Genetic and Molecular Analysis of a *Caenorhabditis elegans* β-Tubulin That Conveys Benzimidazole Sensitivity

Monica Driscoll, Ellen Dean, Evelyn Reilly, Elke Bergholz, and Martin Chalfie

Department of Biological Sciences, Columbia University, New York 10027

Abstract. Benzimidazole anti-microtubule drugs, such as benomyl, induce paralysis and slow the growth of the nematode *Caenorhabditis elegans*. We have identified 28 mutations in *C. elegans* that confer resistance to benzimidazoles. All resistant mutations map to a single locus, *ben-1*. Virtually all these mutations are genetically dominant. Molecular cloning and DNA sequence analysis established that *ben-1* encodes a β-tubulin. Some resistant mutants are completely deleted for the *ben-1* gene. Since the deletion strains appear to be fully resistant to the drugs, the *ben-1* product appears to be the only benzimidazole-sensitive β-tubulin in *C. elegans*. Furthermore, since animals lacking *ben-1* are viable and coordinated, the *ben-1* β-tubulin appears to be nonessential for growth and movement. The *ben-1* function is likely to be redundant in the nematode genome.

In most multicellular organisms α- and β-tubulin genes are encoded in gene families (for review see Cleveland and Sullivan, 1985). The specific roles that the individual α- and β-tubulins of an organism serve are not well understood. One possibility is that different isotypes could have functional specificity. Support for this "multitubulin hypothesis," first suggested by Fulton and Simpson (1976), derives from findings that a *Physarum* β-tubulin is exclusively used in the mitotic spindle of plasmodium (Burland et al., 1988) and a minor neuronal β-tubulin isotype is spatially specialized in its subcellular pattern of expression (Asai and Remolona, 1989). That some variable region substitutions are conserved across species also suggests that these changes could have functional significance (Sullivan and Cleveland, 1986; Rudolph et al., 1987). A second possibility is that different isotypes could function in a cell-specific manner, each carrying out diverse functions within a given cell type. In *Drosophila*, for example, a sperm-specific β_2-tubulin isotype participates in the meiotic spindle, cytoplasm, axonemal central pair, and axonemal outer doublets (Kemphues et al., 1982). A third possibility is that the tubulins are functionally redundant, each capable of participating in many microtubule-controlled processes in several cell types. Functional redundancy has been demonstrated for some α- and β-tubulins (Adachi et al., 1986; Schatz et al., 1986; May et al., 1985; Weatherbee et al., 1985).

We have been characterizing the β-tubulin genes in *Caenorhabditis elegans* to determine the roles of β-tubulin family members in this organism. Microtubules in *C. elegans* are distinctive. In contrast to the 13-protofilament microtubules present in most eukaryotes (Tilney et al., 1973), the majority of the cytoplasmic microtubules of *C. elegans* have 11 protofilaments (Chalfie and Thomson, 1982). Six cells, the touch receptor neurons, have 15-protofilament microtubules. The α- and β-tubulin gene families in *C. elegans* are small (there are three to five α-tubulins and three to four β-tubulins; Gremke, 1986). To date, two β-tubulin genes from *C. elegans*, *mec-7* and *tub-1*, have been cloned and sequenced (Savage et al., 1989; Gremke, 1986). The *mec-7* β-tubulin is required for the 15-protofilament microtubules of the touch receptor neurons. The role of the *tub-1* β-tubulin is unknown.

To identify tubulin genes genetically, we first characterized the effects of benzimidazoles on *C. elegans*. Benzimidazoles exert toxic effects on nematodes by binding to tubulin and inhibiting polymerization of heterodimer subunits into microtubules (Ireland et al., 1979; Laclette et al., 1980; Lacey and Prichard, 1986). These anti-microtubule agents have been used in the selection of resistant mutations in *Neurospora*, *Aspergillus*, *Physarum*, *Schizosaccharomyces*, and *Saccharomyces* (Borck and Braymer, 1974; Orbach et al., 1986; Sheir-Neiss et al., 1978; Burland et al., 1984; Schedl et al., 1984; Yamamoto, 1980; Hiraoka et al., 1984; Thomas et al., 1985). Most often the identified mutations map to β-tubulin genes.

When *C. elegans* is reared in the presence of benzimidazoles, growth is slowed and locomotion is severely uncoordinated (Chalfie and Thomson, 1982). In this study we screened for benomyl-resistant mutants. We find that drug resistance can be imparted by mutations in a single gene, *ben-1* (for *ben*zimidazole-sensitivity), which encodes a β-tubulin. Some resistant alleles are complete deletions of the gene, revealing an unexpected mechanism for benzimidazole resistance. Since the resistant deletion mutants are otherwise wild type, our results demonstrate functional redundancy within the *C. elegans* β-tubulin gene family: *ben-1* is expressed in wild-type animals but is not essential for viability or coordination.

Materials and Methods

Growth and Maintenance of Nematode Strains

In initial experiments, wild-type *C. elegans* var. Bristol (strain N2) and mutant animals were grown on the OP50 strain of *Escherichia coli* on NGM agar as described by Brenner (1974). In later experiments, we used a streptomycin-resistant variant of this strain (OP50-1; a gift from A. Stretton, University of Wisconsin, Madison, WI) that was plated onto modified NGM agar containing 0.2 g/liter streptomycin and 10 mg/liter nystatin (added to inhibit fungal contamination). Animals were usually grown at 25°C.

In addition to the mutations affecting benomyl resistance (listed in Table I), the following mutations were used: on linkage group III, *daf-2(e1370)* (Riddle et al., 1981) and *unc-93(e1500)* (Greenwald and Horvitz, 1980); on linkage group X, *sup-7(st5)* (Waterston, 1981) and *dpy-7(e1324)* (Brenner, 1974). The *C. elegans* hybrid strain TR679, which displays an increase in germ line transposition due to the mutator defect *mut-2(r459)* (Collins et al., 1987), was used in some mutant screens.

Measurement of Drug Sensitivity

Benomyl (methyl 1-[butyl-carbamoyl]-2-benzimidazole-carbamate) and other benzimidazoles and benzimidazole carbamates were dissolved in DMSO and then added to melted modified NGM agar. The final concentration of DMSO in the plates (0.1%) had no detectable effect on *C. elegans* movement or development. Benomyl and carbendazim were gifts from Dr. E. Beyer (DuPont Co., Wilmington, DE). Thiabendazole, mebendazole, and nocodazole were purchased from Janssen Pharmaceutica (Piscataway, NJ). Colchicine (Sigma Chemical Co., St. Louis, MO) was added as dry powder to melted NGM agar to a final concentration of 1 mM. Sensitivity to drugs was assayed by comparison of movement and body posture in the presence and absence of drugs. All larval stages and adults were observed for drug effects. Touch sensitivity was scored as described by Chalfie and Sulston (1981).

Mutageneses

Young adult wild-type animals were mutagenized at 20°C with ethyl methanesulfonate (EMS)[1] (Brenner, 1974) or γ-ray irradiation from a ^{137}Cs source (8,100 rads). Single mutagenized animals were placed on NGM agar plates containing 7 μM benomyl at 25°C (wild-type animals grown under these conditions are fertile but virtually paralyzed; Chalfie and Thomson, 1982). Animals from the mutator strain TR679 were plated on benomyl-containing plates at 20°C. Since *C. elegans* is a self-fertilizing hermaphrodite, the F1 generation produced on the selection plates may include heterozygous mutations and the F2 generation includes the mutations in the homozygous state. After F2 progeny had been produced, the plates were examined for moving resistant animals. This was facilitated by flooding the plates with a few milliliters of M9 buffer (Miller, 1972). Only resistant animals thrash about in liquid. Putative resistant mutants were removed and replated onto benomyl-containing plates, and their progeny were scored for resistance. Homozygous mutant stocks were maintained.

Genetic Analyses

Because of the temperature-sensitive dominance of many of the *ben-1* mutations (see below), complementation tests were done at 15°C. In three-factor crosses, all of the *ben-1* mutations tested (*e1880, e1910, e1911, u101–u117, u134, u135,* and *u347*) mapped between the two closest identified markers on chromosome III, *daf-2(e1370)* and *unc-93(e1500)*. Of the recombinants derived from *daf-2* + *unc-93*/+ *ben-1* + heterozygotes, 200 of 293 Daf recombinants and 96 of 312 Unc recombinants were benomyl resistant. Temperature sensitivity of homozygous and heterozygous mutants was tested by growing animals on benomyl-containing plates at 15 and 25°C.

All of the *ben-1* mutations from EMS mutagenesis were tested for suppression with the amber tRNA suppressor mutation *sup-7* (Waterston, 1981; Wills et al., 1983). *ben-1*/+; *dpy-7 sup-7*/+ + heterozygotes were constructed and suppression was assayed by the absence of Dpy Ben progeny at 25°C. None of the *ben-1* mutations were detectably suppressed.

Recombinant DNA Techniques

C. elegans DNA was prepared as described by Emmons et al. (1979). Restriction enzymes were purchased from New England Biolabs (Beverly, MA), and digests were performed according to the supplier's specifications except that 5 mM spermidine was included in the reactions. Agarose-gel electrophoresis was as described by Maniatis et al. (1982). DNA blot analysis was as described by Southern (1975) except that the transfer buffer was 10× SSC. Filters were hybridized in 6× SSC, 5× Denhart's solution, 0.5% SDS at 65°C. Washes were for 2 h in 0.1× SSC, 0.1% SDS at 65°C. Under these conditions, some hybridization of *ben-1* DNA and other cross-homologous *C. elegans* β-tubulin sequences occurs.

Labeling of DNA for hybridization probes was done by nick translation (Rigby et al., 1977) or random oligonucleotide priming (Feinberg and Vogelstein, 1983; oligonucleotides supplied by Pharmacia Fine Chemicals, Piscataway, NJ). Protocols for subcloning, DNA transformation, phage growth, plaque and colony screening, and other molecular techniques were from Maniatis et al. (1982).

Plasmids and Vectors

The chicken β-tubulin cDNA clone pT2 (Cleveland et al., 1980; Valenzuela et al., 1981) was a gift from N. Cowan, New York University Medical School (New York). pCe2002, a plasmid containing DNA from the *C. elegans* transposon Tc1, was described by Ruan and Emmons (1984). λ cloning vectors, EMBL4 (Frischauf et al., 1983) and λ ZAP (Short et al., 1988), were obtained from Stratagene, Inc. (La Jolla, CA). Subclones were made in plasmids pUC18, pUC19, (Yanisch-Perron et al., 1985), and pI48 (a gift from R. Yocum, Biotechnica, Cambridge, MA).

Table I. Properties of ben-1 Alleles

Allele	Source	Expression 15°C	Expression 25°C	Lowest paralyzing dose of benomyl at 25°C
				μg/ml
+				2.5
e1880	EMS	R	D	>80
e1910	EMS	R	D	>80
e1911	*	R	D	>80
u101	EMS	R	SD	20
u102	EMS	R	D	>80
u103	EMS	R	D	>80
u104	EMS	R	D	>80
u105	EMS	R	D	>80
u106	EMS	R	D	>80
u107	EMS	R	D	40
u108	EMS	R	D	>80
u109	EMS	R	D	>80
u110	EMS	R	SD	40
u111	EMS	SD	D	>80
u112	EMS	R	D	>80
u113	EMS	R	SD	>80
u114	EMS	R	R-SD	40
u115	EMS	R	D	>80
u116	EMS	R	D	>80
u117	EMS	R	R-SD	40
u134	TR679	R	R-SD	>20
u135	TR679	R	D	>80
u347	TR679	R	D	>80
u462	γ-ray	R	D	>80
u463	γ-ray	R	D	>80
u464	γ-ray	R	D	>80
u465	γ-ray	R	D	>80
u471	γ-ray	R	D	>80

The *ben-1* alleles used in this study are listed. Mutagens that were sources of mutations were EMS, the *C. elegans* mutator strain TR679, and γ-ray irradiation. Allele *e1911* (*) was obtained from a strain harboring an independent EMS-induced mutation. *ben-1* alleles were tested for dominance by mating in wild-type males and examining *ben-1*/+ male progeny for coordinated movement. *R*, recessive; *SD*, semidominant; *D*, dominant.

1. *Abbreviation used in this paper*: EMS, ethyl methanesulfonate.

Cloning ben-1 Alleles

The ben-1 Gene. DNA blot analysis identified a 7.4-kb genomic Eco RI restriction fragment homologous to chicken β-tubulin that had altered electrophoretic mobility in *ben-1* alleles derived from the mutator strain TR679. In progeny from *daf-2 + unc-93/+ ben-1(u134 or u347) +* heterozygotes this novel fragment segregated only into Daf or Unc recombinants that were also benomyl resistant (present in 5/5 *ben-1* mutant strains, absent in 10/10 *ben-1*(+) strains). To clone the wild-type fragment, genomic DNA was digested with Eco RI and fractionated by gel electrophoresis. DNA 7–8 kb in size was electroeluted and used to construct a partial library of *C. elegans* DNA in plasmid pI48, a vector that confers tetracycline resistance if it harbors an insertion. The library was transformed into *E. coli* strain HB101 (Boyer and Roulland-Dussoix, 1969) and a clone homologous to chicken β-tubulin, pTU58, was isolated. Hybridization experiments and restriction mapping confirmed that this clone contained the DNA of interest.

Since hybridization experiments and preliminary DNA sequence analysis demonstrated that the 3′ end of the *ben-1* gene was beyond the Eco RI site of pTU58, a genomic 4.6-kb Xba I restriction fragment that appeared to contain the entire coding sequence was identified and cloned. *C. elegans* DNA was digested with Xba I, the 4–5-kb fraction was ligated into λ ZAP to generate a partial library, and a β-tubulin–homologous clone that hybridized to *ben-1* sequences was identified. pTU59 has the 4.6-kb Xba I fragment from this phage in pUC19.

Cloning of Allele u347. The *ben-1* Eco RI restriction fragment from insertion allele *u347* is ~9.2 kb. 9–10-kb fragments from Eco RI–digested DNA prepared from *ben-1(u347)* were ligated into λ ZAP, and a *ben-1*–homologous clone was isolated from the partial library. Transposon Tc1 (Emmons et al., 1983; Liao et al., 1983) is inserted in *ben-1(u347)*: Tc1 sequences hybridize to the mutant clone but not the wild-type clone pTU58. Detailed restriction mapping, facilitated by DNA sequence information, mapped the insertion to position 1,230 ± 100 bp on the DNA sequence (Figs. 2 and 3). Hybridization experiments established that Tc1 is also the transposon in *ben-1(u134)* and positioned the insertion to nucleotide -90 ± 100 bp.

Cloning of ben-1 cDNAs. Approximately 30,000 plaques from a mixed stage *C. elegans* cDNA library (complexity of ~5 × 10^6, constructed in λgt10 by J. Ahringer and J. Kimble, University of Wisconsin, Madison, WI) were screened for homology to *ben-1* sequences. Five clones were isolated, two of which proved to contain *ben-1* sequences after partial DNA sequence analysis (cDNA1 and cDNA2). Both clones end at the Eco RI site near the 3′ end (position 3,052) and neither extends to the 5′ terminus of the gene.

DNA Sequence Analysis

To sequence the 4.6-kb Xba I fragment contained in pTU59, the 2.1-kb Bgl II fragment and the 1.4-kb Eco RI-Bgl II fragment were subcloned into pUC18 and the 0.7-kb Eco RI–Xba I fragment was subcloned into pUC19 (for a restriction map of the region see Fig. 2). For each parent plasmid, a series of deletions from either end of the inserted DNA was generated using nuclease Bal 31 (Gray et al., 1975) or exonuclease III (Henicoff, 1984). Deletion plasmids were religated or, when necessary, truncated fragments were subcloned into pUC18 or pUC19. Deletion subclones differing successively by ~250 bp were selected for double-stranded DNA sequencing by the dideoxy chain termination method of Sanger et al. (1977) using modified T7 DNA polymerase (Tabor and Richardson, 1987; Sequenase and M13 universal and reverse primers were from United States Biochemical Corp., Cleveland, OH). A few additional subclones were constructed to sequence across junctions of parent subclones and in regions of ambiguity. All DNA sequence data was obtained from both strands of the 4.6-kb Xba I fragment. cDNA1 and cDNA2 inserts were partially sequenced by cloning them into pUC19 and sequencing from universal and reverse primers. Sequencing reactions were electrophoresed in 6% acrylamide gels containing 7 M urea and 0.5× TBE (Maniatis et al., 1982).

Computer analysis of DNA sequence data was performed with MicroGenie programs (Queen and Korn, 1984; purchased from Beckman Instruments, Inc., Palo Alto, CA). Homology searches were done with the GenBank(R) database, version 58 (Bilofsky and Burks, 1988).

Results

Benzimidazole Sensitivity in C. elegans

Our studies have primarily used benomyl to characterize benzimidazole sensitivity in *C. elegans*. Larvae freshly hatched in the presence of benomyl look normal, probably because the drug does not enter the eggshell. L2 (second larval) stage and older *C. elegans* are severely paralyzed when reared on benomyl-containing plates (Chalfie and Thomson, 1982). Although head movement is nearly normal, the posterior of the body is immobile and appears constricted. Since a significant portion of the nematode body appears affected by benzimidazoles, it can be inferred that many cells of *C. elegans* express one or more benzimidazole-sensitive tubulins. Some of the sensitive cells are neurons: there are fewer neuronal processes in the ventral nerve chords of animals grown on benomyl (Chalfie and Thomson, 1982).

Benomyl sensitivity is temperature dependent. At low temperature (15°C), 1 μg/ml benomyl is the lowest paralyzing dose and, at high temperature (25°C), 2.5 μg/ml benomyl is required for full paralysis of wild-type animals. Presumably, the deleterious benomyl–tubulin interaction is more stable at low temperature, and at higher temperatures the drug becomes less effective at microtubule disruption. Temperature-dependent inhibition by benomyl has been observed in yeast (Thomas et al., 1985).

C. elegans is sensitive to many benzimidazoles (Table II). Carbendazim, mebendazole, nocodazole, and thiabendazole slow growth and induce an uncoordinated motion similar to that caused by benomyl.

Identification and Characterization of Benomyl-resistant Mutations

We identified benomyl-resistant mutants by mutagenizing wild-type animals and screening for offspring that move normally when grown on 7 μm benomyl. Two mutants were derived in a preliminary EMS mutagenesis, and one arose spontaneously from an EMS-derived strain harboring an independent mutation. We subsequently obtained seventeen independent benomyl-resistant mutations among the progeny of 6,300 F1 animals after EMS mutagenesis, three mutations among the progeny of 510 F1 animals from the *C. elegans* mutator strain TR679 (a strain that exhibits a high frequency of transposition; Collins et al., 1987), and five mutations among the progeny of 57,500 F1 animals after γ-ray muta-

Table II. Response of ben-1 Mutants to Benzimidazoles

Allele	Lowest paralyzing dose			
	Carbendazim	Mebendazole	Nocodazole	Thiabendazole
	μg/ml	μg/ml	μg/ml	μg/ml
+	1.2	5	0.6	10
e1880	>80	>80	>80	>80
u114	10	>80	1.2	>80
u115	>80	>80	>80	>80
u134	>80	>80	>80	>40
u135	>80	>80	>80	>80
u347	>80	>80	>80	>80
u462	>80	>80	>80	>80
u463	>80	>80	>80	>80
u464	>80	>80	>80	>80
u465	>80	>80	>80	>80
u471	>80	>80	>80	>80

Wild-type and *ben-1* mutants were grown at 25°C on drug-containing plates. Mutants that were completely resistant at 25°C were also resistant to all concentrations of drugs tested at 15°C.

genesis. Benomyl-resistant mutations did not confer conditional lethality or dependence on benomyl for coordination or viability. Characteristics of resistant strains are listed in Table I.

All 28 mutations were found to be dominant or semidominant at 25°C. Dominance, however, was conditional. Heterozygote resistance was not seen at 15°C, nor could heterozygote resistance be observed at concentrations of benomyl >12.5 μM. Dominance thus seems correlated with the previously observed effects of the drug: at high temperatures (when drug interaction with the microtubule is less stable) or at low drug doses, benomyl is a less effective poison. Under such conditions, a single copy of the resistant allele allows the animal to thrive in the presence of the drug.

The benomyl-resistant alleles constitute a single complementation group that maps between *daf-2* and *unc-93* on linkage group III. We have designated this gene *ben-1* (for *ben*zimidazole sensitivity).

The *ben-1* mutants are resistant to a variety of benzimidazoles and benzimidazole carbamates (Table II). For many mutants, even saturating concentrations of drugs have no effect on movement. The *ben-1* mutants do not express general resistance to anti-microtubule agents since they become touch insensitive when grown in the presence of 0.5 μM colchicine as do wild-type animals (Chalfie and Thomson, 1982; data not shown).

Isolation of ben-1, a β-Tubulin Gene

Since mutations that confer resistance to benomyl often map to β-tubulin genes and since β-tubulin genes are highly conserved (Cleveland et al., 1980; Sanchez et al., 1980), we used cloned chicken β_2-tubulin DNA as a probe to look for DNA polymorphisms in *ben-1* strains. DNA was prepared from *ben-1(u134)* and *ben-1(u347)* animals, strains derived in the *C. elegans* mutator background that were likely to harbor transposon insertions at the *ben-1* locus. Southern blot analysis demonstrated that a restriction fragment homologous to β-tubulin was polymorphic in these strains (Fig. 1, lanes *1–3*). This polymorphism cosegregated with benomyl resistance in genetic crosses.

The 7.4-kb Eco RI genomic restriction fragment corresponding to the candidate β-tubulin gene was cloned (Fig. 2, *pTU58*). Mapping of β-tubulin-homologous sequences established that the DNA insert in pTU58 contained most of the gene but lacked the 3′ end. Subsequently, a 4.6-kb Xba I genomic DNA restriction fragment that encompassed the entire gene was isolated (pTU59). Cloning of the *ben-1* allele *u347* established that it contained an insertion of the *C. elegans* transposon Tc1 within β-tubulin coding sequences. Southern blot analysis showed that allele *u134* resulted from a Tc1 insertion at the 5′ end of the coding region. As shown in Table I, this allele produces a partially resistant phenotype: mutants remain sensitive to high concentrations of benomyl, and resistance at low benomyl concentrations is incompletely dominant.

Using cloned genomic *ben-1* DNA as a probe, we isolated two *ben-1* cDNA clones. Although neither clone spanned the entire coding sequence, isolation of these clones demonstrates that the *ben-1* gene is expressed and suggests that transcripts from this locus are moderately abundant. This is substantiated by Northern blot analysis in which *ben-1* transcripts

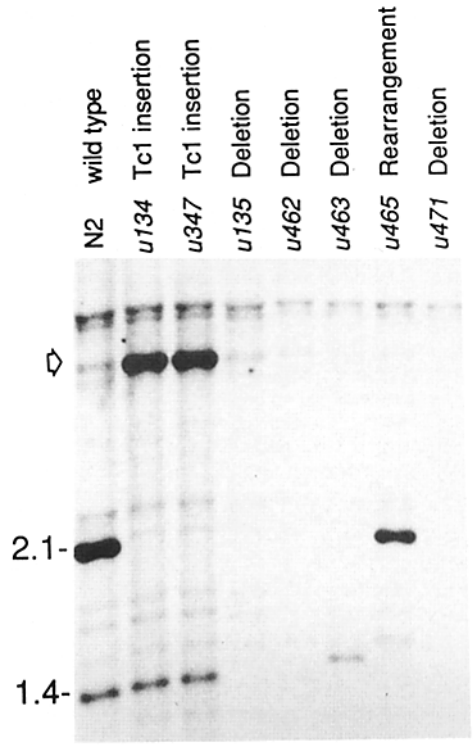

Figure 1. DNA polymorphisms at the *ben-1* locus. DNA was prepared from wild-type N2 as well as mutator-derived *ben-1(u134)*, *ben-1(u135)*, and *ben-1(u347)* and γ-ray–induced *ben-1(u462)*, *ben-1(u463)*, *ben-1(u465)*, and *ben-1(u471)* mutants. DNA was digested with Eco RI and Bgl II, electrophoresed in 0.7% agarose, transferred onto nitrocellulose paper, and hybridized to ^{32}P-labeled sequences from the 1.4-kb Bgl II–Eco RI and the 2.1-kb Bgl II restriction fragments of the *ben-1* locus. The arrow indicates the polymorphic fragment present in alleles *u134* and *u347*. Other visible bands are restriction fragments that share cross-homology with the *ben-1* β-tubulin.

are readily detectable in total RNA preparations. Although *ben-1* does not appear to be a minor β-tubulin, *ben-1* transcripts are approximately fivefold less abundant than *tub-1* transcripts observed in total RNA preparations (data not shown).

Characterization of ben-1 Deletion Alleles Demonstrates ben-1 Is a Nonessential β-Tubulin

To confirm our identification of the *ben-1* locus, we used

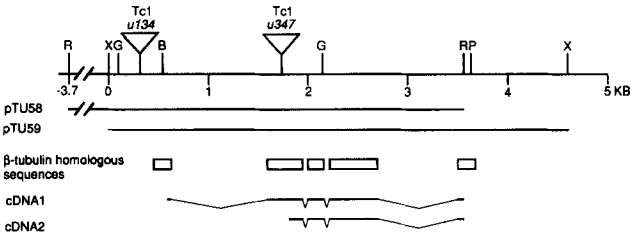

Figure 2. Restriction map and clones from the *ben-1* region. Restriction sites depicted are Bam HI (*B*), Bgl II (*G*), Eco RI (*R*), Pst I (*P*), and Xba I (*X*). Positions of Tc1 insertions in *ben-1(u134)* and *ben-1(u347)* are indicated by triangles. DNA included in pTU58, pTU59, cDNA1, and cDNA2 are represented by bold lines; lighter lines indicate sequences spliced out in cDNAs. β-tubulin-homologous regions are indicated by open bars.

cloned *C. elegans ben-1* sequences to look for polymorphisms in other *ben-1* alleles. Five *ben-1* mutations, one derived from TR679 and four derived from γ-ray mutagenesis, were found to be deleted for some or all *ben-1* sequences (Fig. 1, lanes *4–8*). These data support the identity of the *ben-1* gene and, more importantly, demonstrate that deletion of this β-tubulin gene can confer resistance to benomyl. Furthermore, since animals harboring *ben-1* deletions are viable and exhibit no phenotype other than benomyl resistance, the *ben-1* gene can be said to be nonessential in *C. elegans* under laboratory conditions.

The Nucleotide and Deduced Amino Acid Sequence of the ben-1 Gene

The nucleotide sequence of the entire 4.6-kb genomic Xba I fragment in pTU59 was determined on both DNA strands by sequencing a set of overlapping deletion subclones. Fig. 3 depicts the genomic DNA sequence and the predicted amino acid sequence of the *ben-1* β-tubulin. Coding regions were deduced by comparison of homology with sequenced β-tubulins, identification of *C. elegans* consensus splice junctions (Emmons, 1988), and partial DNA sequence analysis of the cDNA clones. The *ben-1* gene contains four introns. Two of the introns are short (52 and 57 bases for introns II and III, respectively), a feature of many *C. elegans* introns (Blumenthal and Thomas, 1988).

300 bases of 5′ flanking sequence are depicted with the *ben-1* sequence in Fig. 3. The sequences TATAA (−27) and CAAT (−44, −51, −187, −241), commonly used eukaryotic promoter elements, precede the initiation codon (+1). However, the presence of a *C. elegans* splice acceptor sequence at −39 raises the possibility that functional promoter elements lie further upstream than the TATAA box at −27 and that an untranslated mRNA leader might be joined onto the *ben-1* transcript. This leader could be spliced in *cis* from upstream sequences or in *trans* from elsewhere in the genome, as occurs with several other highly expressed *C. elegans* genes (Krause and Hirsh, 1987). In the 3′ region of the *ben-1* gene, the consensus polyadenylation signal AATAAA is present 322 bases after the termination codon (+3,551).

The *ben-1* gene encodes a 444-amino acid β-tubulin. We have compared the deduced protein sequence with that of two other *C. elegans* β-tubulins for which sequence data is available: *ben-1* shares 96% identity with *tub-1* (Gremke, 1986) and 92% identity with *mec-7* (Savage et al., 1989). A homology search of the GenBank(R) version 58 database indicates that *ben-1* is between 74 and 92% identical to other metazoan β-tubulins, with the closest match to the human β-tubulin gene, β_2-tubulin (Lewis et al., 1985). As is the case with other β-tubulin sequences, most homology precedes amino acid 430. After position 430, the carboxy termini of β-tubulins are variable in length and sequence (Cleveland and Sullivan, 1985). The predicted amino acid sequences of the three *C. elegans* β-tubulins and the human β_2-tubulin are compared in Fig. 4 and discussed below.

Discussion

ben-1 Encodes a Benzimidazole-sensitive β-Tubulin

There are three to four β-tubulin genes in *C. elegans* (Gremke, 1986), yet all 28 mutations that confer resistance to benzimidazoles map to one gene, *ben-1*. Because apparently complete resistance to benzimidazoles is observed in animals lacking the *ben-1* locus, it seems that this gene encodes a β-tubulin that is uniquely sensitive to the effects of these drugs. Further support for this conclusion comes from the finding that mutations of *C. elegans* that confer resistance to another benzimidazole, mebendazole (Woods et al., 1989), are allelic to *ben-1* (Reilly, E., and M. Chalfie, unpublished data). It remains possible, however, that rare mutations conferring benzimidazole resistance by effecting interacting proteins (occurring at a frequency of $>10^{-4}$) could be isolated in a more extensive screen.

Benomyl-resistant alleles were isolated at high frequency ($\sim 10^{-3}$) in our EMS mutagenesis. Since this is the frequency at which mutations that eliminate gene function arise in *C. elegans* (Brenner, 1974; Greenwald and Horvitz, 1980), we expected that many of the *ben-1* alleles were loss of function alleles. In support of this expectation, the mebendazole-resistant mutants of Woods et al. (1989) were found to lack one β-tubulin isotype. Five of the *ben-1* alleles identified in our screen were, in fact, deletions. Since *ben-1*(deletion)/+ heterozygotes exhibit drug resistance (the mutant phenotype), *ben-1* can be termed a haploinsufficient locus: i.e., having only a single copy of the wild-type gene is sufficient to create the mutant phenotype. (We originally concluded that *ben-1* was not haploinsufficient [Chalfie et al., 1986]; however, those experiments used preexisting deletions that are incorrectly listed to include the *ben-1* gene [see genetic map in Wood, 1988].)

The simplest explanation for the dominant effects of the *ben-1* deletion mutations is that they lower the intracellular amount of a sensitive β-tubulin, rendering the drugs ineffective. The high frequency of isolation of EMS mutations at the *ben-1* locus and the existence of deletion alleles producing the same phenotype as the EMS alleles suggest that most, if not all, of the dominant alleles result from a loss of *ben-1* activity and not from elevated expression of the wild-type *ben-1* gene to dilute out the deleterious effects of the drugs. Some alleles, which retain partial sensitivity, may be mutations that lessen the effectiveness of benomyl binding.

It is possible that interactions among tubulins could explain benzimidazole resistance in animals harboring *ben-1* deletions. In yeast, increased α-tubulin gene dosage increases benzimidazole resistance, whereas lowered α-tubulin gene dosage enhances sensitivity to these drugs (Schatz et al., 1986). Thus, the intracellular ratio of α- to β-tubulin subunits might be crucial in determining drug sensitivity. We consider this mechanism less likely than the one discussed above because we observe complete drug resistance rather than enhancement or diminution.

The Function of the ben-1 β-Tubulin Is Redundant

The severe effects of benomyl on wild-type *C. elegans* and the abundance of *ben-1* transcripts imply that a significant number of cells normally express the *ben-1* gene. Thus, it is striking that animals harboring deletions of this gene are wild type in behavior, coordination, and viability. As first suggested by Suzuki (1970) and more recently by Greenwald and Horvitz (1980), the absence of an important gene product could lead to a wild-type phenotype if the gene was part of a gene family and the function of the gene product was

```
CCGTCCTTGTAAAAGTAATTTAGCCGCGCGCGGTGTCACCGCGACCCCATGGCCATCTGCCAATTTTGGCCCCTGGCTAGT  -221
TCAAACGAAGAGAAGGACGGAGCTCCCAATTTCCAATCGCTTTTTTCCCTTTTTGTTTCACATTCTCACTCGTTGTTTGC  -141
GAGTTTAAAATTTAAAATTCAACGAATTTTACCTTTTTGTTTCCCCTCTACGTGACCCTTCTCGTTTTGCACTCCGGTTA   -61
CAAAATATTCAATATTCAATATTTCAGTTCTCATATAACTTCAAAAAGAACTTGGAAAAA ATG AGA GAA ATT GTT    15
                                                              Met Arg Glu Ile Val

CAC GTT CAA GCC GGA CAA TGT GGT AAT CAA ATC GGA GCC AAG TTC TGG GAA GTG ATA TCC     75
His Val Gln Ala Gly Gln Cys Gly Asn Gln Ile Gly Ala Lys Phe Trp Glu Val Ile Ser

GAT GAG CAT GGG ATC CAG CCT GAT GGA ACT TAT AAG GGA GAA AGT GAT TTG CAG TTG GAA    135
Asp Glu His Gly Ile Gln Pro Asp Gly Thr Tyr Lys Gly Glu Ser Asp Leu Gln Leu Glu

AGA ATC AAT GTC TAC TAT AAT GAG GCT AAT GGTGAGAAATTTAGCTTTTTTATTCGATTTTCAGATTCTG   205
Arg Ile Asn Val Tyr Tyr Asn Glu Ala Asn

TTTGAATATAATAAAGCAAAATGTTCCGAAATTTTCTTTAACTTCCAATTTTCAATATTTATTGCTCAATCGCAAAATTT   285
GTTCGAGTGTTGCGCAATAAATACGGTGCCCGGTCTCGACACGACTTTTGTTGACAACGAAAGGTCGTGCGCCTTTAAAG   365
GATACTGTAGCTTCAAACTTTTGTGGCAGCGGGCTTTGCATTAGTTTTCATAGTTTTTTGATTGATAAATGTGTACTTAT   445
GTATTTTTTTATTAAAAACTCAAATATTTATTACACATTTTAACAAATTAATTCTGCAAATTATGAGAATGAACGGAAGA   525
TATATTGCCAGAGACACTATTACCGGTACAGAGAGTGTAGATAGTTAGAGAGTGACAGACATACGGGAACCTATGGGGCG   605
GGGCGCGCGGAAGAGAAGATTTCGTAAATCGACACAAATCGTCTCTTCCGCGCGCCCCGCCCCATTGGTCCCGGATGTCT   685
GTCACTCTCTAACTATATACACTCTCTGTACCGGTATTACGAACGTAGGAATCGTGGTATTTTGAGAGACAAAACCACCG   765
GGGTATACTAGTTTCTTGCACACTTTTCGGTTGAATTAGGATTTTAGTTAGTCAAACAAAAGCTCAAAGACAGCTTTCT   845
GAAATTCACGTTTACAAATTCACGAATAGTTATTTTTATAGACCCATTCTGATGAAATTTCAGTATATCTGTTTCTCCCA   925
TTTTTCGATTCTATAAACGTGTGGTGTCTCTTCGCATTCGCGCTCTATAGCAACCAATTTTTTTCAATTTTTATTTTTTC  1005
TTTCCAAAGATAAATCTTCGATTAAGACCTCATTTTTGTTGGTTTTAAAAAAAAAATTTTTTAAATTAATTTAAAATTTA  1085
AAATTTAATTCTCTCAATGTGAATTACCAAAATTCACTAATTGTTACCATTTTCA GGC GGC AAA TAT GTC CCA   1158
                                                          Gly Gly Lys Tyr Val Pro

CGC GCT GTT CTC GTC GAT CTT GAG CCA GGA ACC ATG GAT TCT GTC CGC TCT GGA CCA TTC   1218
Arg Ala Val Leu Val Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ser Gly Pro Phe

GGC CAG CTG TTC CGT CCA GAT AAC TTT GTG TTC GGA CAA TCC GGA GCC GGA AAC AAC TGG   1278
Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Ser Gly Ala Gly Asn Asn Trp

GCC AAG GGT CAC TAC ACC GAA GGA GCC GAA CTT GTC GAT AAT GTG CTC GAC GTA GTT CGA   1338
Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu Val Asp Asn Val Leu Asp Val Val Arg

AAA GAG GCT GAA GGA TGT GAT TGT CTT CAA GGA TTC CAG TTG ACT CAT TCA CTT GGA GGA   1398
Lys Glu Ala Glu Gly Cys Asp Cys Leu Gln Gly Phe Gln Leu Thr His Ser Leu Gly Gly


GGA ACT GGA TCT GGA ATG GGA ACT CTT CTC ATT TCG AAA ATC CGT GAA GAG TAT CCA GAT   1458
Gly Thr Gly Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Tyr Pro Asp

AGA ATT ATG AGT TCT TTC TCG GTT GTT CCG TCG CCA AAG GTTGGATTAATTGAATTTAATGAATATT   1525
Arg Ile Met Ser Ser Phe Ser Val Val Pro Ser Pro Lys

TTAAAACTAATAATTAAAATTCAG GTC TCG GAC ACA GTC GTC GAG CCA TAC AAC GCT ACT CTT TCT   1591
                        Val Ser Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser

GTC CAC CAG CTC GTT GAA AAT ACC GAT GAG ACT TTC TGC ATT GAC AAC GAG GCT CTT TAT   1651
Val His Gln Leu Val Glu Asn Thr Asp Glu Thr Phe Cys Ile Asp Asn Glu Ala Leu Tyr

GAT ATC TGC TTC AGA ACC CTC AAG CTT TCA AAT CCA ACT TAT GGA GAT CTC AAT CAT CTT   1711
Asp Ile Cys Phe Arg Thr Leu Lys Leu Ser Asn Pro Thr Tyr Gly Asp Leu Asn His Leu
```

Figure 3. Complete nucleotide and deduced amino acid sequence of the *ben-1* locus. Numbering of the nucleic acid sequence begins at the initiation codon (+1). CAAT and TATAA sequences at the 5′ end of the gene and the AATAAA sequence at the 3′ end are underlined. *C. elegans* consensus splice donor cores (AG/GT) and consensus acceptor cores (TTTCAG/) are italicized. The potential consensus splice donor site located 5′ to the coding sequence is double underlined.

redundant. Other *C. elegans* genes have been shown to be functionally redundant, including tRNA genes (Waterston, 1981; Wills et al., 1983) and actin genes (Landel et al., 1984).

Since the *ben-1* gene is nonessential, its function is likely to be replaced by other members of the β-tubulin family. These other genes might be silent in *ben-1*–expressing cells, but might become transcriptionally active as a consequence of the *ben-1* deletion. Alternatively, these genes could be coexpressed with *ben-1* and upregulated when *ben-1* activity is reduced. We favor the latter hypothesis from a consideration of tubulin autoregulation and the assumption that au-

```
GGTAAGATTTTCTTTTATTTTTTATTATTTTTTCTATTTTAACTTTCAATTATTTCA GTT TCC GTT ACA ATG     1783
                                                            Val Ser Val Thr Met

TCC GGA GTC ACC ACG TGC CTC CGC TTC CCA GGA CAA CTC AAT GCT GAT CTC CGC AAA CTT   1843
Ser Gly Val Thr Thr Cys Leu Arg Phe Pro Gly Gln Leu Asn Ala Asp Leu Arg Lys Leu


GCA GTC AAC ATG GTT CCA TTC CCA CGT CTT CAC TTC TTT ATG CCA GGA TTT GCT CCA TTG   1903
Ala Val Asn Met Val Pro Phe Pro Arg Leu His Phe Phe Met Pro Gly Phe Ala Pro Leu

TCA GCT AAA GGA GCA CAA GCG TAC CGT GCA CTT ACG GTC GCC GAG CTT ACC CAG CAG ATG   1963

Ser Ala Lys Gly Ala Gln Ala Tyr Arg Ala Leu Thr Val Ala Glu Leu Thr Gln Gln Met

TTT GAC GCC AAG AAT ATG ATG GCT GCC TGT GAT CCG AGA CAC GGA CGT TAC CTG ACT GTA   2023
Phe Asp Ala Lys Asn Met Met Ala Ala Cys Asp Pro Arg His Gly Arg Tyr Leu Thr Val

GCT GCA ATG TTC CGT GGA CGT ATG TCA ATG AGA GAA GTC GAT GAT CAA ATG ATG AAT GTG   2083

Ala Ala Met Phe Arg Gly Arg Met Ser Met Arg Glu Val Asp Asp Gln Met Met Asn Val

CAG AAT AAG AAC TCT TCG TAT TTT GTT GAA TGG ATT CCG AAT AAT GTG AAG ACT GCG GTC   2143
Gln Asn Lys Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Val Lys Thr Ala Val

TGT GAT ATT CCA CCA CGT GGG CTC AAG ATG TCG GCC ACT TTT ATT GGA AAC TCG ACA GCT   2203
Cys Asp Ile Pro Pro Arg Gly Leu Lys Met Ser Ala Thr Phe Ile Gly Asn Ser Thr Ala


ATT CAG GAG TTG TTC AAG AGA ATC TCT GAA CAA TTT ACA GGTACTATTATGGAGACGCGGAAAAGTT  2270
Ile Gln Glu Leu Phe Lys Arg Ile Ser Glu Gln Phe Thr

CATTTTATTGCGGATTCGAAAGCGAAAACGCGAAAAAATTATCGACTTTTCAGAAAATCAAAAAATTTAAAAAAATTAAA  2350
ATGTTTATTATATTTTGTATCCAAAAATTACAAAAATTCTCATTAAAGGCTTTTTGAAAATTAAATATTGAAAAAAGTTG  2430
CTGGAAACGTGCTCCACCGCCAATTTACAACGCTCCGCCCTAATCAGTTGGGTCTCGTTAGGTATTTGGAGGCGAAATTG  2510
TGAATCCAAATGTTTTACCAAATTTCAGCCATTTTTCGTCTTTTTTGTGATTTTTTTCCGTTTAAAAAAAAATTTCGTGT  2590
CAAAACGATTTTTTCACCAATTTCGACATAATTTTTTCAACGAACAGCATCACAAAGTTGAATAAAATCGGCAAAAAAAT  2670
TCAAAAAACCTTTTAATTTTCAGTTGTTTTTTTATTGAAAATTGGCGAAACTAATTGAAACATTCTAAATTTACGGTATT  2750
AAATATCCAACATGTCGGATTTTATAGCGAAAATTATCGATTTTTCAGAACATAAAAAAAAAACGAGAAAAATTTCAATAT  2830
TTTATCGAATTTGGTATATTTTAAATTTAAAAAAATCATAATTTTTCTCAATTTTATGAAATTTTCAGCAATTTTTTGGT  2910
TAAAGTTTGGTAAATTTGGGTTAAAAACATGAATTTAGGCGAAATTCGAAAAAGAATTTCCAATTATCGAAAAATCTAAA  2990
AAAAAAAAAAATTTTCTATATTTTTTTTTCATAATTTTCCCCTAACTCTGTCCAAATTTCA GCA ATG TTC CGC     3063
                                                                Ala Met Phe Arg

CGC AAG GCT TTC CTC CAT TGG TAT ACT GGC GAA GGA ATG GAC GAG ATG GAA TTC ACT GAA   3123
Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu Gly Met Asp Glu Met Glu Phe Thr Glu

GCT GAG AGT AAT ATG AAC GAT CTT GTC TCA GAA TAC CAG CAA TAC CAG GAA GCA ACT GCA   3183
Ala Glu Ser Asn Met Asn Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Glu Ala Thr Ala

GAG GAA GAT GGA GAA CTT GAT GGA ACT GAT GGA GAT GCT GAA TAGAATAGAAAACTATGATTTGAA  3249
Glu Glu Asp Gly Glu Leu Asp Gly Thr Asp Gly Asp Ala Glu

CAATTATTTATGAAAAAAAAACTTGAAATTTCTAAAATTTGTATCGCCTGCTTCCTGCCATAATTTGTTCTTTTTTTAAC  3329
TTCAAATCTCCCTTATTTCTATCGTTATTGCCAGAAATGTTCGGTACATTTACAAAATTTCCCGAAGTATTTCCCAGAAA  3409
TTTAGAGGGAAAAATATTTAATTTTCGGTTTTCCGGAAGATCAAAAAATCGAAAAAAAAAAATTATTTATAATTTTTCGA  3489
TTTAGTTTCAAAAAAACTGCCCAAAATCGATTTTTAAGCAAATTTTTTGGCTTTTTTGCACAATAAATCGAAAAACGAAA  3569
ATTTATATTTTTAGATTTACCGAAAAGTCGACAAATGTTCAGAACATTAACAAAAATTCTTTTTTTTTAAATCAAATTGC  3649
```

toregulation is operative in *C. elegans*. Autoregulation of cellular β-tubulin levels via mRNA stability has been elegantly characterized by Cleveland and colleagues (for review see Cleveland, 1989). A crucial element in this regulation is the concentration of the nascent amino-terminal tubulin tetrapeptide met-arg-glu-ile associated with ribosomes. Since all sequenced *C. elegans* β-tubulins have this highly conserved amino terminus, such a mechansim could control the intracellular concentration of β-tubulin but would not distinguish between the expression of different isotypes. If *ben-1* were the only β-tubulin expressed in a cell, autoregulation should elevate *ben-1* expression and compensate for the loss of one copy of the *ben-1* gene. The resulting animal would be expected to have benzimidazole sensitivity similar to wild-type animals: i.e., loss of function of *ben-1* would produce recessive, not dominant, mutations. However, if other β-tubulin genes are normally coexpressed with *ben-1*, autoregulation would elevate expression of *ben-1* in proportion with

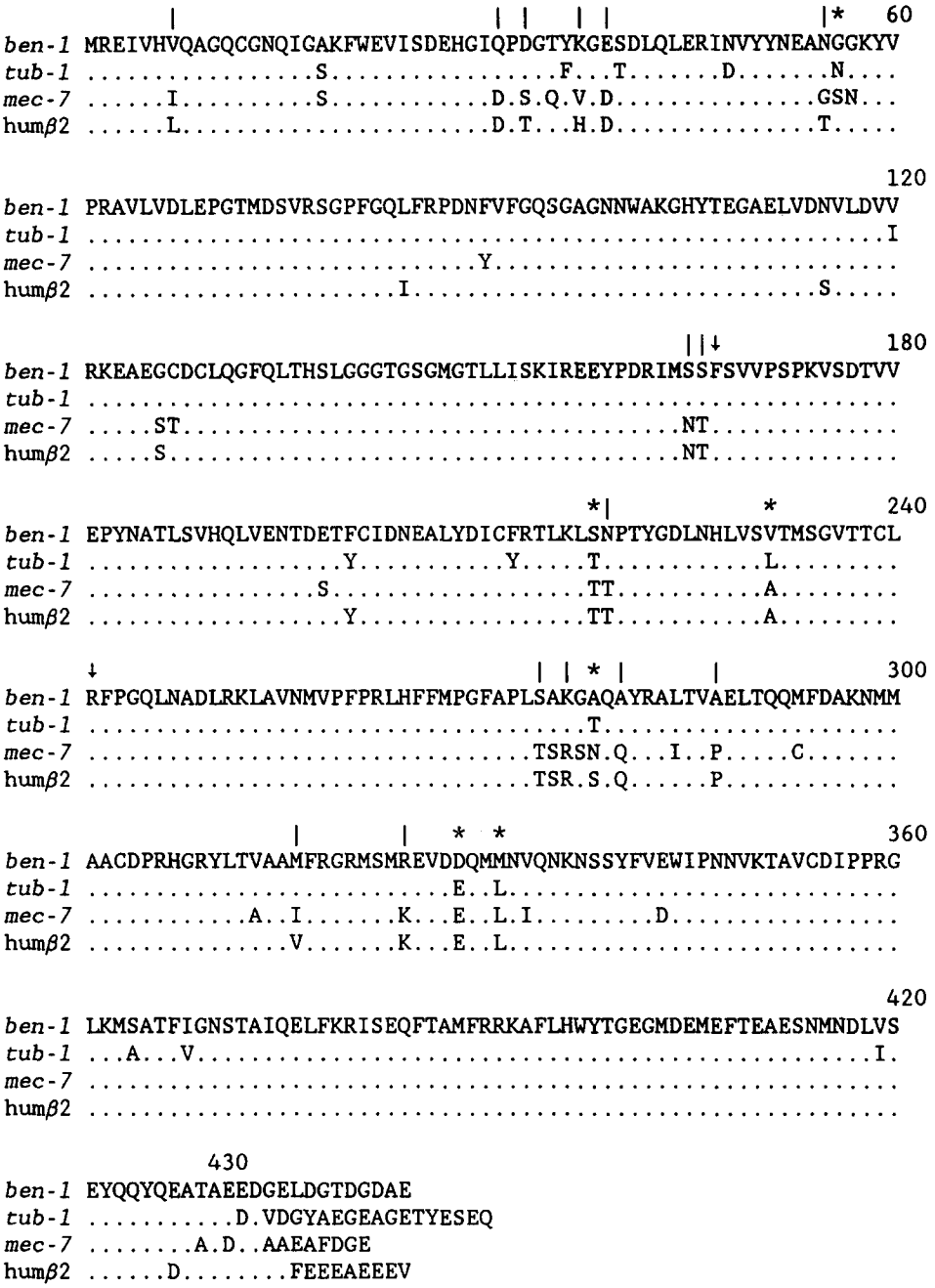

Figure 4. Comparison of *C. elegans* β-tubulin sequences. Single letter code amino acid sequences of *C. elegans* β-tubulins *ben-1*, *tub-1* (Gremke, 1986), and *mec-7* (Savage et al., 1989) and human β_2-tubulin (Lewis et al., 1985) are aligned. The *ben-1* sequence is depicted in its entirety; dots indicate positions of amino acid identity in the other β-tubulins. Vertical lines mark amino acid residues before position 430 that are unique to *ben-1* and *tub-1*, the β-tubulins likely to form 11-protofilament microtubules; asterisks mark amino acid residues unique to the benomyl-sensitive β-tubulin, *ben-1*. Arrows at positions 167 and 241 indicate residues that have been mutated in fungi to confer benomyl resistance (Orbach et al., 1986; Thomas et al., 1985).

the other β-tubulins and the *ben-1* product would not be expected to reach wild-type levels, a condition that would impart partial resistance to benzimidazoles.

Redundancy of *ben-1* function also explains the rather unusual nature of benzimidazole resistance in *C. elegans*. Instead of altering the interaction of the β-tubulin with the drugs, resistance is often achieved by eliminating the only significantly drug-sensitive β-tubulin in the animal. Although resistance to toxic substances via elimination of gene activity has been observed in several systems (e.g., commonly used selections in tissue culture depend on the elimination of hypoxanthine phosphoribosyltransferase or thymidine kinase activity), in these cases the loss of functional activity renders the selected cell dependent upon nutrient supplementation for viability. In the case of the *ben-1* tubulin, redundancy allows for viability. Moreover, because the number of mutable sites needed to eliminate or reduce gene function is likely to be much greater than those needed to change drug affinity, redundancy of β-tubulins allows for a much higher frequency of drug-resistant mutations.

Our genetic analysis failed to identify an essential function for the *ben-1* β-tubulin: we detect no growth advantage of wild-type animals compared with animals lacking the *ben-1* gene nor do we detect evidence of chromosomal instability such as high incidence of males produced by increased chromosomal nondisjunction in *ben-1* deletion strains. Still, it is difficult to rule out the possibility that this β-tubulin provides a required activity in the wild. It is interesting to note that a drug-sensitive β-tubulin appears present throughout the nematode phylum: benzimidazoles are effective as broad-

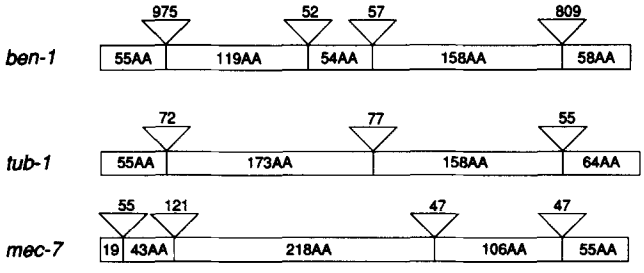

Figure 5. Genomic organization of *C. elegans* β-tubulin genes. The coding regions of *ben-1*, *tub-1* (Gremke, 1986), and *mec-7* (Savage et al., 1989), deduced from genomic DNA sequence data, are aligned. Open bars represent exons drawn to scale (in numbers of amino acids). Positions of introns are indicated by triangles. Intron lengths (in number of nucleotides, not drawn to scale) are indicated above the triangles.

spectrum anti-nematode agents. Because benzimidazole resistance is common among parasitic populations (Prichard et al., 1980), we speculate that the benzimidazole-sensitive β-tubulin may not be essential in the wild in many nematode species. Perhaps the reason for the maintenance of this β-tubulin is that redundancy itself has a selective advantage.

Finally, it should be noted that in *C. elegans* cell biology two tubulin isotypes are used differently. *mec-7*, encoding a β-tubulin that appears to be expressed only in the touch receptor neurons and required for the production of 15-protofilament microtubules (Savage et al., 1989), exhibits functional specificity. The *ben-1* β-tubulin, expressed in many cells, is functionally redundant.

The Structures of C. elegans β-Tubulin Genes

Three *C. elegans* β-tubulin genes have been sequenced. The *ben-1* protein is 96% identical to the *tub-1* β-tubulin (Gremke, 1986) and 92% identical to *mec-7*, the β-tubulin required for the 15-protofilament microtubules of the touch receptor cells (Savage et al., 1989). In Fig. 5, the genomic organization of the three *C. elegans* β-tubulin genes is compared. *ben-1* and *tub-1*, the β-tubulins that share most amino acid homology, are most similar in sequence organization. Although *ben-1* has one more intron than *tub-1*, the locations of the three other introns are identical. Corresponding introns, however, vary both in size and sequence content. In contrast, only the last intron of the *mec-7* gene interrupts the coding sequence at a position similar to *ben-1* and *tub-1*.

Variation in numbers and position of introns is not unusual among invertebrate or fungal β-tubulin genes (Neff et al., 1983; Hiraoka et al., 1984; Orbach et al., 1986; Rudolph et al., 1987). Vertebrate β-tubulin gene families, however, exhibit conservation of intron positions (Hall et al., 1983; Lee et al., 1983, 1984; Sullivan and Cleveland, 1984, 1986; Lewis et al., 1985). Vertebrate introns occur after amino acids 19, 55, and 92. It may be noteworthy that the position of the first intron in *mec-7* and *Drosophila* β_3-tubulin (Rudolph et al., 1987) corresponds to the position of the first vertebrate intron, and the position of the first intron in *ben-1* and *tub-1* corresponds to that of the second vertebrate intron. It seems likely that an ancestral β-tubulin gene had introns at both these positions. The structure of the *C. elegans* β-tubulin genes suggests that in the evolution of this species a gene harboring an intron after amino acid position 386 was duplicated and the resultant genes diverged into *mec-7* and *ben-1/tub-1* progenitors. Another duplication event could have then produced the *ben-1* and *tub-1* precursor genes.

Features of the ben-1 Coding Sequence

The *ben-1* β-tubulin is unusual in that it is incorporated into 11-protofilament microtubules. Although microtubule nucleation can influence protofilament number (Scheele et al., 1982; Evans et al., 1985), characterization of the *mec-7* gene of *C. elegans* suggested that some determinants of protofilament number may be inherent in the primary structure of the β-tubulin itself (Savage et al., 1989). In Fig. 4 we indicate the amino acid residues unique to the 11-protofilament-forming *ben-1* and *tub-1* β-tubulins. (In this discussion, we assume that *tub-1* contributes to an 11-protofilament microtubule; this is likely since all microtubules except those of the six touch cells have 11 protofilaments.) In the amino-terminal region, which has been implicated in protofilament interaction (Kirchner and Mandlekow, 1985), a change in charge distribution is evident in the region of amino acids 31–37. At position 31 (an absolutely invariant residue in other species) Gln is substituted for Asp; at position 33 Asp is substituted for Thr; and at position 35 Lys is substituted for a variable, but always uncharged, residue. Another striking change that may alter the properties of the *ben-1* and *tub-1* β-tubulins is the substitution of Ala for an invariant Pro at position 287. This change occurs adjacent to the site of a single amino acid substitution in a mutant *Drosophila* β-tubulin (Glu for Lys at position 288) that prevents assembly of tubulin protofilaments into microtubules (Rudolph et al., 1987). The domain harboring these changes forms a hinge between the amino- and carboxy-terminal regions of β-tubulin (Kirchner and Mandelkow, 1985; Mandelkow et al., 1985). This domain also includes a residue uniquely present in the *mec-7* β-tubulin (Cys for Met at 293). The role of this region in influencing protofilament number remains to be tested.

Benomyl Sensitivity of the ben-1 β-Tubulin

The ben-1–encoded β-tubulin is the only *C. elegans* β-tubulin sensitive to the effects of benomyl under the conditions of our assay. Since sensitivity might be a consequence of the primary structure of the *ben-1* protein, we looked for amino acid changes that correlate with benzimidazole sensitivity (Fig. 4). However, of the six changes that are unique to *ben-1* in the region before amino acid 430, most are conservative. Perhaps interaction with benzimidazoles is strictly dependent on one or a few of the identified amino acid side chains. Alternatively, the unique *ben-1* carboxy terminus may play a role in *C. elegans* benzimidazole sensitivity. In yeast, although no amino acid changes that alter benzimidazole sensitivity map to the carboxy terminus, deletion of the amino acids after position 430 renders the organism hypersensitive to benomyl (Matsuzaki et al., 1988), suggesting some modulation of drug interaction by the carboxy-terminal end.

Other studies in fungal systems have investigated the molecular nature of benomyl resistance. In *Saccharomyces cerevisiae*, resistance is associated with a change of Arg to His at position 241 (Thomas et al., 1985). In *Neurospora crassa*, resistance is correlated with a change of Phe to Tyr at position 167 (Orbach et al., 1986). All three *C. elegans* β-tubulins, however, have Arg at position 241 and Phe at po-

sition 167. It is difficult, therefore, to formulate a hypothesis concerning the residues required for benomyl interaction by examining primary sequence features. DNA transformation experiments using in vitro–mutagenized or chimeric β-tubulins are needed to address the specificity of benomyl resistance.

Implications of the Mechanism of Benzimidazole Resistance in C. elegans

Benzimidazoles are commonly used to control parasitic nematodes. With the widespread use of these anthelmintics, resistant strains of several nematode species have appeared (Drudge et al., 1964; Prichard et al., 1980; Donald, 1983). Detailed characterization of some resistant strains established that these strains are insensitive to multiple benzimidazoles, and that tubulins purified from these strains exhibit lowered affinity for the drugs (Sangster et al., 1985; Lacey and Prichard, 1986; Lacey and Snowdon, 1988). Resistance to benzimidazoles has been interpreted to result from altered binding sites, prompting some authors to recommend searches for benzimidazoles that bind more avidly. Such a screen would be useful if mutations conferring resistance affected binding site affinity, but our results suggest this may not be the most common mechanism of benzimidazole resistance. Rather, we find that the deletion of a sensitive but dispensable β-tubulin leads to resistance. Since the genomes of various nematode species exhibit extensive conservation of coding sequences (Blumenthal et al., 1984) and since nematodes have strikingly similar microtubule structures (Chalfie and Thomson, 1982), the mechanisms of sensitivity and resistance to benzimidazoles may also be conserved. Indeed, strains of the sheep parasitic nematode, *Haemonchus contortus*, also exhibit dominant benzimidazole resistance (Le Jambre et al., 1979). Moreover, when DNAs from some resistant strains of *H. contortus* were probed with β-tubulin sequences, deletions of β-tubulin genes were observed (Roos, M. [University of Utrecht, Utrecht, The Netherlands], personal communication). Deletion of sensitive genes thus may prove to be a general mechanism of resistance among nematode populations. An appropriate strategy for control of resistant parasitic populations would thus be to seek reagents that act on novel targets, not on presumed modified targets.

We thank S. Brenner for discussions of the significance of redundant genes, G. Chin for valuable comments and critical reading of the manuscript, K. Smith for help with computer analysis, and E. Byard for communication of results before publication.

This work was supported by grants from the U.S. Public Health Service (GM30997 and AI9399) and the U.S. Department of Agriculture (85-CRCR-1-1806). M. Driscoll was supported by postdoctoral fellowships from the Muscular Dystrophy Association, the American Cancer Society (PF-2720), and the National Institutes of Health (NF08595).

Received for publication 19 June 1989 and in revised form 25 July 1989.

References

Adachi, Y., T. Toda, O. Niwa, and M. Yanagida. 1986. Differential expressions of essential and nonessential α-tubulin genes in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* 6:2168–2178.

Asai, D. J., and N. M. Remolona. 1989. Tubulin isotype usage *in vivo*: a unique spatial distribution of the minor neuronal-specific β-tubulin isotype in pheochromocytoma cells. *Dev. Biol.* 132:398–409.

Bilofsky, H. S., and C. Burks. 1988. The GenBank(R) genetic sequence data bank. *Nucleic Acids Res.* 16:1861–1864.

Blumenthal, T., and J. Thomas. 1988. *Cis* and *trans* mRNA splicing in *C. elegans*. *Trends Genet.* 4:305–308.

Blumenthal, T. M., M. Squire, S. Kirtland, J. Cane, J. Donegan, J. Speith, and W. Sharrock. 1984. Cloning of a yolk protein gene family from *Caenorhabditis elegans*. *J. Mol. Biol.* 174:1–18.

Borck, K., and H. D. Braymer. 1974. The genetic analysis of resistance to benomyl in *Neurospora crassa*. *J. Gen. Microbiol.* 85:51–56.

Boyer, H., and D. Roulland-Dussiox. 1969. A complementation analysis of the restriction and modification of DNA in *E. coli*. *J. Mol. Biol.* 41:459–472.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics.* 77:71–94.

Burland, T. G., T. Schedl, K. Gull, and W. F. Dove. 1984. Genetic analysis of resistance to benzimidazoles in *Physarum*: differential expression of β-tubulin genes. *Genetics.* 108:123–141.

Burland, T. G., E. C. A. Paul, M. Oetliker, and W. F. Dove. 1988. A gene encoding the major β-tubulin of the mitotic spindle in *Physarum polycephalum* plasmodia. *Mol. Cell. Biol.* 8:1275–1281.

Chalfie, M., and J. Sulston. 1981. Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* 82:358–370.

Chalfie, M., and J. N. Thomson. 1982. Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. *J. Cell Biol.* 93:15–23.

Chalfie, M., E. Dean, E. Reilly, K. Buck, and J. N. Thomson. 1986. Mutations affecting microtubule structure in *Caenorhabditis elegans*. *J. Cell Sci. Suppl.* 5:257–271.

Cleveland, D. W. 1989. Autoregulated control on tubulin synthesis in animal cells. *Current Opinion in Cell Biology.* 1:10–14.

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

Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner. 1980. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. *Cell.* 20:95–105.

Collins, J., B. Saari, and P. Anderson. 1987. Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*. *Nature (Lond.).* 328:726–728.

Donald, A. D. 1983. The development of anthelmintic resistance in nematodes of grazing animals. *In* Facts and Reflections IV, Resistance of Parasites to Anthelmintics. F. H. M. Borgsteede, S. A. Henriksen, and H. J. Over, editors. Central Veterinary Institute, Lelystad, The Netherlands. 15–28.

Drudge, J. M., J. Szanto, Z. N. Wyant, and G. W. Elam. 1964. Field studies on parasitic control in sheep: comparison of thiabendazole, ruelene, and phenothiazine. *Am. J. Vet. Res.* 25:1512–1518.

Emmons, S. C. 1988. The genome. *In* The Nematode *Caenorhabditis elegans*. W. B. Wood, editor. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 47–79.

Emmons, S. C., M. R. Klass, and D. Hirsh. 1979. Analysis of the constancy of DNA sequences during development and evolution of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA.* 76:1333–1337.

Emmons, S. C., L. Yesner, K.-S. Ruan, and D. Katzenberg. 1983. Evidence for a transposon in *Caenorhabditis elegans*. *Cell.* 32:55–65.

Evans, L., T. Mitchison, and M. Kirschner. 1985. Influence of the centrosome on the structure of nucleated microtubules. *J. Cell Biol.* 100:1185–1191.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6–13.

Frischauf, A. M., H. Lehrach, A. Poustka, and N. Murray. 1983. Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* 170:827–842.

Fulton, C., and P. A. Simpson. 1976. Selective synthesis and utilization of flagellar tubulin: the multitubulin hypothesis. *In* Cell Motility. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 987–1005.

Gray, H. B., D. A. Ostrander, J. L. Hodnett, R. J. Legerski, and D. L. Robberson. 1975. Extracellular nucleases of *Pseudomonas* BAL 31. I. Characterization of single strand-specific deoxyribonuclease and double strand deoxyribonuclease activities. *Nucleic Acids Res.* 2:1459–1492.

Greenwald, I., and H. R. Horvitz. 1980. *unc-93(e1500)*: a behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. *Genetics.* 96:146–164.

Gremke, L. 1986. Cloning and molecular characterization of the tubulin genes of *Caenorhabditis elegans*: nucleotide sequence analysis of a tubulin gene. Ph.D. thesis. Northwestern University, Evanston, IL. 158 pp.

Hall, J. L., P. R. Dudley, P. R. Dobner, S. A. Lewis, and N. J. Cowan. 1983. Identification of two human β-tubulin isotypes. *Mol. Cell. Biol.* 3:854–862.

Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene (Amst.).* 28:351–359.

Hiraoka, Y., T. Toda, and M. Yanagida. 1984. The NDA3 gene of fission yeast encodes β-tubulin: a cold-sensitive nda3 mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell.* 39:349–358.

Ireland, C. M., K. Gull, W. E. Gutteridge, and C. I. Pogson. 1979. The interaction of benzimidazole carbamates with mammalian microtubule protein. *Biochem. Pharmacol.* 28:2680–2682.

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.

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.

Krause, M., and D. Hirsh. 1987. A *trans*-spliced leader sequence on actin mRNA in *C. elegans*. *Cell.* 49:753–761.

Lacey, E., and R. K. Prichard. 1986. Interactions of benzimidazoles (BZ) with tubulin from BZ-sensitive and BZ-resistant isolates of *Haemonchus contortus*. *Mol. Biochem. Parasitol.* 9:171–181.

Lacey, E., and K. L. Snowdon. 1988. A routine diagnostic assay for the detection of benzimidazole resistance in parasitic nematodes using tritiated benzimidazole carbamates. *Vet. Parasitol.* 27:309–324.

Laclette, J. P., G. Guerra, and Zetina, G. 1980. Inhibition of tubulin polymerization by mebendazole. *Biochem. Biophys. Res. Commun.* 92:417–423.

Landel, C. P., M. Krause, R. H. Waterston, and D. Hirsh. 1984. DNA rearrangements of the actin gene cluster in *C. elegans* accompany reversion of three muscle mutants. *J. Mol. Biol.* 180:497–513.

Lee, M. G.-S., S. A. Lewis, C. D. Wilde, and N. J. Cowen. 1983. Evolutionary history of a multigene family: an expressed human β-tubulin gene and three processed pseudogenes. *Cell.* 33:477–487.

Lee, M. G.-S., C. Loomis, and N. J. Cowen. 1984. Sequence of an expressed human β-tubulin gene containing ten *Alu* family members. *Nucleic Acids Res.* 12:5823–5836.

Lewis, S. A., M. E. Gilmartin, J. L. Hall, and N. J. Cowan. 1985. Three expressed sequences within the human β-tubulin multigene family each define a distinct isotype. *J. Mol. Biol.* 182:11–20.

Liao, L. W., B. Rosenzweig, and D. Hirsh. 1983. Analysis of a transposable element in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA.* 80:3585–3589.

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

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.

Matsuzaki, F., S. Matsumoto, and J. Yahara. 1988. Truncation of the carboxyl-terminal domain of yeast β-tubulin causes temperature-sensitive growth and hypersensitivity to antimitotic drugs. *J. Cell Biol.* 107:1427–1435.

May, G. S., J. Gambino, J. A. Weatherbee, and N. R. Morris. 1985. Identification and functional analysis of beta-tubulin genes by site specific integrative transformation in *Aspergillus nidulans*. *J. Cell Biol.* 101:712–719.

Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 466 pp.

Neff, N. F., J. H. Thomas, P. Grisafi, and D. Botstein. 1983. Isolation of the β-tubulin gene from yeast and demonstration of its essential function in vivo. *Cell.* 33:211–219.

Orbach, M. J., E. B. Porro, and C. Yanofsky. 1986. Cloning and characterization of the gene for β-tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. *Mol. Cell. Biol.* 6:2452–2461.

Prichard, R. K., C. A. Hall, J. D. Kelly, I. C. A. Martin, and A. D. Donald. 1980. The problem of anthelmintic resistance in nematodes. *Aust. Vet. J.* 56:239–251.

Queen, C., and L. J. Korn. 1984. A comprehensive sequence analysis program for the IBM personal computer. *Nucleic Acids Res.* 12:581–599.

Riddle, D. L., M. M. Swanson, and P. S. Albert. 1981. Interacting genes in nematode dauer larva formation. *Nature (Lond.).* 290:668–671.

Rigby, P. W., M. Diekman, C. Rhodes, and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237–251.

Ruan, K.-S., and S. C. Emmons. 1984. Extrachromosomal copies of the transposon Tc1 in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA.* 81:4018–4022.

Rudolph, J. E., M. Kimble, H. D. Hoyle, M. A. Subler, and E. C. Raff. 1987. Three *Drosophila* β-tubulin sequences: a developmentally regulated isoform (β3), the testis-specific isoform (β2), and an assembly-defective mutation of the testis-specific isoform ($\beta 2t^8$) reveal both an ancient divergence in metazoan isotypes and structural constraints for β-tubulin function. *Mol. Cell. Biol.* 7:2231–2242.

Sanchez, F., J. E. Natzle, D. W. Cleveland, M. W. Kirchner, and B. J. McCarthy. 1980. A dispersed multigene family encoding tubulin in *Drosophila melanogaster*. *Cell.* 22:845–854.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

Sangster, N. C., R. K. Prichard, and E. Lacey. 1985. Tubulin and benzimidazole-resistance in *Trichostrongylus colubriformis* (Nematoda). *J. Parasitol.* 71:645–651.

Savage, C., M. Hamelin, J. G. Culotti, A. Coulson, D. G. Albertson, and M. Chalfie. 1989. *mec-7*, a β-tubulin gene, is required for the production of 15 protofilament microtubules in *Caenorhabditis elegans*. *Genes & Dev.* 3:870–881.

Schatz, P. J., F. Solomon, and D. Botstein. 1986. Genetically essential and nonessential α-tubulin genes specify functionally interchangeable proteins. *Mol. Cell. Biol.* 6:3722–3733.

Schedl, T., J. Owens, W. F. Dove, and T. G. Burland. 1984. Genetics of the tubulin gene families of *Physarum*. *Genetics.* 108:143–164.

Scheele, R. B., L. G. Bergen, and G. B. Borisy. 1982. Control of the structural fidelity of microtubules by initiation sites. *J. Mol. Biol.* 154:485–500.

Sheir-Neiss, G., M. H. Lai, and N. R. Morris. 1978. Identification of a gene for β-tubulin in *Aspergillus nidulans*. *Cell.* 15:639–647.

Short, J. M., J. M. Fernandez, J. A. Sorge, and W. D. Huse. 1988. λ ZAP: a bacteriophage λ expression vector with in vivo excision properties. *Nucleic Acids Res.* 16:7583–7600.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503–517.

Sullivan, K. F., and D. W. Cleveland. 1984. Sequence of a highly divergent β-tubulin gene reveals regional heterogeneity in the β-tubulin polypeptide. *J. Cell Biol.* 99:1754–1760.

Sullivan, K. F., and D. W. Cleveland. 1986. Identification of conserved isotype-defining variable region sequences for four vertebrate β-tubulin polypeptide classes. *Proc. Natl. Acad. Sci. USA.* 83:4327–4331.

Suzuki, D. T. 1970. Temperature-sensitive mutations in *Drosophila melanogaster*. *Science (Wash. DC).* 170:695–706.

Tabor, S., and C. C. Richardson. 1987. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. USA.* 84:4767–4771.

Thomas, J. H., N. F. Neff, and D. Botstein. 1985. Isolation and characterization of mutations in the β-tubulin gene of *Saccharomyces cerevisiae*. *Genetics.* 112:715–734.

Tilney, L. G., J. Bryan, D. J. Bush, K. Fujiwara, M. S. Mooseker, D. R. Murphy, and D. H. Snyder. 1973. Microtubules: evidence for 13 protofilaments. *J. Cell Biol.* 59:267–275.

Valenzuela, P., M. Quiroga, J. Zaldivar, W. J. Rutter, M. W. Kirschner, and D. W. Cleveland. 1981. Nucleotide and corresponding amino acid sequences encoded by α-tubulin and β-tubulin mRNAs. *Nature (Lond.).* 289:650–655.

Waterson, R. H. 1981. A second informational suppressor, *sup-7X*, in *Caenorhabditis elegans*. *Genetics.* 97:307–325.

Weatherbee, J. A., G. S. May, J. Gambino, and N. R. Morris. 1985. Involvement of a particular species of beta-tubulin (beta3) in conidial development of *Aspergillus nidulans*. *J. Cell Biol.* 101:705–711.

Wills, N., R. F. Gesteland, J. Karn, L. Barnett, S. Bolten, and R. H. Waterston. 1983. The genes *sup-7* X and *sup-5* III of *C. elegans* suppress amber nonsense mutations via altered transfer RNA. *Cell.* 33:575–583.

Wood, W. B. 1988. The Nematode *Caenorhabditis elegans*. W. B. Wood, editor. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 667 pp.

Woods, R. A., K. M. Malone, A. M. Spence, W. J. Sigurdson, and E. H. Byard. 1989. The genetics, ultrastructure and tubulin polypeptides of mebendazole-resistant mutants of *Caenorhabditis elegans*. *Can. J. Zool.* In press.

Yamamoto, M. 1980. Genetic analysis of resistant mutants to antimitotic benzimidazole compounds in *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* 180:231–234.

Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene (Amst.).* 33:103–119.