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 β1-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; Hirooka 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 benzimidazole-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 1), the following mutations were used: on linkage group III, *daf-2(e1370)* (Riddle et al., 1981) and *unc-93(el500)* (Greenwald and Horvitz, 1980); on linkage group X, *sup-7(st)* (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*(e459) (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 nocardazole 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) (Brenner, 1974) or γ-ray irradiation from a 137Cs 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. Potative 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-l* mutations (see below), complementation tests were done at 15°C. In three-factor crosses, all of the *ben-l* mutations tested (e1890, e190, e191, ul00-ul17, ul34, ul35, and u347) mapped between the two closest identified markers on chromosome III, *daf-2(e1370)* and *unc-93(el500)*. Of the recombinants derived from *daf-2 + unc-93+* and *ben-l + heterozygotes*, 200 of 293 Daf recombinants and 96 of 312Unc 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-l* mutations from EMS mutagenesis were tested for suppression with the amber RNA suppressor mutation *sup-7* (Waterston, 1981; Wills et al., 1983). *ben-l+t+; dpy-7 sup-7+t* heterozygotes were constructed and suppression was assayed by the absence of Dpy Ben progeny at 25°C. None of the *ben-l* mutations were detectably suppressed.

Recombinant DNA Techniques

*C. elegans* DNA was prepared as described by Emmons et al. (1979). Recombination DNA techniques were used as described in Emmons et al. (1979).

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

### Table 1. Properties of *ben-l* Alleles

| Allele | Source | Expression 15°C | Expression 25°C | Lowest paralyzing dose of benomyl at 25°C |
|--------|--------|----------------|----------------|----------------------------------------|
| +      |        | R              | D              | 2.5                                   |
| e1890  | 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              | D              | >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-l* 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-l* alleles were tested for dominance by mating in wild-type males and examining *ben-l*/+ male progeny for coordinated movement. R, recessive; SD, semidominant; D, dominant.

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Striction enzymes were purchased from New England Biolabs (Beverly, MA), and digestes 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-l* 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). pC2002, a plasmid containing DNA from the *C. elegans* transposon *Tc1*, was described by Ruan and Emmons (1984). λ cloning vectors, EMBL3 (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 pJ48 (a gift from R. Yocum, Biotechnica, Cambridge, MA).
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 mutant strain TR679. In progeny of daf-2 + unc-93/ + ben-1(u347 or u437) + heterozygotes this novel fragment segregated only into Daf or Unc recombinants that were trophotically in to generate a partial library, and a ~-tubulin-homologous clone that hybridized 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 pBI48, a vector that confers tetracycline resistance if it harbors an insertion. The library was transformed into E. coli strain HB101 (Bryant et al., 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. pTU58 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 Tcl (Emmons et al., 1983; Liao et al., 1983) is inserted in ben-1(u347): Tcl 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 Tcl is also the transposon in ben-1(u347) 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 x 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 at the Eco RI site near the 3' end (position 3052) and neither extends to the 5' terminus of the gene.

DNA Sequence Analysis

To sequence the 4.6-kb Xba I fragment contained in pTU58, 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 (Henickoff, 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 MI3). 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 α-endorphin chain termination method of Sanger et al. (1977) using modified T7 DNA polymerase (Tabor and Richardson, 1987; Sequenase and MI3). DNA sequences after partial DNA sequence analysis. Those sequences were screened for homology to Benomyi-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 Benomyi-resistant mutations among the progeny of 6,300 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 mutagenesis.

| Allele | Carbendazim | Mebendazole | Nocodazole | Thiabendazole |
|--------|-------------|-------------|------------|---------------|
| +      | 1.2 5 0.6 10 | >80 >80 >80 >80 | >80 >80 >80 >80 | | |
| e1880  | >80         | >80         | >80         | >80           |
| u14    | 10          | >80         | 1.2         | >80           |
| u15    | >80         | >80         | >80         | >80           |
| u34    | >80         | >80         | >80         | >80           |
| u35    | >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.

Results

Benzmanidazole Sensitivity in C. elegans

Our studies have primarily used benomyl to characterize benzmanidazole 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 benzmanidazoles, it can be inferred that many cells of C. elegans express one or more benzmanidazole-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 benzmanidazoles (Table II). Carbendazim, mebendazole, nocardazole, and thiabendazole slow growth and induce an uncoordinated motion similar to that caused by benomyl.

Identification and Characterization of Benzmanidazole-resistant Mutations

Table II. Response of ben-1 Mutants to Benzmanidazoles

| Allele | Carbendazim | Mebendazole | Nocodazole | Thiabendazole |
|--------|-------------|-------------|------------|---------------|
| +      | 1.2 5 0.6 10 | >80 >80 >80 >80 | >80 >80 >80 >80 | | |
| e1880  | >80         | >80         | >80         | >80           |
| u14    | 10          | >80         | 1.2         | >80           |
| u15    | >80         | >80         | >80         | >80           |
| u34    | >80         | >80         | >80         | >80           |
| u35    | >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           |

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Driscoll et al. A Benzmanidazole-sensitive β-Tubulin in C. elegans
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-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 R I 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 Tcl within β-tubulin coding sequences. Southern blot analysis showed that allele u34 resulted from a Tcl 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 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
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-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-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^-6) could be isolated in a more extensive screen.

Benomyl-resistant alleles were isolated at high frequency (∼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...
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 bend and upregulated when bend activity is reduced. We favor the latter hypothesis from a consideration of tubulin autoregulation and the assumption that autoregulation

冗余。其他C. elegans基因已被证明在功能上是冗余的，包括tRNA基因（Waterston, 1981; Wills et al., 1983）和actin基因（Landel et al., 1984）。

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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-mg-glu-ile associated with ribosomes. Since all sequenced C. elegans β-tubulins have this highly conserved amino terminus, such a mechanism 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 the levels of the other β-tubulins.
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 β2-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 I1-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.

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 Mandelkow, 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 charged, 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., 1984; 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.

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