

Tissue-specific and constitutive α -tubulin genes of *Drosophila melanogaster* code for structurally distinct proteins

(gene family)

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ABSTRACT We have determined the nucleotide sequences of all four *Drosophila* α -tubulin genes ($\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$). Two of the genes, $\alpha 1$ and $\alpha 3$, are constitutively expressed and code for proteins that are very similar to previously sequenced α -tubulins. They differ from each other by only two amino acid substitutions. These two genes also have blocks of homology between the noncoding leader regions of their transcription units. In contrast to these constitutive genes, the tissue-specific $\alpha 2$ and $\alpha 4$ genes code for tubulins with different structures. The $\alpha 2$ mRNA is male-specific in adults and codes for a tubulin that differs from $\alpha 1$ at 21 of the 450 residues. Six nonconservative substitutions are clustered within the 14 carboxyl-terminal amino acids, a region implicated in the regulation of microtubule assembly. The $\alpha 4$ mRNA is maternal and is found only in ovarian nurse cells, eggs, and early embryos. It codes for the most highly divergent α -tubulin yet reported and differs from $\alpha 1$ at 149 positions.

Microtubules are the major structural elements of cilia, flagella, the cytoskeleton, and the mitotic and meiotic spindles. As might be expected from their presence in these structures, the disruption of normal microtubule assembly affects cell motility, division, and secretion, as well as intracellular transport (reviewed in ref. 1). This diversity in microtubular structure and function suggests that different microtubule subunits assemble into specialized microtubules (2). The basic subunit of all microtubules is a heterodimer of α - and β -tubulin polypeptides. This $\alpha\beta$ subunit coassembles with species- and tissue-specific microtubule-associated proteins (MAPs) (3) to form microtubules *in vivo*. Thus, functional specialization should be reflected in the structures of the α - and β -tubulins, the MAPs, or both.

Studies of tubulin proteins and genes from a wide variety of species indicate that although tubulin structure is highly conserved among species, there is some minor variation between the tubulins found in a single organism. This variation is due to both posttranslational modification of the tubulin heterodimer (4, 5) and primary-structure differences encoded in multiple tubulin genes (reviewed in ref. 6). The degree to which primary-structure differences contribute to tubulin heterogeneity is only beginning to emerge, because complete sequence analysis of an entire tubulin gene family of a higher eukaryote has not been reported. The available sequence data indicate that multiple tubulin genes do code for structurally different tubulins. For example, among the known sequences of chicken β -tubulins the greatest difference is 8.7%. This clearly represents a minimum estimate of the range of differences in a single organism because only four of the nine or more β -tubulin genes from chicken have been analyzed.

The sequences of all the α - or β -tubulin genes from a higher eukaryote would indicate the entire range of tubulin primary structures utilized by the organism and facilitate molecular approaches to the study of tubulin specialization. In this paper we describe the sequence of the entire α -tubulin gene family of the higher eukaryote *Drosophila melanogaster*.

There are four α -tubulin genes in *Drosophila*, referred to as $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$,[†] at chromosomal locations 84B3-6, 85E6-10, 84D4-8, and 67C4-6, respectively (8). The $\alpha 1$ and $\alpha 3$ genes appear to be constitutively expressed, whereas the other two genes have highly specialized developmental patterns of expression (9-11). In adults, $\alpha 2$ transcripts are found only in males, where they may be testes-specific (9). The $\alpha 4$ transcripts accumulate only in very early embryos and in adult female ovaries (9). *In situ* hybridization to ovarian tissue indicates that the $\alpha 4$ message is maternal and is synthesized in the nurse cells (M. Harris and P.C.W., unpublished data). This suggests that $\alpha 1$ and $\alpha 3$ may have functions, such as providing cytoskeletal structure, that are common to most cells, whereas $\alpha 2$ and $\alpha 4$ may provide tubulins with specialized functions.

In this paper we report the complete nucleotide sequences and the transcript maps of all four *Drosophila* α -tubulin genes. The constitutively and coordinately expressed $\alpha 1$ and $\alpha 3$ genes code for nearly identical proteins that are very similar to the known sequences of α -tubulins from other species. The two sex-specific α -tubulin genes, $\alpha 2$ and $\alpha 4$, code for quite different polypeptides that may be functionally specialized.

MATERIALS AND METHODS

Sequencing. DNA fragments containing *Drosophila* α -tubulin genes were excised from plasmids pDmT $\alpha 1$ -4 (8, 12) and subcloned into plasmids pUC8 and pUC9 for sequence analysis. A series of deletions in the pUC subclones were prepared using the DNase I method of Hong (13), modified for use with double-stranded plasmid DNA. Plasmid DNA was prepared by the alkaline-lysis method (14). Deletions were sized by gel electrophoresis rather than by the sequencing protocol of Hong.

Single-stranded templates for dideoxy sequencing (15) were prepared by digesting plasmid DNA with a restriction endonuclease that cut within 2.0 kilobases downstream (relative to the direction of primer extension) of the primer site. The DNA was then precipitated with ethanol, washed in 70% (vol/vol) ethanol, dried, and resuspended in 50 μ l of 70 mM Tris-HCl, pH 8.0/1 mM MgCl₂/10 mM dithiothreitol. This DNA was digested (16) with 25 units of exonuclease III at 37°C for 3 hr or at 25°C overnight. Digestions were terminated and protein was removed by three extractions

Abbreviation: MAPs, microtubule-associated proteins.

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[†]Recommended symbols (7) for these genes are α Tub84B, α Tub85E, α Tub84D, and α Tub67C.

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with phenol/chloroform (1:1). This DNA was precipitated, washed, and dried as above and then resuspended in 15 μ l of 10 mM Tris-HCl, pH 8.0/1 mM EDTA. The resuspended templates were sequenced by the dideoxy procedure (15), but using 32 P-end-labeled primer (universal sequencing primer, New England Biolabs). End-labeled primer was essential for generating unambiguous sequence data from these templates. Products of the reactions were electrophoresed in 6% polyacrylamide buffer-gradient sequencing gels (17).

Other Methods. DNA restriction fragments were radiolabeled using either bacteriophage T4 polynucleotide kinase or avian myeloblastosis virus reverse transcriptase as described (18). RNA isolation, plasmid purification, agarose gel electrophoresis, ligations, and bacterial transformations were done as described (14, 19). Restriction endonucleases and other DNA-modifying enzymes were purchased from

Boehringer Mannheim and New England Biolabs. Radiolabeled nucleotides were obtained from ICN.

RESULTS AND DISCUSSION

Nucleotide Sequence and Transcript Maps of the *Drosophila* α -Tubulin Genes. The strategy of Hong (13), adapted for use with double-stranded plasmid vectors (see *Materials and Methods*), was used to generate deletions in subcloned fragments of the four α -tubulin genes. DNA neighboring the deletion breakpoints was then sequenced by the dideoxy method (15), resulting in the sequences shown in Figs. 1 and 2.

The intron/exon structures of the four genes are shown in Figs. 1 and 2. The structures of $\alpha 1$, $\alpha 2$, and $\alpha 4$ are similar. The first exon of each encodes only the first amino acid of the protein. In each case the codon for the first amino acid is located within a consensus splice-donor sequence and the

$\alpha 1$

GTGACAGCTTGGCGTCTCTAGTCCGGTGCCTATATAAAGCAGCCCGCTTCCACATTTCATATTCGTTTTACGTTTGTCAAGCCTCATAGCCGGCAGTTCGAAC +45
 GTATACGCTCTCTGAGTACAGACCTCGAAATCGTAGCTCTACACAAATCTGTGAAATTTTCCTTGTGCGCTGTGAAACACTTCCAATAAAAACTCAATATGTGAGTA +150
 CTTAAAAAAAATCTAGTGAATAATGCTGAAAAGAAATTTGTGTTGGGCAAAATCAATGGGCAAAAACGCGATGCGGCTTTTCTCAAATGGCCGGCCGCTG +255
 CGTTTTTCTCAAAGTATGACGTCATGCCGTGTTTTTTTTTTTTTTTGTGTCGCAATAGGAAATGGCTCTTAAATCTAGATAAAAAAATATTCATTATTTCTAT +360
 GCTGCTGGAACGCTTCATTAATCTTAAAAATCTAAATTCGGTTACCATGATACCTTCGACGCATAACTGTAGATTTTGGATAGAAATTAAGAGAAAAATGGCGAGAG +465
 AGTAAATTCGGCGTCCGCAAGTAGACAAAAAATCAGTATACCATTAGCTACCTCTCCTCCTCGCAGCAGTCCCGGCTCAAGTTGGGCGCGGCTCTGCA +570
 TTATCGATTTCTTGGGGTGTGTAACATAATCATCCGTTTTCCCTTCCCTCATCCACAGCGTGAATGTATCTCTATCCATGTTGGTCAAGGCTGGTGTCCAGATTG +675
 GAAACGCTGCTGGGAGCTCTACTGCTTGGAGCACGGCATCCAGCCGATGGCCAGATGCCGCTGTGACAGACCCTGGGCGGAGGTGATGACTCGTTCAACACCTT +780
 CTTCCAGCGACTGGAGCTGGCAGCAGCTGCCCGCCGCGCTGTTTGTGGATCTGGAACCCACTGTGGTCCGATGAGGTCCGTACCCGGAACCTACCGTCAAGCTGTT +885
 CACCCTGAGCAGCTGATCACTGGTAAGGAGGATGGCGCAACAACCTACGCCGCTGGCCACTACCCATCGGCAAGGAGATCGTCGATCTGGTCTGGACAGGATCC +990
 CCAAGCTGGCCGATCAGTGCACCGCTCAGGGCTTCCCTCATCTTCCACTCGTTCGGTGGTCCAGCCGCTCCGGCTTCACTCGTGTGATGGAGCCCTCTCTC +1095
 CGTGACTACGGCAAGAAGTCCAAGCTGGAGTTCGCCATCTACCCAGCCCCAGGTGTCCTACTGCCCTGGTTCGAGCCCTACAACCTCCAGCCACCCACCC +1200
 ACCCTGGAGCATTCGACTGCGCTTCATGGTGCAGCAAGGAGCTATCTACGACATCTGCCGCCCAACCTGGACATGAGCGCCCAACCTACACCACTGAAAC +1305
 GTCTGATTTGGCCAGATCGGTGCTCGATACCCGCTCTCTGCGATTCCGATGGTGGCCCTTAACGTTGATCTGACTGAGTTCAGACCAACTTGGTGCCTACCCAGG +1410
 TATCACTTCCCTCTGGTACCTACGCCCGCTTATCTCCGCGAGAAGCCCTACCCAGCAGCTGTCCGGTGGCTGAGATCAACAACCGCTGCTTCGAGCCGCGCC +1515
 AACCAGATGGTCAAGTCCGATCCCGCTCAGCGCAAGTACATGGCCCTGCTGCATGCTGACCGCGTGTATGTTGTGCGCCAAAGGACGTCACGCCGCTATTGCCACCA +1620
 TCAAGACCAAGCGCACCATTAATTCGTCGACTGGTGCCTCAGGCTCAAGTGGCATCACTACCAGCCACCCACCGTGGTGCCTGGAGGATTTGGCCAA +1725
 GGTGACGCTGGCGTGGCATGTTGTCCAACACCAGGCCATCCGCGAGGCCCTGGCCCGCTTGGACCAAGTTCGATCTGATGTACGCCAAGCGTGCCTTCGTC +1830
 CACTGATCGTTGGTGGGATGAGGAGGAGGAGTTCCTCCGAGGCCGCTGAGGATTTGGCTGCCCTCCGAGAGGACTACGAGGAGGCTGGCATGGACTCCGGTG +1935
 ACGCCGAGGCTGAGGCGCTGAGGAGTACTAAGCGTCCGCCACTTCAACGCTCGATGGGAGCGTCAATGGTGGGCGGGTAAACCGTCAAAATCAGTGTTCAGCT +2040
 TCCAATCGCAACAAAAAATTCAGTCAAGCAATACGAAAGCATACGAAAGCATGAAGATTTGACGAGAAACCATAAAGTATTTTATCCACBAAGACAGCTATAGCAG +2145
 AAAAGCCAGTTAATCCGCGATAAGTTGTGTACACAAGATAAATTCGCCAGATTCAGTGTGTCAGAAATAAGAAAAACCCACTATGTTTTTCTTTCCTTTT +2250

$\alpha 2$

CGGCTCCACATTTACATAACTTCCACATATAGCATGTAGCATATAGCATATGCTACATATGCTGTCTTGGGGTCTTATAAAAAGAACCGGTATCGTCAAGATTTCG -4
 GTTAGTTGACTCCAGCTGTGTGCGCATCACAGACCAGTGTAGGATTTTCGTTGATTGAATCTGCTTTTTTAAACTTCAGCTTAAACTTTTGATCATCATGTGTA +100
 GGATAATAGGCACTTTCAATGATTTAGCTGGACTTTTGAATTTTCGTCGGCTGCACAGAATATGGATCCTTAAAAGTCTTATATGCGATTTTCATGCAAACTAG +205
 ACCATATGGTGCATATTTTATCATTTTTCAGAGTATTAATTAATGGTTTTAAAAAATAAAGTCTGTGTAAGTATATCTTAAATGAATACCAAGAAGTAC +310
 AGCGATCAAAATGTTTGTAGCATATGGTCCAAATCACAGATTTGCAACCGGAAGTCCAAAGTAAATTTTAGAATTTACCCAAATTTACAGCGACATGAGTCACAT +415
 TATTATAAATCTGTTCCAGAGGAATGCATTTCCGTTCCATTTGGCCAAAGCTGGTTCAGATCGGCAATGCCCTGCTGGGAACCTTACTGCTGGAGCACGGCAT +520
 CCAAGCCGATGGCCATATCCCGCTCCGATAAAAACCGTGGCGGAGGCGATGACTCCTTCCAGCAGCTTCTTCCAGGAGACTGGAGCTGGTAAGCATGTGCCCGCTGCC +625
 GTGTTGTTGATCTGGAGCCACTGTGGTGGATGAGGTGCGGACAGGAACCTACCCGAGCTGTTCATCCGGAGCAACTGATCACCGGTAAGGAGGATGCGGCCA +730
 ACAACTATGCCCGCGCCACTATACCATCGGTAAGGAGATCGTCGACGCTGGTCCGACAGGATTCGCAAGTGGCGGATCAGTGCACGGGCTGCAGGAGTTGCT +835
 GGTCTTCCACTCCTTCCGGCGGGCCACTGGCTCTGGATTTACGTCGCTGTGATGGAGCGCCTGCTCCGGACTATGGAAGAAGTCAAAGCTGGAGTTCTCCATT +940
 TACCAGCACCAAGATGTCATTTGCATATGGATATTATCTATTGCTTAGCATTATAGAAGACCAATTAACATAATTAGTAAAAGACATGGGAAATTAGT +1045
 CTTGAAAGGCAATCTAATACTGAACCTTCCGTTAGGTGTCACAGCTGTGGTGGAGCCATACAACCTCAATTAACCCACCAACCGCTGGAGCATCCGAC +1150
 TGTGGTTTTATGGTTGACAAAGGCAATCTACGACATCTGCCCGCGCAACTGGATATCGAGCCACCCTACATGAATCTCAATCCGCTAATTTGGCCAGATCG +1255
 TTTCTCCATTACCGGCTCGTTGCGCTTCGATGGCGCCCTGATGATGACTGACGAGCTCCAGACCAATTTGGTGCCTACCCAGCGATTTCATTTCCCACTGGC +1360
 GACATACGCTCCCGCTATTTCCGTTGGAGAAGGCTACCATGAGCACTGACCGTGGCCGAGATCACCATGCCCTGCTTCGAGCCGCGCAACCCAGATGGTCAAGTGT +1465
 GATCCGCGCTGGTCAAGTACTGGCTGCTGCATGCTTACCCGCGGTGATGGTGGCCAAAGGATGTAACCGGACATTCGCCACCATTAAGACCAAGCGCTCTA +1570
 TCCAGTTCGTCGACTGGTGTCCACGGCTTCAAGTGGGATCAACTATCAGCCACCACCGTGTTCCTGGCGGAGATCTGGCCAAAGTGCAGCGCGCGCTCTG +1675
 CATGCTGTCCAATACCACTGCCATTGCCGAGGCTGGGCGGCTGTGATCAAGATTCGATCTGATGTACGCCAAGAGGGGCTTCGTCCTGTTAGCTGGTGGG +1780
 GGCATGGAGGAGGGGAGTTCGCGGAGGCTCGCGAGGATCTCGCTGCCCTCGAAGGACTACGAGGAGGTAGGCATCGACTCCACCACCGAAGTGGGGGAAGATG +1885
 AGGAATACCTAGGAAACCTTGTCAACAATGATACAGCCATTAACAACCTTATAAACCATCACTTCTTTTACCAAAATCAACATGTCATAAAACAGTTTCGCCAATAA +1990
 AACAGTTTAAACAAATTTGCTTAAAAATGGCTTATTTAAATGGGAAATATTCGCTTTAGAT

Fig. 1. Nucleotide sequences and transcript maps of the $\alpha 1$ - and $\alpha 2$ -tubulin genes of *Drosophila*. Exons are underlined. The "TATA boxes," the first and last codons, and the polyadenylation consensus sequences are overlined. Position +1 is the transcription start site. The intron/exon structures of the four genes were predicted by localizing open reading frames coding for proteins homologous to porcine brain α -tubulin (20) and by identifying regions that match consensus sequences found at initiation, termination, and processing sites (21). In ambiguous cases, the exon boundaries were determined by nuclease protection, primer-extension, or RNA blot hybridization analysis and are described in detail elsewhere (11, 22). Blot hybridization analysis of electrophoretically fractionated RNA localizes the first exon of $\alpha 2$ between -325 and +415. In the figure, this exon is positioned according to the best fit to consensus sequences for the TATA box and the donor splice junction within this region.

The conservation of sequences in the 5' leaders of the two coordinately expressed genes suggests a regulatory function for the leader region. Tubulin mRNA levels appear to be subject to feedback regulation in a number of cultured cell lines (25, 26). Drugs that promote depolymerization of microtubules and increase the tubulin subunit pool size also cause a decrease in the steady-state levels of α - and β -tubulin mRNA. This regulatory mechanism appears to act posttranscriptionally (27, 28). We speculate that the homologies in the 5' leader sequences of $\alpha 1$ and $\alpha 3$ are involved in a post-transcriptional regulatory circuit, perhaps interacting with cytoplasmic factors to reduce stability of the message. It is also possible that these sequences are involved in transcriptional regulation of the two genes.

Amino Acid Sequence Homologies and Heterogeneities. The sequences of the four genes were used to predict the primary structure of the *Drosophila* α -tubulins (Fig. 4). The $\alpha 1$ - and $\alpha 3$ -tubulins are nearly identical and are very similar to the known sequences of α -tubulins from other species. However, the two tissue- and sex-specific α -tubulin genes, $\alpha 2$ and $\alpha 4$, code for clearly different polypeptides.

The $\alpha 1$ and $\alpha 3$ proteins differ by two substitutions. Isoleucine-170 and cysteine-305 of $\alpha 1$ are replaced by valine residues in $\alpha 3$ (Fig. 4). These substitutions do not cause

differences between the predicted secondary structures (Fig. 5), indicating that the products of these two genes are likely to have identical functions. They resemble previously characterized α -tubulins. As an example, $\alpha 1$ differs at 16 of 450 residues relative to a porcine α -tubulin, and of those 16 differences, only 6 are nonconservative (Fig. 4).

The sequence of the $\alpha 2$ protein differs from $\alpha 1$ at 21 of 450 residues (Fig. 4). While most of these are conservative differences, a few do change the predicted secondary structures (Fig. 5). The most striking cluster of nonconservative substitutions is at the carboxyl terminus of the protein, between residues 439 and 450 (Fig. 4).

The $\alpha 4$ protein is clearly different from the other *Drosophila* α -tubulins and from the sequenced α -tubulins of other species (Fig. 4). The $\alpha 1$ and $\alpha 4$ proteins differ at 149 residues. Such high divergence has not been observed between any two previously described α -tubulins. Substitutions are found throughout the polypeptide, with two major clusters near the amino and carboxyl termini, respectively. The amino-terminal cluster lies between residues 34 and 61, and includes an 11 amino acid insertion in $\alpha 4$ relative to other α -tubulins. The $\alpha 1$ tubulin from *S. pombe* has a 4 amino acid insertion at this same site (29). The predicted secondary structure of the protein is quite different from those of the

D1	MRECTSIHVGGQAGVQIGNACWELYCLEHGIQPDGQMPKSDKTVGGGDDSFN-----TFFSETGAGKHVPRAVFVDLEPTVVDEVRTGTGRQLFHP	89
D2	V I H S	
D3		
D4	VV QI C I L NL SLKTKKEELTAGS ASVGHDT SANDAR T N Q SI I D N C M E Y	
Y1	V V G FPTENSE HKNSYL DGFG Q F SIY N I Q P KD	
Y2	I T N Y NP TASNQS GG S Q Y SIY N I Q DP D	
P	I I	
D1	EQLITGKEDAANNYARGHYTIGKEIVDLVLDRIKRLADQCTGLQGLIFHSFGGGTSGFTSLLMERLSVDYGKKSLEFAIYPAPQVSTAVVEPYNSIL	189
D2	V V S	
D3		
D4	S R S V I R TS LQ I E DS L V T S C D V S K AL	
Y1	MV S V M I S E RM NS V LGT L NME N Q SV S V	
Y2	S V L E T K RIG NS V GA L AME T Q SV S V	
P	I SV S	
D1	TTHTTLEHSDCAFMDVNEAIYDICRRNLDIERPTYTNLNRILQIVSSITASLRFDGALNVDLTFEQTNLVPYPRIHFPVLYAPVISA EKAYHEQLSVA	289
D2	M A V T	
D3		
D4	S MD V NNS GVD A R A T S SM N F A LM RSA HAIT	
Y1	A DN T C E A V A S N S IV A F SN Q	
Y2	A DLA V L L KKS D S A VA VI L T D A IN N IV A F SN Q	
P	A	
D1	EITNACFEPANQMKCDPRHGKYMCCMLYRGDVPKDVNAAIATIKTKRTIQFVDWCPTGFKVGINYPPTVVPGGDLAKVQAVCMLSNTTAIAEAWA	389
D2	R S	
D3	V	
D4	TL SS M A F VSA S H I EK AF D TS C SV FS	
Y1	Q Q Y T R T L I R Q VTS SR I C E QH SGI N S S	
Y2	Q Q Y A R T L I R Q VT A I CDR QHIE SEI D S S	
P	L E	
D1	RLDHKFDLMYAKRAFVHWVYVGGMEEGEFSEAREDLAALEKDYEEVGMDSGDGEGE GA-EEY	450
D2	A I TTEL D-	
D3		
D4	N SY FK T NI V R F L NAE EG DEDFD F	
Y1	S R Q M N MYE D	
Y2	S R Q MEVDYME-	
P	M V VE EEG	

Fig. 4. Comparison of amino acid sequences of α -tubulins. The species abbreviations are D1, D2, D3, and D4 for *D. melanogaster* $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$ proteins, respectively; Y1 and Y2 for *Schizosaccharomyces pombe* (NDA2)1 and (NDA2)2, respectively (29); and P for porcine brain α -tubulin (20). The amino acids are written from left to right and top to bottom in an amino-terminal to carboxyl-terminal direction. Standard one-letter abbreviations are used. Only differences from the $\alpha 1$ sequence are shown. A dash represents the position of an insertion relative to $\alpha 1$.



FIG. 5. Secondary structure predictions for *Drosophila* α -tubulins. Chou and Fasman (30) predictions are diagrammed. α -Helical regions are represented by solid boxes; β -sheets, by cross-hatching; β -turns, by hatching; and unpredicted structures, by open boxes. The dashed lines in $\alpha 1$, $\alpha 2$, and $\alpha 3$ are the positions of deletions relative to $\alpha 4$.

other *Drosophila* α -tubulins (Fig. 5). There are, however, regions of predicted secondary structure that are shared by all four α -tubulins. Many of the α -helices and β -turns predicted for the $\alpha 1$, $\alpha 2$, and $\alpha 3$ proteins have corresponding structures in $\alpha 4$ -tubulin (Fig. 5). This analysis indicates that the $\alpha 4$ protein, although clearly different, is similar in length, shares most of the sequence, and may fold to form a protein with many of the structural features of other α -tubulins.

The highly divergent $\alpha 4$ polypeptide ends with a carboxyl-terminal phenylalanine residue instead of the tyrosine found at the termini of all other α -tubulins sequenced to date. The terminal tyrosine of α -tubulins is involved in a novel posttranslational modification. This residue can be removed from and religated to α -tubulin by a specific carboxypeptidase and tyrosine ligase (4). This modification may be involved in regulation of microtubule function (31). The carboxypeptidase and ligase that catalyze this cyclic reaction have been found to use phenylalanine as an alternate substrate, indicating that $\alpha 4$ -tubulin may undergo a similar modification.

One cluster of differences between the constitutive and developmentally regulated genes lies at the carboxyl terminus of the proteins. This region has been implicated in regulation of microtubule assembly and interactions with MAPs (32, 33). It is possible that this region interacts with specific MAPs to form specialized microtubular structures. This hypothesis is particularly attractive for $\alpha 2$ -tubulin. In adult flies the $\alpha 2$ gene is expressed only in males, where it may be testes-specific (9). The testes are the only tissue in the fly that are known to assemble flagella. This correlation suggests that the $\alpha 2$ polypeptide may represent a testes-specific tubulin that is selected for assembly into the flagellar axoneme.

The tissue-specific distribution of the $\alpha 4$ transcript suggests that the $\alpha 4$ polypeptide may also have specialized functions. The $\alpha 4$ polypeptide is the product of a maternal transcript that is detected only in ovarian nurse cells and 0- to 3-hr embryos (ref. 9; M. Harris and P.C.W., unpublished data). During the first 2.5 hr of embryonic development, nuclei are undergoing 13 synchronous divisions without the formation of cell membranes (34, 35). These divisions are extremely rapid, averaging 9 min each. Concurrent with some of these nuclear divisions are the movement of nuclei to the periphery of the embryo, contraction of the yolk sac, and nuclear elongation. All of these events require microtubules (35) and appear to be unique in the life cycle of *Drosophila*. Some or all of these microtubule-dependent events may require the divergent $\alpha 4$ polypeptide.

The two tissue- and sex-specific α -tubulins differ from each other and from the constitutively expressed α -tubulins. The divergence of the $\alpha 4$ gene product is particularly striking. It is unclear whether the degree of heterogeneity observed within the *Drosophila* α -tubulin gene family reflects the unique requirements of this species or the incomplete nature of the structural data on tubulin gene families of other eukaryotes.

We speculate that the tissue-specific α -tubulin genes code for functionally specialized proteins. While this is an attractive hypothesis, it clearly need not be the case. Genetic

analysis of the testes-specific β -tubulin of *Drosophila* clearly demonstrates that this tissue-specific protein participates in assembly of several different microtubule-based structures (36). The nucleotide sequences of all four α -tubulins of *Drosophila* should facilitate direct analysis of the regulation and function of the members of this gene family.

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