semi-dominant effect of *B2t*^{8} is noticeable in the reduced number of progeny produced by *B2t*^{8}/+ males (Table I). The defect in axoneme structure caused by the *B2t*^{6} mutation appears to have no semi-dominant effects and is fully complemented by *B2t*^{7}, *B2t*^{8} and wild type.

B2t^6 and *B2t*^{8} show strong interallelic complementation. *B2t*^{6}/*B2t*^{8} males assemble morphologically normal flagellar axonemes, produce mature, individualized, motile sperm and are fertile when mated (Table I). Although the fertility of *B2t*^{6}/*B2t*^{8} males is reduced compared with wild-type, it is not much lower than that of *B2t*^{8}/+. Axonemes from *B2t*^{6}/*B2t*^{8} do not have the filled in outer doublets characteristic of *B2t*^{6} homozygotes. Mature sperm from *B2t*^{6}/*B2t*^{8} males contain substantial amounts of each of the two variant β_2 tubulin subunits and have no wild-type β_2 tubulin (Figure 8). Thus, although the *B2t*^{8} mutant subunit forms aberrant microtubules in homozygous males, it appears to be able to participate in assembly of morphologically normal axonemes in combination with wild type (Fuller et al., 1987) or certain variant forms of β_2 tubulin. Mature sperm from *B2t*^{6}/+ or *B2t*^{7}/+ heterozygotes also contain both mutant and wildtype β_2 subunits in roughly equal amounts.

Discussion

This and the previous study (Fuller et al., 1987; Rudolph et al., 1987) demonstrate the feasibility of using genetic analysis in *Drosophila* to investigate how the function of microtubules in vivo is directed by the primary structure of tubulin subunits. The *B2t* gene of *Drosophila* offers an excellent system for genetic analysis of the relationship between tubulin structure and function in architecturally different microtubule arrays, through ultrastructural and cytological analysis of the effects of mutations induced in vivo on the structure and function of the several kinds of microtubule arrays used during spermatogenesis. The class II β_2 tubulin mutations encode partially functional subunits at least able to participate in the $\alpha\beta$ tubulin heterodimer. Thus this category excludes those alleles that cause extreme defects in the β_2 tubulin subunit, and is preselected for mutations likely to affect assembly or function of microtubules, as opposed to protein folding or dimer formation.

The class II mutation *B2t*^{8} disrupts a general property of β_2 tubulin, the ability to form closed microtubules, and

Table III. Characteristic Phenotypes of β_2 Tubulin Mutants

Allele	Meiosis	Onion stage	Axoneme morphology	Nuclear shaping
Class I*				
	No chromosome movement No cytokinesis	4N Nucleus Large cells Large mitochondrial derivatives and fragments	No axonemes	No shaping
Class II				
B2t^6	Chromosome movement normal. Occasional failure of cytokinesis.	Nuclei regular in size. Most cells normal. A few large mitochondrial derivatives with two or four nuclei.	Outer doublets filled with dense central material.	Elongation
B2t^7	Chromosome movement normal. Cytokinesis often fails.	Nuclei usually regular in size. Many cells normal. Large mitochondrial derivatives with two or four nuclei common. Fragments of mitochondrial derivatives common.	Axonemes short and fray apart. Lack of central pair tubules.	Elongation
B2t^8	Homologous chromosomes and sister chromatids separate, but remain scattered across the spindle. No cytokinesis.	Multiple nuclei vary in size. Large cells. Large mitochondrial derivatives and fragments.	Abnormal microtubules, many S-shaped. Axonemes fall apart within a few microns of the basal body.	No shaping

* The class I *B2t* mutations and are described in detail in Kemphues et al. (1982, 1983). The phenotype of the null allele, *B2t*^{n}, is similar to the class I alleles.

causes defects in all known β_2 tubulin functions—meiosis, axoneme assembly and nuclear elongation (Table III). The lesion in this mutation results in substitution of lysine for the highly conserved glutamic acid at amino acid residue 288 (Rudolph et al., 1987). When tubulin dimers are treated by limited proteolysis with chymotrypsin, β tubulin is cleaved at *tyr* 281 and also near the CH_2 terminal end. If the dimers are then exposed to assembly conditions in vitro, they form S-shaped and hooked microtubules similar to those seen in *B2t*^{8} in vivo (Mandelkow et al., 1985; Kirchner and Mandelkow, 1985). Together, these results indicate that the region near *tyr* 281 and *glu* 288 could be important for normal side-to-side interactions between protofilaments (Fuller et al., 1987).

Some β_2 Tubulin Mutations Preferentially Affect Function in Specific Microtubule Arrays

The *B2t*^{6} mutation causes a subtle defect in a specific subset of microtubules (Table III). Males homozygous for *B2t*^{6} have normal chromosome movement during meiosis, assemble morphologically normal cytoplasmic microtubules, have normal nuclear shaping, and assemble the characteristic nine-plus-two flagellar axoneme. However, the *B2t*^{6} mutation appears to alter the inner surface of flagellar outer doublet microtubules so that they become decorated with a dense central core usually found only in the central pair and accessory microtubules. Thus *B2t*^{6} could identify an aspect of β_2 tubulin primary structure important for differentiation between doublet and singlet microtubules.

Some of the defects observed in *B2t*^{7} males might be accounted for by a decrease in the structural integrity or stability of organized microtubule arrays. For example, *B2t*^{7} could identify an aspect of β_2 tubulin primary structure required for the binding of accessory proteins involved in crosslinking microtubules in the axoneme. Central pair microtubules may be especially sensitive to the effects of *B2t*^{7} due to inherently lower stability in the axoneme. During meiosis, the *B2t*^{7} lesion could impair the ability of the spindle asters to trigger cytokinesis. If so, astral microtubules would appear to be more sensitive to the effects of the *B2t*^{7} lesion than the microtubules responsible for chromosome disjunction.

The class II mutations *B2t*^{6} and *B2t*^{7} differentially affect β_2 tubulin function in specific subsets of microtubules (Table III), and thus could identify amino acids more critical for function in certain types of microtubule based organelles than in others. Analysis of many more such mutations may eventually define regions of β tubulin primary structure important for specific microtubule functions. At least fourteen other class II *B2t* alleles have already been isolated and await phenotypic and molecular analysis (Fuller, M. T., P. R. Hocstenbach, S. Buhrman, J. Hutchens, K. Kemphues, and E. C. Raff and co-workers, unpublished results). Ultimately, solution of the crystal structure of tubulin is needed to give three-dimensional context to hypotheses about the relationship between the primary structure of tubulin and its function in microtubules. However, even given a crystal structure, analysis of the effects of changes in primary structure due to mutations will be required to test such hypotheses.

Intragenic Complementation Among β_2 Tubulin Alleles

Many of the defects in spermatogenesis characteristic of class II mutations are partially alleviated in heterozygous combinations between different alleles. Complementation between *B2t*^{6} and *B2t*^{8} is especially striking—the heterozygous males are fertile and produce sperm that contain no wild-type β_2 tubulin.

Classical models for interallelic complementation derived from studies of enzyme systems in microorganisms invoke mechanisms such as mutations in functionally independent structural domains or correction of conformation during subunit interactions in multimeric assemblies (Zabin and Villarejo, 1975). Both of these mechanisms could contribute to interallelic complementation between the partially func-

tional β_2 tubulin mutant alleles. Complementing mutations could affect different functional regions of the molecule even though they do not reside in obvious independent structural domains. If a mutation affects the binding site for an accessory protein required in only certain kinds of microtubule arrays, but does not alter the structure of the rest of the β_2 tubulin molecule, the resulting subunit should be capable of many of the interactions normally carried out by tubulin, but deficient in the specific subset of functions mediated through the defective binding site.

An additional mechanism for interallelic complementation may stem from the fact that microtubules consist of a regular array of many α and β tubulin subunits and are often decorated at regular intervals with accessory proteins present in ratios less than or equal to 1 accessory protein:12 tubulin dimers (reviewed by Olmsted, 1986). In males heterozygous for two β_2 tubulin mutations, individual microtubules may be assembled from a mixture of the two variant subunits. If the defect in one allele involves failure to bind a specific accessory protein, a functional microtubule might still be assembled if the other variant subunit is near the normal binding site for that accessory protein in the microtubule. The same argument can be made for microtubules assembled from a mixture of mutant and wild-type subunits in mutant/+ heterozygotes. In general, a structure assembled from a large number of repeating subunits may provide extensive opportunities for partially defective mutant subunits to substitute for each other, resulting in intragenic complementation.

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References

Cleveland, D. W., and K. F. Sullivan. 1985. Molecular biology and genetics of tubulin. *Annu. Rev. Biochem.* 54:331–365.

Fuller, M. T. 1986. Genetic analysis of spermatogenesis in *Drosophila*: the role of the testis-specific β tubulin and interacting genes in cellular morphogenesis. *In* Gametogenesis and the Early Embryo. J. Gall, editor. 44th Symposium of the Society for Developmental Biology, Alan R. Liss, Inc. New York. 19–41.

Fuller, M. T., J. Caulton, J. A. Hutchens, T. C. Kaufman, and E. C. Raff. 1987. Genetic analysis of microtubule structure: a β tubulin mutation causes the formation of aberrant microtubules *in vivo* and *in vitro*. *J. Cell Biol.* 104:385–394.

Gonzales, C., J. Casal, and P. Ripoll. 1988. Functional monopolar spindles caused by mutation in *mgr*, a cell division gene of *Drosophila melanogaster*. *J. Cell Sci.* 89:39–47.

Hardy, R. W. 1975. The influence of chromosome content on the size and shape of sperm heads in *Drosophila melanogaster* and the demonstration of chromosome loss during spermiogenesis. *Genetics.* 79:231–264.

Kemphues, K. J., E. C. Raff, and T. C. Kaufman. 1983. Genetic analysis of *B2t*, the structural gene for a testis-specific β-tubulin subunit in *Drosophila melanogaster*. *Genetics.* 104:345–356.

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.

Kemphues, 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.

Kiefer, B. I. 1970. Development, organization, and degeneration of the *Drosophila* sperm flagellum. *J. Cell Sci.* 6:177–194.

Kirchner, K., and E.-M. Mandelkow. 1985. Tubulin domains responsible for assembly of dimers and protofilaments. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2397–2402.

Lewis, E. B., and F. Bacher. 1968. Method of feeding ethyl methanesulfonate (EMS) to *Drosophila* males. Drosophila Information Service. 43:193.

Lifschytz, E., and D. Hareven. 1977. Gene expression and the control of spermatid morphogenesis in *Drosophila melanogaster*. *Dev. Biol.* 58:276–299.

Lindsley, D. L., and E. H. Grell. 1968. Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington.

Lindsley, D. L., and K. T. Tokuyasu. 1980. Spermatogenesis. *In* Genetics and Biology of *Drosophila*, Vol. 2*d*. M. Ashburner and T. R. F. Wright, editors. Academic Press. New York. 225–294.

Mandelkow, E.-M., M. Herrmann, and U. Rühl. 1985. Tubulin domains probed by limited proteolysis and subunit-specific antibodies. *J. Mol. Biol.* 185:311–327.

O'Brien, S. J., and R. J. MacIntyre. 1978. Genetics and biochemistry of enzymes and specific proteins of *Drosophila*. *In* Genetics and Biology of *Drosophila*, Vol. 2*a*. M. Ashburner and T. R. F. Wright, editors. Academic Press. New York. 395–551.

Olmsted, J. B. 1986. Microtubule-associated proteins. *Annu. Rev. Cell Biol.* 2:419–455.

Raff, E. C., and M. T. Fuller. 1984. Genetic analysis of microtubule function in *Drosophila*. *In* Molecular Biology of the Cytoskeleton. G. G. Borisy, D. W. Cleveland, and D. B. Murphy, editors. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY. 293–304.

Rudolph, J. E., M. Kimble, H. D. Hoyle, M. A. Subler, and E. C. Raff. 1987. Three *Drosophila* beta-tubulin sequences: a developmentally regulated isoform (β_3), the testis-specific isoform (β_2) and an assembly-defective mutation of the testis-specific isoform (*B2t*^{8}) reveal both an ancient divergence in metazoan isotypes and structural constraints for beta-tubulin function. *Mol. Cell. Biol.* 7:2231–2242.

Tates, A. D. 1971. Cytodifferentiation during spermatogenesis in *Drosophila melanogaster*. Ph.D. Thesis, Rijksuniversiteit, Leiden.

Tokuyasu, K. T. 1974. Dynamics of spermiogenesis in *Drosophila melanogaster*. IV. Nuclear transformation. *J. Ultrastruct. Res.* 48:284–303.

Tokuyasu, K. T. 1975. Dynamics of spermiogenesis in *Drosophila melanogaster*. VI. Significance of "onion" nebenkern formation. *J. Ultrastruct. Res.* 53:93–112.

Zabin, I., and M. R. Villarejo. 1975. Protein complementation. *Annu. Rev. Biochem.* 44:295–313.