Supplemental Data

Axoneme β-Tubulin Sequence Determines Attachment of Outer Dynein Arms

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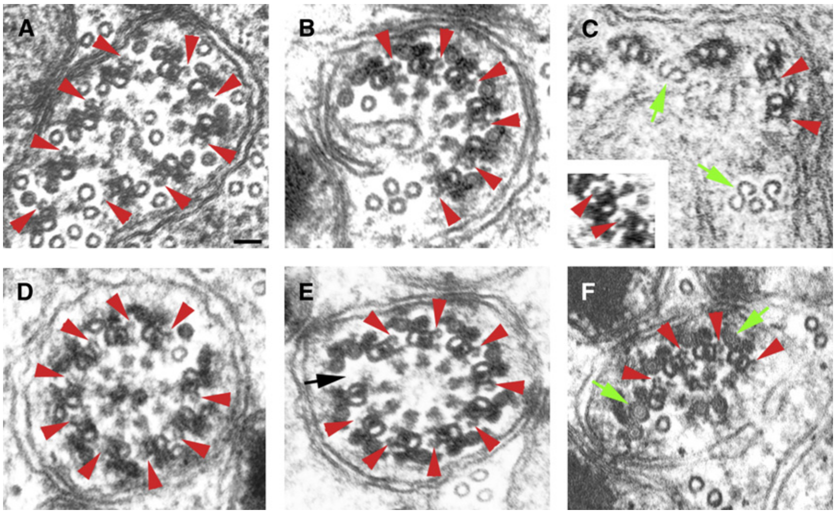

Figure S1. Axonemes Utilizing Variant β-Tubulins Have Outer Dynein Arms If Amino Acid 57 Is Glycine, Even If Other Aspects of Axoneme Architecture Are Defective

(A–C) Axonemes in males expressing mutant alleles of *Drosophila* β2-tubulin.

(A) Abnormal axoneme in a sterile male that is heterozygous for $B2t^D$, a dominant β2 mutation that causes profound disruption of axoneme assembly [S1, S2]. Although axoneme architecture is disorganized, outer dynein arms (ODAs) are present on all doublets (indicated by red arrowheads). The $B2t^D$ mutation is a lysine substitution for glutamic acid 194 [S3].

(B) Partial axoneme in a sterile homozygous $B2t^7$ male; ODAs are present on all doublets (indicated by red arrowheads). $B2t^7$ axonemes are nonfunctional and often lack the central pair [S4]. The $B2t^7$ mutation is an asparagine substitution for aspartic acid 114 [S3].

(C) Abortive axonemal doublets in a homozygous $B2t^8$ male. The $B2t^8$ protein disrupts all categories of microtubules in which β2 normally functions [S3, S4]. The most notable defect is formation of unclosed or S-shaped microtubules (examples indicated by green arrows). In rare morphologically normal doublets, ODAs are present, as shown here (indicated by red arrowheads). The inset shows doublets in another $B2t^8$ male; ODAs are present (indicated by red arrowheads). The $B2t^8$ mutation is a lysine substitution for glutamic acid 288 [S5].

(D–F) Axonemes in males expressing chimeric or heterologous β-tubulins.

(D) Axoneme in a sterile male expressing two transgenic copies of the chimeric protein β2β1C16 with no wild-type β2 present. In β2β1C16, the 16 amino acids of the β1 variable C-terminal tail replace the 15 amino acids of the β2 C-terminal tail (see CTT sequences in Table S2) [S6]. β2β1C16 can support axoneme assembly, but intact axonemes are rare; the great majority of axonemes in β2β1C16 males are fragmented or disorganized [S6]. In the example here, all nine doublets are present, but the axonemal ring is opened; ODAs are present on all doublets (indicated by red arrowheads).

(E) Defective 9 + 0 axoneme in a sterile male expressing one transgenic copy of *Drosophila* β1-tubulin in late stages of spermatogenesis in which β1 is not normally expressed, with no wild-type β2 present. β1 can support assembly of all aspects of axonemes except for the central pair; other associated structures, including dynein arms, spokes, and linkers, are present [S7, S8]. β1 axonemes fail to maintain longitudinal stability and are very short (only approximately 1% of the normal 2 mm length of the wild-type *Drosophila* sperm tail). The ring of the β1 axoneme here is slightly disrupted; all doublets have ODAs (indicated by red arrowheads), except for the doublet at the opening in the ring, which lacks an ODA (indicated by black arrow).

(F) Partial axoneme in a sterile male in which the β2 homolog from the moth *Heliothis virescens* (*H.v.*) is coexpressed with β2. *H.v.* β2 can not alone support axoneme assembly at all and exerts strong dominant disruption when it is coexpressed with β2 [S9]. The axoneme shown is from a male in which the moth β2 constitutes approximately 60% of the total β-tubulin. The most striking phenotype resulting from expression of the moth β2 homolog in the *Drosophila* testis is the conversion of some of the accessory microtubules from the normal 13 protofilament architecture of the fly to the moth's 16 protofilament architecture (indicated by green arrows). However, even in fragmented or partial axonemes, the doublets have ODAs, as shown here (indicated by red arrowheads). All of the β-tubulins utilized in the axonemes shown in (A)–(F) have glycine 56 in the internal variable region (see sequences in Table 1). The scale bar represents 50 nm.

Table S1. Addition of Outer and Inner Dynein Arms and Ectopic Luminal Filaments in Doublet Microtubules of Axonemes in Wild-Type Males and Males Expressing β-Tubulins with Variant Internal Variable Regions

		Dynein Arms			Luminal Filaments in Doublet MTs		
β-Tubulins Utilized in Axonemes	Number of Males	Total Axonemes Scored	Average ODA/ Axoneme	Average IDA/ Axoneme	Total Axonemes Scored	Average A Tubes with LF/Axoneme	Average B Tubes with LF/Axoneme
Males Expressing Only Wild-Type β2							
Wild-type males (two copies β2: males have only wild-type β2; normal β-tubulin pool size)	4	64	9	9	35	0	0
Hemizygous β2 (one copy β2: males have only wild-type β2, but half the normal β-tubulin pool size)	10	74	8.96	8.96	69	0	0
Males Expressing $B2t^6$							
Homozygous $B2t^6$ (two copies $B2t^6$: males have only mutant $\beta 2_6$; normal β-tubulin-pool size)	13	100	3.2	8.7	86	7.1	0.8
Hemizygous $B2t^6$ (one copy $B2t^6$; males have only mutant $\beta 2_6$; half the normal β-tubulin-pool size)	4	23	3.1	8.8	9	6.2	2.9
Heterozygous $B2t^6$ (one copy $B2t^6$ + one copy β2; 50% mutant $\beta 2_6$; normal β-tubulin-pool size)	14	111	8.4	8.8	95	0.4	0
Males Coexpressing β3β2C & β2 (% β3β2C in total β-tubulin pool)							
β3β2C + β2 (50% β3β2C)	4	50	1.7	8.8	50	7.0	0.1
β3β2C + β2 (33% β3β2C)	6	105	7.3	8.96	100	1.8	0
β3β2C + β2 (25% β3β2C)	4	46	7.7	8.96	45	1.4	0
β3*+ β2 (50% β3*)	4	33	3.8	8.9	33	4.4	0.03
Males Coexpressing β3 or β3* and β2 (% β3 or β3* in total β-tubulin pool)							
β3* + β2 (33% β3*)	4	45	5.4	8.98	45	1.8	0.02
β3 + β2 (31% β3)	9	58	2.3	8.95	58	5.0	0.78
β3* + β2 (25% β3*)	1	11	7.6	9	11	0.5	0
β3 + β2 (25% β3)	1	6	3.5	8.7	6	4.3	0.17
β3 + β2 (18% β3)	2	18	6.2	8.94	18	2.5	0

Complete data sets are shown for the studies presented in Figures 1 and 2 and Tables 2 and 3, documenting the dependence of addition of outer dynein arms (ODAs) on sequence in the axoneme β-tubulin internal variable region (IVR). Axoneme morphology and number of ODAs and inner dynein arms (IDAs) per axoneme were scored as described in Experimental Procedures. In *Drosophila* spermatogenesis, luminal filaments (LFs) are added to central- pair and accessory microtubules in late intermediate stages, well after dynein arms are added. To avoid false negatives, we scored LFs in doublet microtubules only in axonemes in which LFs were present in the accessory and central-pair microtubules.

In *Drosophila* males, β2 is the only β-tubulin utilized in sperm axonemes [S1, S2]. The transgenic constructs for male germline expression of other β-tubulins β3, β3*, and β3β2C, shown in Table 2 and Table S1, were as follows: (1) *p{β3}* contains the 5′ UTR of β2 joined to the coding sequence of *Drosophila* β3-tubulin, plus 3′ UTR from β3. This construct drives β3 in the male germline with the same temporal and spatial specificity as β2, but at lower levels than wild-type β2 [S10, S11]. β3 is not normally expressed in the male germline [S12, S13]. (2) *p{β3*}* contains the 5′UTR and first 19 codons of β2 joined to the remainder of the β3 coding sequence, inserted into 3′ UTR sequences from β2 [S11]. (3) *p{β3β2C}* encodes a chimeric β-tubulin in which the final 104 amino acids of β2 replace the equivalent C-terminal residues in β3*, with 3′ UTR sequences from β2 [S11]. β2′s 5′ UTR specifies pattern of expression; the β2 3′ UTR is necessary to obtain the correct level of expression [S11]. *p{β3*}* and *p{β3β2C}* drive the respective proteins in the male germline with the same temporal and spatial specificity and at the same level as β2. β3 and β3* differ in sequence in two conservative amino acids in the first exon; at equivalent levels of expression, the two proteins exhibit the same phenotypes in spermatogenesis [S10, S11].

Expression levels of variant tubulins in the male germline were determined by gel electrophoresis as previously described. $B2t^6$ is an EMS-induced mutation in the endogenous β2 gene; the mutant $B2t^6$ protein is expressed in the same pattern and level as wild-type β2 [S3, S4, S14, S15]. β3* and β3β2C are also expressed at the same levels as wild-type β2; thus, expression level directly reflects gene copy number [S11]. Gene copy numbers in males expressing different relative amounts of wild-type β2 and one of the transgenic proteins are as follows: Transgenic protein is 50% of the total β-tubulin pool in males with one copy each of the β2 gene and the transgene. Transgenic protein is 33% of the total β-tubulin pool in males with two copies of β2 and one copy of the transgene. Transgenic protein is 25% of the total β-tubulin pool in males with three copies of β2 and one copy of the transgene. Transgenic β3 is expressed at approximately 22% the normal β2 level [S10, S11]. Transgenic β3 is 31% of the total β-tubulin pool in males with one copy of the β2 gene and two copies of the *p{β3}* transgene or an equivalent gene ratio. Transgenic β3 is 25% of the total β-tubulin pool in males with two copies of the β2 gene and three copies of the *p{β3}* transgene. Transgenic β3 is 18% of the total β-tubulin pool in males with one copy of the β2 gene and one copy of the *p{β3}* transgene or an equivalent gene ratio.

Male fertility depends on both the number of ODAs and the C-terminal tail, as illustrated by males expressing β3, β3*, or β3β2C. As shown in the table, deficits in the number of ODAs per axoneme and presence of ectopic LFs in doublets are approximately the same in males expressing β3, β3*, or β3β2C at equivalent proportions in the male germline β-tubulin pool. However, the ability to generate functional axonemes depends on the presence of β2 C-terminal sequences [S11]. When β3 or β3* constitute 25% or more of the β-tubulin pool, males are completely sterile. However, β3β2C exerts a less severe dominant phenotype: Males are sterile when β3β2C constitutes 50% of the β-tubulin pool, weakly fertile when β3β2C constitutes 33% of the β-tubulin pool, and fertile when β3β2C constitutes 25% of the β-tubulin pool.

Table S2. Internal Variable Region and C-Terminal Sequences for Axonemal and Nonaxonemal β-Tubulins Compiled in Table 4

	IVR[a]	C Terminus[b]	References and/or NCBI Protein Database Accessions
I. Axonemal β-Tubulins[c]			
Animals			
D. melanogaster, β2 (85D) (identical in all *Drosophila* species)	YNEA TGA KYV	EATAD EEGEFDEDEEGGGDE$_{446}$	[S5], A27810; [S16], NP_524290 [S17]
Moth, *Heliothis virescens*, testis β (β2 homolog)	YNEA SGG KYV	DATAD DEGEFDEEAEGEGLE	[S9], AAC47425
Moth, *Bombyx mori*, β2 homolog	YNEA SGG KYV	DATAD DEGEFDEEAEGEGLE	[S18], AB072310
Mosquito, *Anopheles gambiae*, β2 homolog	YNEA SGG KYV	EATAD DEGEMDEEEEGGED	[S19], EAA10161
Honey bee, *Apis mellifera*, β2 homolog	YNEA SGG KYV	EATAE EEGEFDEEEEGEGEHP	[S20], XP_394038
Beetle, *Tribolium castaneum*, β2 homolog	YNEA TGG KYV	DATAE EEGEFDEEEEGDNEGEN	[S21], XP_969993
Vertebrate class IVb[d], major testis isotype; expressed in many tissues	YNEA AGG KYV	DATAE EEGEFEEEAEEEAE	Chicken [S22]
	YNEA TGG KYV	DATAE EEGEFEEEAEEEVA	Human, AAN87335; Mouse, D25437
Zebra fish, *Danio rubrio*, similar to class IV (two sequences with the same IVR)	YNEA TGG KYV	DATAE EEGEFEEEGEKELA	[S23], NP_942104
	YNEA TGG KYV	DATAE EEGEFEEEAEDDA	NP_942113
Antarctic yellowbelly rockcod, *Notothenia coriiceps*, Ncβ1–3	YNEA SGG KYV	DATAE EEGEFEEEGEDLA (Ncβ1)	[S24]
	YNEA SGG KYV	DATAE EEGEFEDEGEDLA (Ncβ2)	
	YNEA SGG KYV	DATAE EEGEFEEEGYEDGA (Ncβ3)	
Sea urchin, *Strongylocentrotus purpuratus*	FNEA TGG KYV	DATAE EEGEFEEEEEEDLEQ	[S25], XP_001192274
Sea urchin, *Paracentrotus lividus*	YNEA TGG KYV	DATAE EEGEFDEEEEGDEEAA	[S26], P11833
Ascidian, *Halocynthia roretzi*	YNEA TGG KYV	DATAE EEGEFDEEEGEDEEV	[S27], BAA22382
Plants[e]			
Moss (Bryophyte), *Physcomitrella patens,* Beta 1-6 (six sequences with the same IVR)	YNEA SGG RYV	DASAE EEGEYEEDLEEA (B1,2,6)	[S28], AAQ88113–AAQ88118
	YNEA SGG RYV	DASAE EEGEYEDDPEEA (B3)	
	YNEA SGG RYV	DASAE EEGEYEDDLEEA (B5)	
	FDEA SGG RYV	DASAE EEGEYEEDLEEA (B4)	
Spikemoss (Lycophyte), *Selaginella moellendorffi*, (five sequences with the same IVR)	FNEA SGG RYV	DASAD DEGEFEEEA	[S29]
	FNEA SGG RYV	DATAD EEGEFEEEE	
	FNEA SGG RYV	DASAD EEGEFEEEEGEG	
	FNEA SGG RYV	DASAD EEGEFEEEGGEA	
	FNEA SGG RYV	DASAD EEGEFEEEGAEA	
Fungi			
Blastocladiella emersonii	YNEA TGG KYV	DATAD DEGELDEEEGEGEAEAA	[S30], ABK34895
Protists			
Chlamydomonas reinhardti	FNEA TGG RYV	DASAE EEGEFEGEEEEA	[S31], EDP02933
Tetrahymena pyriformis	YNEA TGG RYV	DATAE EEGEFEEEEGEN	[S32], CAA31258
Paramecium tetraurelia	YNEA TGG RYV	DATAE EEGEFEEEGEQ	[S33, S34], AA47663
Euglena gracilis	YNEA TGG RYV	DATVE EEGEFDEEEDVEQY	[S35], AAK37837
Polytomella agilis	FNEA TGS RYV	DASAE EEGEFGEEEEEN	[S36], P22852
Volvox, *Carteri f. nagariensis*	FNEA TGG RYV	DASAE EEGEFEGEEEEN	[S37], CAA31334
Diatom, *Thalassiosira weissflogii*	FNEA TGG RYV	DATAD EEGEFDEDEMEG	[S38]; Q9LKI8
Brown alga, *Ectocarpus variabilis*, β5	FNEA TGG RYV	DATAE EEGEFDEDEELDDAMG	[S38, S39]; P30156
Stylonychia lemnae	YNEA TGG RYV	DATAE D*EEEMDEE*QME	[S40]
Trypanosoma brucei	FDEA TGG RYV	DATIE EEGEFDEEEQY	[S41], CAJ16365
Plasmodium falciparum	YNEA TGG RYV	DATAE EEGEFEEEEGDVEA	[S42], CAA34207
Toxoplasma gondii	YNEA TGG RFV	DATAE EEGEFDEEEGEMGAEEGA	[S43], P10878
Leishmania (sequences from 11 species with two different IVRs)	GDES TGG RYV	DATVE EEGEYDEEQEAY (one species)	[S44]
	GDES TGG RYV	DATVE EEGEYEEEQEAY (one species)	
	GDES TGG RYV	DATVE EEGEYDEEEEAY (two species)	
	GDES TGG RYV	DATVE EEGEFDEEEEAY (one species)	
	GDES AGG RYV	DATVE EEGEYDEEQEAY (three species)	
	GDES AGG RYV	DATVE EEGEYEEEQEAY (one species)	
	GDES AGG RYV	DATVE EEGEYDEEEEAY (two species)	
Physarum polycephalum, β1	YNEA TGG KYV	DATID D*EEGGEEE*EGGAEEEARQRKH YVIDYVPSVCVILIR	[S45]
Naegleria gruberi	YNEA TGG RYV	DATAE EEGEFDENEGAEGEEQPADY	[S46], P34108

(*Continued on next page*)

Table S2. *Continued*

	IVR[a]	C Terminus[b]	References and/or NCBI Protein Database Accessions
Giardia lamblia	FNEA AGG RYV	EAGVD EGEEFEEEEDFGDEYA	[S47], EDO79714
Choanoflagelate, *Monosiga brevicollis*	YNEA TGG KYV	DATAE EEGEFDEEEEEME	[S48], EDQ92151
II. Nonaxonemal β-Tubulins[f]			
Animals			
D. melanogaster, β1	YNEA SGG KYV	EATAD EDAEFEEEQEAEVDEN	[S49], Q24560; [S16], NP_523795
C. elegans, Mec 7 (neural specific)	YNEA GSN KYV	EAAAD EDAAEAFDGE	[S50], CAA33320
Vertebrate class I[d], constitutive; many tissues	YNEA TGG KYV	DATAE EEEDFGEEAEEEA	Chicken, NP_990646; Human, AAB59507
Vertebrate class II[d], major neuronal; many tissues	YNEA TGN KYV	DATAD EQGEFEEEGEEDEA	Chicken, P32882; *Xenopus*, NP_001079533
	YNEA AGN KYV	DATAD EQGEFEEEEGEDEA	Human, AAN85571
Vert. Class III[d], minor neuronal; neuron specific	YNEA SSH KYV	DATAE EEGEMYEDDEEESEQGAK	Chicken, P09652
	YNEA SSH KYV	DATAE EEGEMYEDDEEESEAQGPK	Human, AAC52035
Vertebrate class IVa[d], major neural; brain specific	YNEA TGG KYV	DATAE EQGEFEEEAEEEVA	Human, X00734
Vertebrate class V[d], minor constitutive; absent from neurons	YNES SSQ KYV	EATAN DGEEEEAFEDDEEEINE	Chicken, P09653
	YNES SSQ KYV	DATVN DGEEAFEDEDEEEINE	Chinese hamster, AAZ14959
Vertebrate class VI[d], major erythrocyte/ platelets; hematopoiesis specific	FNEA YSH KYV	DATAD VEEYEEAEASPEKET	Chicken, P09207
	YNEA YGK KYV	DVRAG LEDSEEDAEEAEVEEDKDH	Mouse, CAM26336
	YNEA YGR KYV	DAKAV LEEDEEVTEEAEMEPEDKGH	Human, AAH33679
Zebra fish, *Danio rubrio*, similar to vertebrate class VI	FNEA HGG KYV	DATAD MDYEEEEVMEEESSIAH STQVEIKTEVVTETSVSE	[S23], XP_686874
Plants[e]			
Zea Maize, Beta-1, not expressed in pollen	YNEA SCG RFV	DATAD EEGEYEDEEEGDLQD	[S51], CAA37060
Zea Maize, Beta-2, expressed in pollen (not major)	YNEA SCG RFV	DATAD EEADYEEEEAAAE	[S51], P18026
Zea Maize, Beta-3, major pollen expression	YNEA GGG RYV	DATAE EYDEEEQDGEEEHD	[S52], Q43695
Zea Maize, Beta-4, major pollen expression	YNEA GGG RYV	DATAE EYEEEEHDGEEEHA	[S52], CAA52719
Zea Maize, Beta-5, expressed in pollen; abundant in vegetative tissues	YNEA SCG RFV	DATAD EEAEYEDEEAIQDE	[S52], CAA52720
Rice, *Oryza sativa*	YNEA SCG RFV	DATAD DEEEDYGDEEEDEVAA	[S53], CAA55912
Arabidopsis thaliana, Beta 8	YNEA SCG RFV	DATAD EEEGYEYEEDEVEVQEEQ	[S54], BAB10059
Arabidopsis thaliana, Beta 9	FNEA SGG KYV	DATVG EEEYEEDEEEEEA	[S55], CAB45884
Eucalyptus grandes	YNEA SGG RYV	DATAD EELEYEDEEDAEQDM	[S56], ABS50669
Lichen, *Parmelia serrana*	A SNN KYV	Not available	[S57], AAO20049
Spike moss (Lycophyte), *Selaginella moellendorffi* (six sequences with three different IVRs)	YNEA SGG RFV	DASAE EEYDEEAEELEQ	[S29]
	YNEA SGG RYV	DAAVE ADYEDDDEEQAA	
	YNEA TGG RYV	DASAE EEGEYEDEAEELQA	
	YNEA TGG RYV	DASAD DEYEEEEPEEELQS	
	FTEA TGG RYV	DARPD DEGDYGEEEEQEGEYSQMS	
	FNEA SAG RYV	DASID DDFGEDPTCSSVQSTA	
Fungi			
Candida albicans	FNEA TSG KYV	EASID EEELEYADEIPLEDAAME	[S58], AAA34375
Saccharomyces cerevisiae	FNEA SSG KWV	EATVE DDEEVDENGDFGAPQNQ DEPITENFE	[S59], P02557
Beauveria bassiana	FNEA SGN KYV	DAGID DEEEEYEEELPIEGEEPL	[S60], ABD61945
Schizosaccharomyces pombi	FNEA AGG KYV	EAGID EGDEDYEIEEEKEPLEY	[S61], NP_596650
Aspergillus nidullans, BenA	FNEA SGN KYV	DASIS EGEEEYAEEEIMEGEE	[S62], 1312295A
Aspergillus nidullans, TubC	FNEA GGN KYV	EATVS DGEGAYDAEEGEAYEQEE	[S62], 1312295B
Septoria nodorum	FNEA SGN KFV	EASIS EGEEEYDEE APLEAEE	[S63], AAB25800
Neurospora crassa	FNEA SGN KYV	DAGVD EEEEEYEEEAPLEGEE	[S64], AAA33617
Flax rust, *Melampsora lini*	YNEV AGN KYV	EAHVD EDEVDEEVYEDEAPPEE	[S65], AAG33239
Uromyces viciae-fabae	YNEV AAN KYV	EAHMD DEEAEEAYEDEAPPEE	[S66]
Dissophora decumbens	FNEA AGA KYV	Not available	AAY40433
Ellisomyces anomalus	YNEG SAG KYV	Not available	AAY40413

Table S2. *Continued*

	IVR[a]	C Terminus[b]	References and/or NCBI Protein Database Accessions
Protists			
Physarum polycephalum, β2, major spindle beta	YNEV AGS KYV	EASVD DEAMEDDAEAEGGAGQNEAVEEF	[S67], AAA29977
Social amoeba, *Dictyostelium discoideum*	YNEA RDG KYV	ETEED GGEYQEEHEEHEEQAEN	[S68], P32256
III. Nonaxonemal β-Tubulins with More Than Three Amino Acids in the IVR			
Animals			
D. melanogaster, β3 (60C)	YNEA SAVTRSSGG KYV	EATAD DEFDPEVNQEEVEGDCI	[S5], P08841
Apis mellifera, β3 homolog	YNEA SVATSTNGG KYV	EATAE EDFEAEECADDFETCDQE	[S20], XP-394469
Bombyx mori, TUB3, β3 homolog	YNEA SVATAESGG KYV	EATAE DDTEFDQEDLEELAQDEHHD	[S18], NP_001036888
Anopheles gambiae, β3 homolog	YNEA SAVSRASGG KYV	EATAD DEFEQEDCQDEMEGECV	[S19], EAA09971
Anatarctic yellowbelly rockcod, *Notothenia coriiceps*, Similar to vertebrate class III	YNEA SGSTGS KYV	DATAD EMGEYEEDEIEDEEEVRDHVRH	[S24], AAG15317

Sequences are shown for representative axonemal (I) and nonaxonemal (II and III) β-tubulins in animals, plants, fungi, and protists. (Single-cell organisms are listed together for convenience, although protist species fall into many different phylogenetic groups [S69].)

[a] Internal variable regions: Amino acids 55–57 in most β-tubulins are underlined. Nonaxonemal β-tubulins with more than three amino acids in the IVR are listed separately (III). The IVR is flanked by more conserved sequences, as shown.

[b] C-terminal sequences: Shown after a highly conserved EYQQYQ sequence common to all β-tubulins. The variable C-terminal tail (CTT) starts at the position equivalent to residue 432 in *Drosophila* β2, indicated by spacing of the sequences shown.

[c] Axoneme β-tubulins: Conserved C-terminal consensus axoneme motifs are underlined (EGEF and then three additional acidic residues [S9]). Axoneme tubulins shown include known axoneme-utilized β-tubulins (including β-tubulins from species with multiple β-tubulin genes for which expression pattern is known and β-tubulins in ciliated or flagellated organisms with only one β-tubulin sequence) and also presumptive axonemal sequences identified by the presence of the CTT axoneme motif. A few known axonemal β-tubulins vary in one position (for example, EGEMDEE in mosquito). The axoneme-motif region is underlined and italicized in identified axonemal β-tubulins in which the sequence differs from the consensus motif sequence at more than one position. The proximal region of the C-terminal tail containing the axoneme motif is essential for axoneme assembly, but not for other kinds of microtubules; the distal region is dispensable [S6, S8, S14, S70, S71]. The axoneme motif appears to function in mediating axoneme structural stability, assembly of the central pair, and motility [S6, S8, S71, S72].

Axonemal β-tubulins have been characterized primarily for animals and protists. Many aquatic fungi have flagellated gametes, but we found only one presumptive axoneme β-tubulin sequence for this group and only two for plant species with flagellated gametes (see note e below). See also [S9, S38, S73] for comparisons of C-terminal sequences in axonemal and nonaxonemal β-tubulins.

[d] Vertebrate β-tubulins: Conserved gene family structure with seven distinct isotype classes that exhibit conserved patterns of expression (see [S22] and [S73–S77] and references therein). The vertebrate β-tubulin classes were defined by studies of β-tubulin gene families in chicken and mammalian species (including human, mouse, rat, rabbit, pig, cattle, and hamster); *Xenopus* β-tubulins also appear to fit the defined classes. However, teleost β-tubulin families may not have the same organization [S24]. Thus, the vertebrate β-tubulin family organization as originally defined may be restricted to tetrapod vertebrates. In the table, we have therefore provided representative examples of each isotype class for tetrapod species and have listed representative teleost β-tubulins separately.

Expression patterns for the vertebrate isotypes are given in the table. The class IVb isotype is the major vertebrate axoneme β-tubulin. Vertebrate isotypes other than class IVb have primarily or only nonaxonemal expression or function. Many species, including *Drosophila*, other insects, and many protists, utilize only a single axonemal type β-tubulin in motile axonemes. However, this is not the case in mammals. In axonemes of mammalian cilia, the major testis class IVb β-tubulin isotype and the class I β-tubulin are coexpressed; class V β-tubulin is also present in some but not all cilia types [S72]. Thus, axonemes may coincorporate β-tubulins of both the axonemal and nonaxonemal type. We have shown experimentally that axoneme assembly in *Drosophila* spermatogenesis can accommodate some coexpression of nonaxonemal β-tubulins along with the testis-specific β2-tubulin isoform, the relative amount compatible with functionality depending on the particular isotype (see Figure 2) [S3, S6, S10, S11, S14, S70, S71].

[e] Plant tubulins: We have listed sequences from *Chlamydomonas* and related species with protists according to the convention used in the table, but note that molecular phylogenetic analyses place these single-cell green algae with multicellular plants [S69]. In multicellular plants, flagellated gametes occur in many nonangiosperm basal groups, including gingko, cycads, ferns and related groups (including equisetum), lycophytes (spikemosses, club mosses, and quillworts), and bryophytes (mosses, hornworts, and liverworts) [S78 and S79]. At present, β-tubulin sequences with both IVR and CTT are available for two species with motile gametes: a bryophyte, the moss *Physcomitrella patens*, and a lycophyte, the spikemoss *Selaginella moellendorffii.* The *P. patens* β-tubulin family consists of six genes with very similar coding sequences [S28]; all six have an IVR sequence of SGG and a CTT axoneme-motif region sequence of EGEYEDD, compatible with other known variant axoneme motifs. We have included the *P. patens* sequence with axonemal β-tubulins (counted as one representative axonemal sequence in Table 4). Eleven complete β-tubulin sequences are presently available from the genome of *S. moellendorffii* [S29]. Of these, five have a canonical CTT axoneme motif of EGEFEEE and an IVR sequence of SGG; these are listed with axonemal β-tubulins (counted as one representative axonemal sequence in Table 4). The remaining six *S. moellendorffii* sequences are listed with nonaxonemal sequences. All angiosperms and gymnosperms other than gingko and cycads lack motile axonemes; thus, their β-tubulins are per force nonaxonemal.

[f] Nonaxonemal sequences: β-tubulins with known nonaxonemal expression and/or utilization, and β-tubulins in species that do not have motile axonemes [S9, S38, S73].

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