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17. We synthesized ^{13}C2-ThDP according to (*25*), using 4-amino-5-aminomethyl-2-methylpyrimidine and $^{13}CS_2$ (Isotec, 99 atom % ^{13}C). We prepared ^{13}C2 ThDP–labeled PDC by recombination of 50 mg apo-PDC with 25 μmol of ^{13}C2-ThDP and 25 μmol of $MgSO_4$ in 1 ml of 0.1 M sodium phosphate buffer (pH 6.0). After separation of excess coenzyme by gel filtration with Sephacryl S 200 HR, the sample was concentrated to 40 mg of PDC per milliliter with a Millipore 10-kD membrane. The ^{1}H-decoupled ^{13}C-NMR spectra were recorded in a 5-mm NMR tube on a Bruker AMX 500-MHz NMR spectrometer at 4°C (140,000 scans). After the NMR experiments, the PDC was precipitated by 3 M $(NH_4)_2SO_4$. No ^{13}C2-ThDP was detected in the supernatant, showing the absence of free ^{13}C2-ThDP in the samples used for spectra B and C (Fig. 2).
18. The exchange reactions were initiated by dilution of a sample solution containing 30 mg of holoenzyme per milliliter or 1 mM coenzyme in 0.1 M sodium phosphate buffer (pH 6.0 for PDC and pH 7.0 for TK) with D_2O at a 1:1 ratio in a quenched-flow apparatus (Model RQF-3; Kin Tek Althouse, USA) for exchange times between 2 and 2000 ms or by manual mixing for longer mixing times. All pH values refer to the respective pH meter reading, which is a mixture between pH and pD. The exchange reactions were stopped by addition of DCl and trichloroacetic acid to final concentrations of 0.1 M and 5%, respectively (pH 0.9). In addition, this procedure rapidly and completely denatured and precipitated the protein and released the cofactor. All reactions were carried out at 4°C. After separation of the denatured protein by centrifugation, the ^{1}H-NMR spectra of the supernatant containing only the ThDP were recorded in a 5-mm NMR tube on a Bruker AMX 500-MHz NMR spectrometer.
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23. The residual catalytic activity of the mutants, which bind ThDP as strongly as the wild-type enzymes, was 0.04% for $Glu^{51} \rightarrow$ Gln (E51Q) PDC (M. Killenberg-Jabs, S. König, I. Eberhardt, S. Hohmann, G. Hübner, *Biochemistry*, in press), 0.1% for $Glu^{418} \rightarrow$ Ala (E418A) TK (*13*) and 4% for H481A TK (C. Wikner, U. Nilsson, L. Meshalkina, Y. Lindqvist, G. Schneider, in preparation).
24. In addition, a comparatively small increase in the deprotonation rate by pyruvamide activation in the mutant PDC emphasizes that the signal transfer from the regulatory to the active center is probably mediated by E51. The structural events responsible for this step remain to be clarified.
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28. First, single-turnover conditions ([enzyme] > [substrate]), which were the basis for their calculations, are not valid because hydrolysis of the activator pyruvamide (100 mM) increases the initial pyruvate concentration (25 μM) above the enzyme concentration (50 μM). Second, the recombination of ThDP with apo-PDC was not complete within the used time, whereas our experiments were done with the intact holoenzyme after separation of excess free coenzyme. Third, our data explain why an isotope effect for C2-hydrogen exchange could not be observed under the experimental conditions used. Within the recombination time of ThDP with PDC (20 s), the deuterium on C2 of ThDP is completely replaced by a proton (Table 1).
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Microtubule Architecture Specified by a β-Tubulin Isoform

Elizabeth C. Raff,* James D. Fackenthal,† Jeffrey A. Hutchens, Henry D. Hoyle, F. Rudolf Turner

In *Drosophila melanogaster*, a testis-specific β-tubulin (β2) is required for spermatogenesis. A sequence motif was identified in carboxyl termini of axonemal β-tubulins in diverse taxa. As a test of whether orthologous β-tubulins from different species are functionally equivalent, the moth *Heliothis virescens* β2 homolog was expressed in *Drosophila* testes. When coexpressed with β2, the moth isoform imposed the 16-protofilament structure characteristic of that found in the moth on the corresponding subset of *Drosophila* microtubules, which normally contain only 13-protofilament microtubules. Thus, the architecture of the microtubule cytoskeleton can be directed by a component β-tubulin.

In eukaryotic cells, microtubules form diverse structures that are used for many different functions. Within each microtubule array, there are two levels of supramolecular organization: the architecture of each individual microtubule, determined by the number and arrangement of protofilaments, and the overall morphology of the microtubule array, for example, an axoneme or a spindle. Morphogenesis of each structure depends both on interactions between α- and β-tubulin heterodimers and on interactions between tubulins and other proteins. In vertebrate β-tubulins, isotype-defining variable regions (in particular the COOH-terminus), which have diverged among different isoforms in a gene family but are conserved in orthologs from different species, have been postulated to have an important role in conferring the functional specificity of each class of isoform (*1*). We previously demonstrated the validity of this hypothesis by showing that the unique COOH-terminus of the *Drosophila melanogaster* testis-specific β2-tubulin isoform is required for tissue-specific functions, including morphogenesis of the motile axoneme (*2*, *3*). Spermatogenic-specific microtubule functions cannot be provided by β3, another *Drosophila* β-tubulin isoform normally used during differentiation of a variety of somatic cells (*3*, *4*).

Vertebrate β-tubulin orthologs in different species are conserved in structure and have similar expression patterns, suggesting that they perform similar functions (*1*). If this model is true for other groups of organisms of similar evolutionary relationship, then the β2 ortholog from another insect should be better able to function in the *Drosophila* male germ cells than the paralogous β3 isoform. A cDNA from the moth *Heliothis virescens* was reported that represents the gene for a testis-specific β-tubulin (Hvβt) whose expression pattern suggested it to be the moth β2 ortholog (*5*). We sequenced the Hvβt clone and compared the predicted amino acid sequence with *Drosophila* β-tubulins. The COOH-termini of Hvβt and β2 were more similar to each other than to the other *Drosophila* β-tubulins and exhibited the same relative similarities to the other *Drosophila* isoforms (Fig. 1A), substantiating that Hvβt is orthologous to β2. How-

Department of Biology and Indiana Molecular Biology Institute, Indiana University, Bloomington, IN 47405, USA.

*To whom correspondence should be addressed. E-mail: eraff@bio.indiana.edu

†Present address: Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA.

ever, unlike the highly conserved orthologs in vertebrate β-tubulin families [96 to 99% identity (*1*)], Hvβt in its entirety was equally distant from all of the *Drosophila* β-tubulins (approximately 80% identity, 90% similarity). To confirm the relation between β2 and Hvβt, we therefore compared other axonemal β-tubulins with isoforms not used in motile axonemes. Axonemal β-tubulins from diverse taxa have a motif consisting of the consensus sequence EGEF followed by three acidic residues present at the same position in the COOH-terminus (Fig. 1B). Presence of the sequence motif does not preclude function in other kinds of microtubules, but the motif is absent in many β-tubulins that are not used for motile axonemes. Conservation of the axoneme motif throughout eukaryotic phyla is consistent with the hypothesis that assembly of a motile axoneme imposes structural constraints that limit evolutionary divergence of β-tubulins (*6*, *7*). Presence of the motif supports the conclusion that Hvβt is the ortholog for β2. However, the sequence divergence and the functional differences we report below between moth and fly testis β-tubulins demonstrate that the conservation of the β-tubulin gene families in vertebrates is not the general rule for β-tubulin families in other groups with similar times of evolutionary separation. Spermatogenesis in the moth is similar to the process in *Drosophila* and uses similar sets of microtubules (*8*). To test whether the testis β-tubulin orthologs have equivalent functional properties, we generated transgenic stocks that express Hvβt in the *Drosophila* male germ cells (Fig. 2). We examined Hvβt function when it was the sole β-tubulin in *Drosophila* spermatids and when it was coexpressed with β2. Contrary to our model, Hvβt failed to support any microtubule assembly at all in homozygous $\beta2^{null}$ male flies (*9*). Furthermore, when it was coexpressed with β2, Hvβt poisoned all microtubule-mediated processes (including meiosis, spermatid alignment, nuclear shaping, mitochondrial derivative elongation, and axoneme assembly), such that transgenic males in which Hvβt composed more than about 6% of the total β-tubulin pool were sterile (*10*). The severity of the dominant phenotype reflected the contribution of the moth protein to the germline tubulin pool; additional copies of the β2 gene ameliorated defects in all classes of microtubules.

Figure 3 illustrates the profound failure of cytoplasmic microtubule function (A and B) and axoneme assembly (C to L) that resulted from coexpression of Hvβt with β2. Wild-type axonemes in sperm of moths and flies have the morphology typical for insects, consisting of the highly conserved pattern of nine doublet microtubules surrounding a central pair of two singlet microtubules, plus an additional outer circle of nine singlet accessory microtubules (Fig. 3, C to E). Another unique feature of the insect axoneme is that in the mature sperm, the lumen of each central pair and accessory microtubule contains a filament (*2*, *3*) that in cross section appears as an electron-dense structure in the center of each microtubule (Fig. 3, C and E). The major morphological difference between fly and moth axonemes is that the accessory microtubules in the fly are the same diameter as the central pair, whereas the accessory microtubules in the moth are larger than the central pair (most clearly seen in Fig. 3D in the immature moth axoneme before the luminal structure is present). Tannic acid staining revealed that the difference in diameter reflects a difference in protofilament (pf) number: Moth accessory microtubules are 16-pf compared with 13-pf in the fly (Fig. 3, H to J and L).

In sterile transgenic males, axonemal microtubules were assembled, but axonemes in most spermatids were fragmentary (Fig. 3, F, G, and K). In many of the abortive axonemes, one or more of the accessory microtubules were larger than normal (Fig. 3, G and K), similar to moth accessory microtubules. The larger accessory microtubules in transgenic males were

A

	Dmβ1	Dmβ2	Dmβ3	Dmβ4
Hvβt	44	67	18	37
Dmβ1		44	18	26
Dmβ2			12	21
Dmβ3				16
Dmβ4				

B

Pig βII	-	DEQGEFEEEGEEDEA
Hum βIII	-	EEEGEMYEDDEEESESQGPK
Chi βIVb	+	EEEGEFEEEAEEEAE
Chi βVI	-	DVEEYEEAEASPEKET
Dmβ1	-	DEDAEFEEEQEAEVDEN
Dmβ2	+	DEEGEFDEDEEGGGDE
Dmβ3	-	DDEFDPEVNQEEVEGDCI
Dmβ4	-	DDEVEFDDEQAEQEGYESEVLQNGNGE
Hvβt	+	DDEGEFDEEAEGEGLE
C.e. mec7	-	DEDAAEAFDGE
S. pombe	-	DEGDEDYEIEEEKEPLDY
S. cer.	-	EDDEEVDENGDFGAPQNDEPITENFE
Asp tubC	-	SDGEGAYDAEEGEAYEQEE
Para	+	EEEGEFEEEGQ
Chlamy	+	EEEGEFDEGEEEEA
Euplo	+	EEEGEMDEEEGAME
Giardia	+	-DEGEEFEEEED

Fig. 1. (**A**) Percent identity of the COOH-terminal sequences of Hvβt and *Drosophila* (Dm) β-tubulins. (**B**) An axoneme motif (underlined) in the COOH-termini of β-tubulins used in motile axonemes: plus sign, used in motile axonemes; minus sign, not used in axonemes. Sequences shown begin at residue 431 in most β-tubulins, following a highly conserved region penultimate to the COOH-terminus. Top group, metazoan β-tubulins with well-defined expression patterns (*14*). Hum, human; Chi, chicken; C.e. mec7, *C. elegans* touch neuron–specific isoform (*11*). Middle group, β-tubulins from fungal species in which motile axonemes are not made. S. cer., *Saccharomyces cerevisiae*; S. pombe, *Schizosaccharomyces pombe*; Asp, *Aspergillus nidulans* (*15*). Bottom group, sequences from ciliated or flagellated protists with only a single β-tubulin (*16*). Para, *Paramecium tetraurelia*; Chlamy, *Chlamydomonas reinhardtii*; Euplo, *Euplotes octocarinatus*; Giardia, *Giardia lamblia*. Complete sequences are published and available in databases, except for *Drosophila* β4 (*17*). The Hvβt sequence is available under GenBank accession number U75868. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

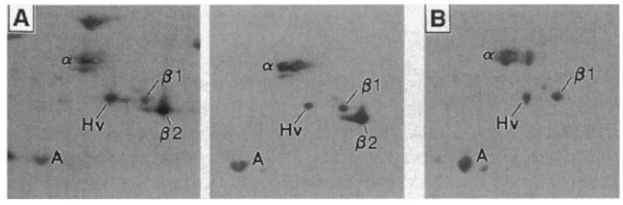

Fig. 2. Expression of the *Heliothis* testis-specific β-tubulin in *Drosophila* males. The Hvβt coding sequence was expressed in the postmitotic male germ cells under the control of regulatory sequences from the *Drosophila* β2 gene (*18*). Hvβt was identified as a novel testis β-tubulin that migrated in two-dimensional gels at the electrophoretic position predicted from the protein sequence. (**A**) Testis tubulins in a sterile male with one copy of the Hvβt transgene and two copies of the wild-type β2 gene. Left, autoradiogram showing incorporation of ^{35}S-labeled methionine into newly synthesized testis proteins. Right, protein immunoblot of the same gel showing the relative contribution of Hvβt and β2 to the testis tubulin pool. (**B**) Protein immunoblot showing testis tubulins in a sterile male with one copy of the Hvβt transgene in a $\beta2^{null}$ background. Immunoblots were probed with antisera to β-tubulin, α-tubulin, and actin. In addition to Hvβt (Hv) and endogenous β2 expressed in the postmitotic male germ cells, positions of β1-tubulin, expressed in earlier spermatogenic stages, α-tubulin (α), and actin (A) are also indicated. β3-Tubulin, expressed in somatic testis cells (*3*, *4*), is visible as a small spot above β1. Quantitation of [^{35}S]methionine incorporation showed that Hvβt and β2 synthesis is proportional to the gene copy number. Stability of Hvβt is the same in the absence or presence of β2.

16-pf instead of 13-pf (Fig. 3, K and L), just as in normal moth axonemes. The unusual protofilament architecture was specific to the axoneme accessory microtubules; no other microtubules in transgenic males had this architecture. The moth protein thus imposed the moth-specific accessory microtubule architecture on the equivalent structures in fly cells, even though it composed only a small percent of the total β-tubulin pool.

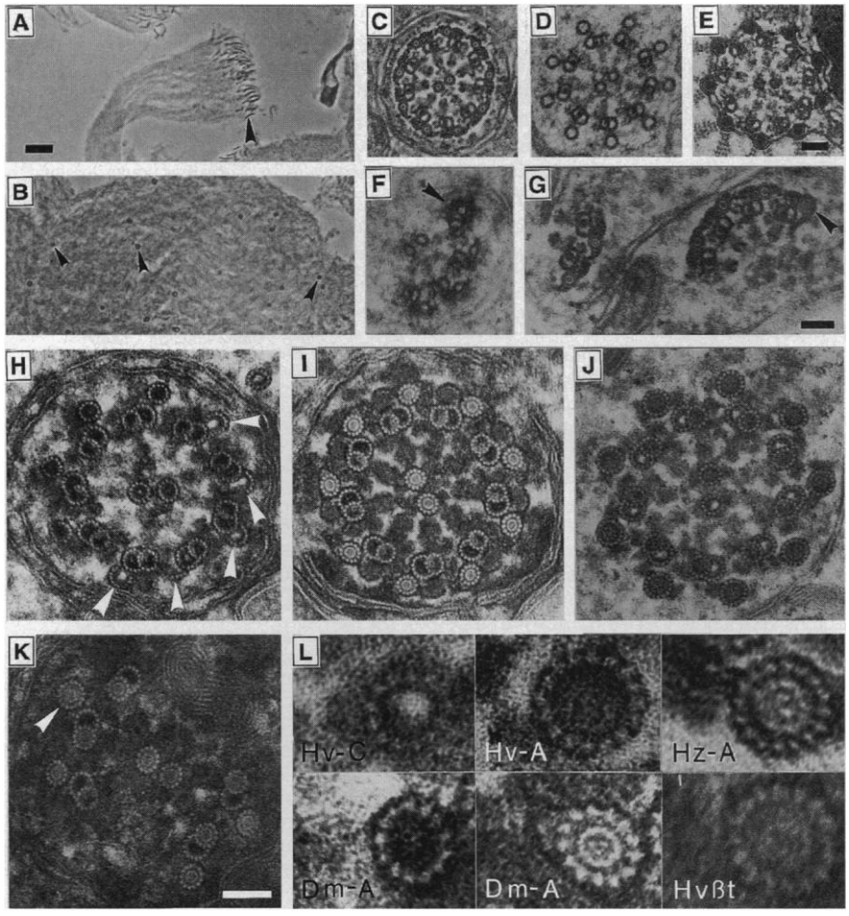

Fig. 3. Spermatogenesis in wild-type *D. melanogaster* and *H. virescens* males and in transgenic *Drosophila* males in which Hvβt is coexpressed with endogenous β2. In (A) and (B) light micrographs of orcein-stained testes show spermatid alignment and nuclear shaping (microtubule-mediated processes). Bar in (A) = 20 μm. (**A**) Spermatids in a wild-type *Drosophila* male. Nuclei are shaped and aligned at the tip of the developing bundle (arrow). (**B**) Spermatids in a sterile transgenic male in which Hvβt constituted 10 to 15% of the β-tubulin pool. Nuclei (arrows) are not shaped and spermatids are not aligned. Spermatid elongation is defective, reflecting failure of axoneme assembly and mitochondrial derivative elongation. Axoneme ultrastructure is shown in (C) to (L) (*19*). Bars = 50 nm [in (E) for panels (C) to (E); in (G) for panels (F) and (G); and in (K) for panels (H) to (K)]. (**C**) Mature axoneme with wild-type morphology from a fertile transgenic male in which low amounts of Hvβt were present. (**D**) Immature *Heliothis* axoneme; accessory and central pair microtubules do not yet contain luminal structures. (**E**) Mature *Heliothis* axoneme. (**F** to **G**) Aberrant axonemes in sterile transgenic males in which Hvβt constituted 8 to 10% of the β-tubulin pool. Partial axoneme with accessory microtubules in the process of assembly is shown in (F); formation of one is abnormal (arrow). Fragmented axoneme in a mature spermatid is shown in (G). One accessory microtubule is of large diameter (arrow). In (**H**) to (**L**) testes are stained with tannic acid to display the microtubule pf number (*20*). (**H**) Immature *Drosophila* axoneme. Accessory microtubules are completed on the left side of the axoneme but are in various stages of assembly (arrows) on the right side (*21*). (**I**) Mature *Drosophila* axoneme. (**J**) Immature *Heliothis* axoneme. (**K**) Partial axoneme in a mature spermatid from a sterile transgenic male in which Hvβt constituted 10 to 15% of the β-tubulin pool. The central pair and three of the accessory microtubules are 13-pf, but one of the accessory microtubules is 16-pf (arrow). (**L**) Enlarged views showing pf architecture. Hv-C, 13-pf, from (J); Hv-A, 16-pf, from (J); Hz-A, 16-pf, from a mature axoneme; Dm-A (left), 13-pf, from (H); Dm-A (middle), 13-pf, from (I); Hvβt, 16-pf accessory microtubule in transgenic male, from (K). Abbreviations: A, accessory microtubule; C, central pair microtubule; Hv, *H. virescens*; Dm, *D. melanogaster*; Hz, *Heliothis zea* (spermatogenesis is identical in *H. virescens* and *H. zea*). All panels are at the same magnification (*21*).

How did the moth protein specify protofilament architecture of the accessory microtubules? One possibility would be that in moth spermatids, Hvβt is dedicated to assembling only the 16-pf accessory microtubules. However, this is unlikely, given that, like β2 in the fly, Hvβt is the predominant isoform in the moth testis (*5*). The templating process for the accessory microtubules in the insect sperm axoneme is unique: Each accessory microtubule is initiated as an "outgrowth" from the B-tubule of the associated doublet (*8*) (Fig. 3H). Because Hvβt forced assembly of 16-pf accessory microtubules in fly cells (but not of other microtubules in either fly or moth), the moth protein must form a unique interaction with other components specific to this assembly mechanism, even in *Drosophila* cells. We postulate that although conserved COOH-terminal sequences are required for fundamental axoneme-forming functions, sequences elsewhere in the Hvβt protein are responsible for specification of accessory microtubule architecture, as well as for the dominant phenotype in *Drosophila* spermatids. Thus, sequences other than the COOH-terminus are also likely to play species-specific roles in spermatogenesis. There has been no previous demonstration of microtubule architecture being specific to a particular tubulin. Expression of the *Caenorhabditis elegans* mec7 β-tubulin is associated with 15-pf touch neuron microtubules (*11*), whereas other microtubules in the nematode are 11-pf, but the initiation mechanism is not known. In *Drosophila*, incorporation of β-tubulins into microtubules of differing pf number depends on the cellular context: Specialized 15-pf microtubules that function in wing maturation (*12*) contain β1 and β3, isoforms that in other cells give rise to typical 13-pf microtubules (*4*). The ability of heterologous β-tubulins to assemble into multiple pf arrangements in vitro and into many different structures in vivo provides additional evidence that microtubule architecture can be determined by factors extrinsic to the component tubulins (*7*). For example, the pf number of microtubules assembled in vitro can be controlled by nucleating conditions (*13*). Our data demonstrate that the templating machinery can be directed by a specific tubulin subunit.

Identification of a common structural feature shared by many axonemal β-tubulins provides evidence for strong conservation of features of tubulin structure required for assembly of the motile axoneme. Nonetheless, the finding that microtubule architecture can be intrinsic to the β-tubulin primary sequence demonstrates selection for a previously unknown

control over cellular microtubule assembly. Many studies have shown that transcription factors and other regulatory proteins retain the ability to function in heterologous systems across long phylogenetic distances. Our functional test of Hvβt in the *Drosophila* testis reveals that the cytoskeletal proteins that carry out the instructions of the regulatory genes may have acquired much more stringent species-specific restrictions on function.

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9. Even when the synthesis rate was the same, less Hvβt protein accumulated than β2; the moth protein is thus less stable than the endogenous isoform. However, testes of sterile males carrying multiple inserts of the Hvβt transgene accumulated Hvβt in amounts equivalent to those of β2 that we have shown to be sufficient to support microtubule function. Thus, the functional deficit of the moth isoform is not attributable simply to its relative instability in *Drosophila* cells. The total failure of Hvβt to support microtubule function contrasts with ectopic expression of *Drosophila* β3 in the male germ cells; β3 can support wild-type function of one class of cytoplasmic microtubules, even though it cannot assemble axonemes, spindles, or other microtubule arrays (*3*).
10. In a given spermatid, the threshold may be lower; we did not detect Hvβt in motile sperm in males in which the amount of Hvβt was sufficiently low as to allow fertility.
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14. Vertebrate isoforms are designated by the organism, followed by the isotype class to which the sequence shown belongs (*1*): class II, major neuronal; class III, minor neuronal; class IVb, predominant testis; class VI, hematopoetic. COOH-termini of isoforms of the same class in other species are identical or very similar to those shown. Widely expressed class I and V isotypes also lack the axoneme motif. β-Tubulins in other *Drosophila* species appear to be identical to those in *D. melanogaster* [(*4*); F. Michiels *et al.*, *Chromosoma* **95**, 387 (1987)].
15. *Aspergillus nidulans* has two β-tubulin genes; benA also lacks the axoneme motif.
16. The *Tetrahymena thermophila* axoneme motif is identical to *Paramecium*. *Giardia* represents the deepest group in eukaryotic taxa, and *Chlamydomonas* groups with plants [M. L. Sogin, H. G. Morrison, G. Hinkle, J. D. Silberman, *Microbiol. Semin.* **12**, 17 (1996)]. β-Tubulins in higher plants, which do not have ciliated cells, lack axoneme motifs.
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18. To generate the Hvβt transgene, we generated cloning sites 29 base pairs (bp) 5′ and 27 bp 3′ of the Hvβt coding sequence, and the resulting fragment was inserted between 2.1 kb of the 5′ β2 genomic sequences and 1.5 kb of the 3′ β2 genomic sequences previously shown to be sufficient to drive expression of heterologous proteins in the postmitotic male germ cells with correct developmental specificity and at the same level of expression as wild-type β2 (*2*, *3*) (J. Hutchens, H. Hoyle, F. R. Turner, E. C. Raff, *Mol. Biol. Cell*, in press). Intronless and intron-containing versions were constructed; in the latter, an oligonucleotide matching the 59-bp β2 intron sequence was inserted between Hvβt codons 73 and 74. Transgenes were inserted into the CaSpeR vector [V. Pirrotta, *Biotechnology* **10**, 437 (1988)] and introduced into the *Drosophila* genome by *P* element–mediated transformation (*2*, *3*). Multiple transgenic lines were obtained and testis tubulins analyzed on two-dimensional gels as described previously (*2*, *3*); the level of Hvβt expression depended on the site of insertion and presence of the intron. We obtained wild-type β2-like levels of expression (as in Fig. 2) only with an intron-containing insert, suggesting that splicing may be important in normal β2 expression. All transgenic lines exhibited the same suite of defects in spermatogenesis; thus, the phenotype is attributable solely to expression of the moth β-tubulin.
19. Electron microscopy and tannic acid staining were done as previously described (*2*, *3*).
20. The morphology of doublet microtubules and the central pair is the same in moths, flies, and transgenic flies. Doublets have a 13-pf A-tubule and a 10-pf shared-wall B-tubule; central pair microtubules are 13-pf. Accessory microtubules in fly axonemes are 13-pf, but 16-pf in moth. Most accessory microtubules in transgenic males are 13-pf, but the abnormal large-diameter accessory microtubules are 16-pf.
21. Accessory microtubules begin as a projection of a protofilament sheet from the B-tubule of each doublet, but completed accessory microtubules are no longer physically associated with the doublet. Completed accessory microtubules in immature axonemes of flies and moths were of a slightly larger diameter than in mature axonemes; thus, adjacent protofilaments in the walls of the accessory microtubules appear to "tighten up" as they form.

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Recognition of Unique Carboxyl-Terminal Motifs by Distinct PDZ Domains

Z. Songyang,* A. S. Fanning, C. Fu, J. Xu, S. M. Marfatia, A. H. Chishti, A. Crompton, A. C. Chan, J. M. Anderson, L. C. Cantley

The oriented peptide library technique was used to investigate the peptide-binding specificities of nine PDZ domains. Each PDZ domain selected peptides with hydrophobic residues at the carboxyl terminus. Individual PDZ domains selected unique optimal motifs defined primarily by the carboxyl terminal three to seven residues of the peptides. One family of PDZ domains, including those of the Discs Large protein, selected peptides with the consensus motif Glu-(Ser/Thr)-Xxx-(Val/Ile) (where Xxx represents any amino acid) at the carboxyl terminus. In contrast, another family of PDZ domains, including those of LIN-2, p55, and Tiam-1, selected peptides with hydrophobic or aromatic side chains at the carboxyl terminal three residues. On the basis of crystal structures of the PSD-95-3 PDZ domain, the specificities observed with the peptide library can be rationalized.

Many cytosolic signaling proteins and cytoskeletal proteins are composed of modular units of small protein-protein interaction domains that allow reversible and regulated assembly into larger protein complexes. Examples are SRC homology 2 (SH2) and SH3 domains and phosphotyrosine-binding (PTB) domains (*1*). PDZ domains have been observed in more than 40 cytosolic proteins, many of which are located at specific regions of cell-cell contact, such as tight junctions, septate junctions, and synaptic junctions. The name PDZ derives from three proteins that contain repeats of this domain: mammalian postsynaptic density protein, PSD-95; *Drosophila* disc large tumor suppressor, Dlg; and the mammalian tight junction protein, ZO1 (*2*–*5*).

Certain PDZ domain–containing proteins bind directly to the last several (COOH-terminal) residues of transmembrane proteins. For example, the second PDZ domain of PSD-95 binds the *N*-methyl-D-aspartate receptor through interaction with the COOH-terminal Ser/Thr-Xxx-Val se-