Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 6108–6112, June 1994
Developmental Biology

par-2, a gene required for blastomere asymmetry in *Caenorhabditis elegans*, encodes zinc-finger and ATP-binding motifs

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Communicated by William B. Wood, January 3, 1994 (received for review October 20, 1993)

ABSTRACT **The *par-2* gene of *Caenorhabditis elegans* functions in early embryogenesis to ensure an asymmetric first cleavage and the segregation of cytoplasmic factors. Both processes appear to be required to generate daughter blastomeres with distinct developmental potential. We isolated an allele of *par-2* by using a screen for maternal-effect lethal mutations in a strain known for its high frequency of transposition events. A transposable element was found to be linked to this allele. Sequences flanking the site of transposon insertion were cloned and found to rescue the *par-2* mutant phenotype. DNA in the *par-2* region hybridized to a 2.3-kb germ-line-enriched mRNA. The cDNA corresponding to this germ-line-enriched message was cloned, sequenced, and used to identify the molecular lesions associated with three *par-2* alleles. Sequence analysis of the *par-2* cDNA revealed that the predicted protein contained two distinct motifs found in other known proteins: an ATP-binding site and a cysteine-rich motif which identifies the *par-2* gene product as a member of a growing class of putative zinc-binding proteins.**

Early embryogenesis in the nematode *Caenorhabditis elegans* is characterized by a series of asymmetric cleavages. The initial asymmetry of the early embryo becomes apparent during the first cell cycle, at which time cytoplasmic factors thought to be required for the specification of cell fate are segregated. The first cleavage produces a larger, anterior cell (AB) and a smaller, posterior cell (P_1) which differ from each other in many respects including the orientation of subsequent cleavage planes, the timing of cell cycles, and the kinds of differentiated cell types they ultimately produce (1). For instance, germ-line-specific P granules are uniformly dispersed in one-cell embryos but become localized to the posterior cortex midway through the first cell cycle (2). At first cleavage, nearly all of the P granules are partitioned to the P_1 blastomere. Furthermore, studies of gut development (3) have shown that factors required for specification of the intestine are present in P_1, and not AB, at the two-cell stage. Blastomere fusion experiments have shown that these factors can be found in the cytoplasm of P_1 (4).

The nature of the cytoplasmic mechanisms that generate asymmetry in the one-cell embryo are unknown, but microfilaments play a significant role in this process. Many of the manifestations of asymmetry in the early embryo can be disrupted by treatment of embryos with cytochalasin D (5). Treated one-cell embryos typically divide symmetrically and have mislocalized P granules; subsequent cleavage patterns are also abnormal. Presumably, disorganization of the microfilament system between fertilization and pronuclear fusion affects the segregation of factors that govern embryo polarity.

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A class of genes has been identified that is required for the generation of asymmetry in the early embryo and whose mutant phenotype resembles the cytochalasin D-treated embryos described above (6). Mutations in any one of the five *par* genes (for partitioning-defective) result in embryos with defects in the position of the first cleavage furrow, altered orientation of the spindle at the second cleavage, synchronous early cleavages, defects in P-granule localization, and altered expression of intestinal cell differentiation markers. All mutations in these five genes result in strict maternal-effect lethality; therefore maternal expression of the *par* genes is required during oogenesis in order to produce viable embryos.

To gain further insight into the role of the *par* genes in cytoplasmic partitioning, molecular analysis of these genes is essential. As a step toward an understanding of the generation of asymmetry and cytoplasmic localization in the *C. elegans* embryo at the molecular level, we have isolated and sequenced the *par-2* gene.‖

MATERIALS AND METHODS

Genetics. Nematode strains were maintained according to Brenner (7). The strain for identification of a transposon-induced allele of *par-2* was constructed by crossing *egl-23(n601)* into the mutator strain TR679. Mutator strains in *C. elegans* undergo a high rate of transposition. The mutator activity in TR679, *mut-2(r459)*, can be balanced by the chromosomal rearrangement *mnC1* (8). Homozygous *egl-23(n601);mut-2(r459)* animals were derived from the heterozygous strain *egl-23(n601)/+;mnC1/mut-2(r459)*. These *egl-23(n601);mut-2(r459)* animals were the starting strain for the screen for maternal-effect lethal mutations. The screen was performed as described by Kemphues *et al.* (6).

Embryos from candidate strains bearing maternal-effect lethal mutations were examined by Nomarski microscopy and assigned to a linkage group. One mutation identified in this screen, *jb2*, mapped to LGIII. Eggs from homozygous *jb2* mothers exhibited synchronous and symmetric early cleavages. Since mutations in a known gene (*par-2*; see ref. 6) on LGIII result in a similar phenotype, complementation tests were performed between *jb2* and *par-2(it5ts)*. *jb2* failed to complement *par-2(it5ts)*.

Abbreviations: YAC, yeast artificial chromosome; SSCP, single-strand conformation polymorphism.

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‖The sequence reported in this paper has been deposited in the GenBank database (accession no. L26987).

The *jb2* mutation was backcrossed 10 times to homozygous *daf-7(e1372ts)*, which was shown previously to map 1% to the left of *par-2*, to generate the strain of the genotype *egl-23(n601);daf-7(e1372ts)+/+par2(jb2)*. At 25°C, homozygous *daf-7(e1372ts)* animals arrest as dauer larvae and can therefore be used to balance the *par-2(jb2)* mutation.

The following strains were constructed for isolation of recombinants between *par-2(jb2)* and flanking markers: *egl-23(n601);++dpy-1(e1)/daf-7(e1372ts)par-2(jb2)+* and *egl-23(n601);+dpy-1(e1)+/par-2(jb2)+daf-2(e1370ts)*. These parent strains were maintained at 25°C, and homozygous Daf animals were picked to individual plates, allowed to recover at 16°C, and then examined for the production of progeny. Nonrecombinant animals are Daf Par and produce large numbers of dead eggs, whereas recombinant Daf non-Par animals produce many progeny. Recombinant genotypes are either *daf-7+dpy-1/daf-7par-2+* or *+dpy-1daf-2/par-2+daf-2*. Strains homozygous for individual recombinant chromosomes were constructed. DNA was isolated from an expanded population of each strain and examined by Southern hybridization for the presence of a linked transposon.

The *par-2* allele *lw32* is an ethyl methanesulfonate-generated mutation generously provided by Jocelyn Shaw (University of Minnesota, St. Paul, MN). *par-2(it46)* is an ethyl methanesulfonate-generated allele generously provided by Niansheng Cheng (Cornell University, Ithaca, NY). *par-2(zu191)* was isolated in a screen for *mut-6*-induced maternal-effect lethal mutations carried out in the lab of J. Priess (Fred Hutchinson Cancer Research Center, Seattle, WA). (C. Mello, B. Draper, K. Kemphues and J. Priess, personal communication).

Southern and Northern Analysis. For Southern analysis, genomic DNA was prepared by the proteinase K method (1). DNA was digested with restriction enzymes, separated by gel electrophoresis, and transferred to Nytran filters (Schleicher & Schuell) by standard techniques (9). For Northern analysis, RNA was isolated by the glass-bead method (10). Poly(A)$^+$ RNA was purified on poly(U)-Sepharose and was subjected to electrophoresis through ≈0.4 M formaldehyde/1% agarose gels (9). After electrophoresis, gels were rinsed in distilled water and the separated RNAs were transferred to Nytran filters in 10× SSPE (1.8 M NaCl/100 mM sodium phosphate pH 7.8/10 mM EDTA). Radiolabeled DNA probes for both Northern and Southern analysis were prepared by the random-primer labeling method (11).

Cloning of 3.0-kb *Hin*dIII Fragment. A 3.0-kb *Hin*dIII-generated fragment containing the transposon Tc*1* was identified that was present in the *par-2(jb2)* backcrossed strain and both parental strains but was absent from 20 Daf-7 non-Par recombinants in the interval to the left of *par-2* and 70 Daf-2 non-Par recombinants in the interval to the right. Thus, this 3.0-kb *Hin*dIII fragment was tightly linked to *par-2(jb2)*. This *Hin*dIII-generated fragment was isolated from the backcrossed *egl-23(n601);daf-7(e1372)+par-2(jb2)* strain by gel purification of *Hin*dIII-digested genomic DNA of 2.5–4.0.kb and ligation of the purified DNA fraction to *Hin*dIII-cut λNM1149 DNA. The ligation was packaged *in vitro* (Gigapack,Stratagene) and plated on *Escherichia coli* C600 host cells. Plaques were transferred to Nytran filters and hybridized to radiolabeled pCe2001, a plasmid containing Tc*1* sequences (12).

DNA Sequencing. cDNA libraries analyzed during the course of this work were generously provided by Stuart Kim (Massachusetts Institute of Technology, Cambridge, MA), Irene Schauer (University of Colorado, Boulder, CO), Chris Martin (Columbia University, New York, NY), Bob Barstead (University of Missouri, St. Louis, MO), and Julie Ahringer (University of Wisconsin, Madison, WI). Restriction fragments isolated from the cDNA clones were inserted into the plasmid vector pBluescript SK or KS (Stratagene). Clones were sequenced by the chain-termination technique using Sequenase enzyme (United States Biochemical). All sequencing was performed on double-stranded template DNA; both strands of all cDNA subclones were sequenced.

Preparation of Yeast Artificial Chromosome (YAC) DNA for Injection. The Y11F11/rol-6 YAC was generated by first inserting an *Eco*RI fragment containing the dominant *rol-6* gene into the plasmid pSL42-2 (a YIp5 derivative from S. Carl Falco, Dupont), which contains the yeast *LYS2* gene. The resulting plasmid, pL2R6, was linearized at the unique *Sal* I restriction site and transformed into the yeast strain bearing the Y11F11 YAC. The desired recombination product was recovered by selecting for lysine prototrophy. One liter of yeast cells harboring the Y11F11/rol-6 YAC were grown at 30°C under selective conditions. Cells were harvested, lysed, and subjected to centrifugation through two sucrose gradients (see ref. 13 for details) to obtain fractions enriched in YAC DNA. *par-2(it5ts)* animals were injected as described (14).

Polymerase Chain Reaction (PCR), Single-Strand Conformation Polymorphism (SSCP) Analysis, and Sequencing. PCR assays were performed on individual animals as described (15). Reaction conditions differed from those described in that the final reaction mixture contained 1 mM $MgCl_2$, 0.2 μM primers, and 0.1 mM each dATP, dGTP, dCTP, and dTTP. Reactions were cycled 35 times: 94°C for 1 min, 57°C for 2 min, 72°C for 2 min. Primers DL15 (5′-CAACTGCTCGTCCCGCCA-3′) and DL16 (5′-GAACGAGTTGCGCTTCAT-3′), which hybridize to sequences in the fifth exon of the *par-2* cDNA, were used for all reactions. For SSCP analysis (16), 7 μCi (259 kBq) of [α-^{32}P]dATP was added to each reaction mixture. Two microliters of each reaction was added to 18 μl of 95% formamide/1 mM NaOH/2 mM EDTA and was boiled for 10 min; then 2.5 μl was loaded onto a polyacrylamide gel [6% (wt/vol) acrylamide/5% (vol/vol) glycerol/1× TBE (89 mM Tris/89 mM boric acid/2 mM EDTA); running buffer, 0.5× TBE] and subjected to electrophoresis at 52 V for 5 hr. Ten *par-2* alleles were tested for a SSCP with DL15 and DL16. Only PCR DNA from *par-2(lw32)* showed evidence of a polymorphism. The *par-2(zu191)* insertion/duplication was detected by PCR with DL15 and DL16 in single worms. The sequences of the PCR products of both the *par-2(lw32)* and *par-2(zu191)* mutant alleles were determined by the chain-termination technique. PCRs were scaled up to 100 μl and the products were purified by using Geneclean glass beads (Bio 101) according to the manufacturer's protocol. Approximately 1 μg of DNA was added to 0.2 μmol of primer, boiled for 3 min, and then sequenced according to the Sequenase protocol (United States Biochemical). PCR products from at least two individual worms were sequenced separately for each allele.

RESULTS

***par-2* Mutant Phenotype.** The *par-2* mutant phenotype has been described previously (6). Briefly, embryos from homozygous *par-2* mothers exhibit a symmetric first cleavage. The two resulting daughter cells then divide at the same time and in the same orientation and produce a four-cell embryo with blastomeres of equal size. Mutant *par-2* embryos arrest as balls of cells without undergoing morphogenesis, although cellular differentiation of most cell types has been observed (6). Temperature-shift experiments have shown that the temperature-sensitive period of the *par-2* gene product begins ≈12 hr before fertilization and ends before the first cleavage (17).

Cloning of Sequences Linked to *par-2*. We identified an allele of *par-2* in a transposon mutagenesis screen designed to identify maternal-effect lethal mutations (see *Materials and Methods*). *C. elegans* strains have been found that have

increased transposition rates of the transposon Tc*1* (8) and that consequently have an increased rate of spontaneous mutation. The *par-2* allele *jb2* was isolated from such a strain, and was backcrossed 10 times to the wild-type strain *C. elegans* var. Bristol (N2). By recombination analysis, we analyzed this strain for the presence of Tc*1* elements associated with the mutation. We identified a Tc*1*-containing 3.0-kb *Hin*dIII DNA fragment that was located in the interval between 0.05% to the left and 0.1% to the right of *par-2* (see *Materials and Methods* for details). We cloned the 3.0-kb *Hin*dIII-generated fragment containing Tc*1* from a size-selected library made from the backcrossed strain by probing with radiolabeled Tc*1* and analyzing positive clones by restriction analysis. DNA containing the Tc*1* was excised and the flanking DNA was used to probe existing cosmid and YAC libraries. In collaboration with John Sulston, Alan Coulson, and coworkers, who have physically mapped 95% of the worm genome (18), we identified one cosmid, F58B6, and one YAC, Y11F11, that contain DNA flanking the Tc*1* insertion in *par-2(jb2)*.

Rescue of the *par-2* Mutant Phenotype by Microinjection. We used the 80-kb Y11F11 YAC in microinjection experiments designed to rescue the *par-2* mutant phenotype. To identify successful injection of YAC DNA, we first inserted the dominant *rol-6* gene (14) into one arm of the YAC by recombination in yeast. DNA from this YAC, Y11F11/rol-6, was partially purified from yeast chromosomes and was injected at a concentration of 100 ng/μl into *par-2(it5ts)* hermaphrodites raised at the permissive temperature (16°C). Injected animals were placed at the permissive temperature. When the F_1 progeny of the injected animals reached the L3–L4 larval stage, we shifted them to restrictive temperature (25°C). After the F_1 progeny matured and self-fertilized, we examined the plates for the presence of F_2 larvae instead of dead eggs. One out of 42 injected animals produced F_2 larvae at the restrictive temperature; many of these F_2 progeny were rollers. In subsequent generations, only the Rol animals produced progeny at 25°C. This transformed line continued to grow at 25°C for several months. In contrast, when uninjected *par-2(it5ts)* animals or *par-2(it5ts)* animals injected with *rol-6* alone are shifted to 25°C, 96% of the eggs they produce are dead and the small number of eggs that hatch become sterile adults (17). Analysis of DNA isolated from the transformed line confirmed the presence of YAC DNA in the strain (data not shown). The cosmid F58B6 failed to rescue the *par-2* mutant phenotype. Subsequent analysis showed that the cosmid was deleted for portions of the *par-2* gene (data not shown).

Identification of an Allele-Specific *par-2* Polymorphism. The microinjection results described above indicate that the *par-2* gene is present on the Y11F11 YAC but that transformation rescue would not permit us to delimit further the sequences encoding *par-2*. As an alternative approach, we searched for allele-specific DNA polymorphisms by using small subclones of Y11F11 to probe DNA from *par-2* mutant alleles. Using a 1.0-kb *Pst* I–*Xba* I fragment from the cosmid F58B6, we detected a restriction fragment length polymorphism in the strain bearing the *par-2* allele *it46*. DNA isolated from the heterozygous strain bearing *it46* contained a 4.3-kb hybridizing *Cla* I fragment in addition to the wild-type 2.8-kb *Cla* I fragment (Fig. 1, lanes 7 and 8). The polymorphic fragment resulted from fusion of two adjacent *Cla* I fragments by deletion of a *Cla* I site and surrounding sequences in the *it46* mutant allele. Examination of wild-type and mutant DNA digested with *Eco*RI (Fig. 1, lanes 3 and 4) showed that a total of 4.5 kb was deleted. The parental strains from which *it46* was isolated did not exhibit this polymorphism (data not shown). Restriction mapping in this region indicated that the site of the *it46* deletion was ≈12 kb from the site of Tc*1* insertion in the *jb2* allele.

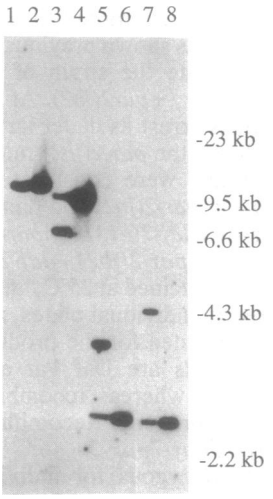

FIG. 1. Identification of an allele-specific restriction fragment length polymorphism. DNA from wild-type animals (lanes 2, 4, 6, and 8) and from the heterozygous strain *daf-7(e1372ts)+/par-2(it46)+* (lanes 1, 3, 5, and 7) was cut with *Xba* I (lanes 1 and 2), *Eco*RI (lanes 3 and 4), *Cla* I and *Eco*RI (lanes 5 and 6), or *Cla* I (lanes 7 and 8), electrophoresed through a 0.5% agarose gel, and blotted to a Nytran filter. The filter was hybridized to a radiolabeled *Pst* I–*Xba* I fragment isolated from the end of the genomic insert DNA in F58B6. The *Pst* I site is located in the vector sequence of F58B6; the *Xba* I site is located in the genomic insert DNA. The positions of molecular size markers are indicated at right.

Northern Analysis. To test whether the *it46*-associated deletion might cover the *par-2* gene, we asked whether there was an mRNA transcribed from the region deleted in *it46* with an expression pattern consistent with our expectations for the *par-2* gene. We predicted that transcription from a strictly maternal-effect lethal gene such as *par-2* should be highest in the female germ line. We compared mRNA isolated from a mutant strain with an exclusively female germ line to mRNA isolated from a mutant strain with a dramatically reduced germ line. The *fem-2(b245ts)* mutation transforms XX animals, which are normally hermaphrodites, into fertile females; i.e., at the restrictive temperature these animals do not make sperm (19). The *glp-1(e2141ts)* mutation affects germline proliferation so that only 4–10 germ cells are formed at restrictive temperature. The somatic gonad is essentially wild type in these animals (20). We used DNA corresponding to the deleted region in the *it46* allele to probe Northern blots containing poly(A)$^+$ RNA from *fem-2(b245ts)* and *glp-1(e2141ts)* animals grown at the restrictive temperature. The probe hybridized to a 2.3-kb RNA from *fem-2* "female" animals (Fig. 2, lane 1). The 2.3-kb RNA was not present in RNA isolated from *glp-1* animals (Fig. 2, lane 2).

Isolation of a *par-2* cDNA. We used DNA corresponding to the deleted region in the *it46* allele to probe cDNA libraries. The largest cDNA we identified was 2.2 kb. When this cDNA was used to probe a Northern blot, it hybridized to a single germ-line-enriched 2.3-kb mRNA, suggesting that this cDNA corresponded to the same mRNA as that identified by the genomic fragment. Primer extension analysis and PCR amplification of a cDNA pool synthesized from RNA by using an oligonucleotide homologous to SL2 [a 22-nucleotide trans-spliced leader sequence (21)] and an oligonucleotide homologous to the sequenced cDNA suggested that the putative *par-2* message was trans-spliced (data not shown).

We hybridized a 5′-specific cDNA probe and a 3′-specific cDNA probe to restriction enzyme digests of the cosmid, YAC, and genomic DNA so that we could orient the cDNA relative to the allele-specific polymorphisms we described earlier. These data are summarized in Fig. 3*A*. The *par-2* gene spans >20 kb of genomic DNA. The *it46* deletion, as de-

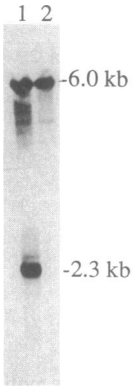

FIG. 2. Northern blot of RNA isolated from *fem-2* and *glp-1* animals. Three to four micrograms of poly(A)$^+$ RNA was run in a formaldehyde/1% agarose gel and blotted to a Nytran filter. The filter was hybridized to radiolabeled DNA isolated from the region of the *par-2(it46)* deletion and to a radiolabeled *unc-54* (myosin heavy-chain gene) to control for amount of RNA loaded in each lane. The filter was washed with 1× SSPE/0.25% SDS at 65°C for 1 hr. Lane 1, RNA from *fem-2(b245ts)* animals grown at the restrictive temperature; lane 2, RNA from *glp-1(e2141ts)* animals grown at the restrictive temperature. The *unc-54* probe hybridized to a 6.0-kb message; the genomic DNA from the region of the *par-2(it46)* deletion hybridized to a 2.3-kb message.

scribed above, encompasses the *Cla* I site that is also present in the 5′ end of the cDNA, and thus deletes coding sequences contained in this germ-line-enriched cDNA clone. The Tc*1* insertion site in the *jb2* allele is located 3′ to sequences in this cDNA.

Identification of Additional Allele-Specific Polymorphisms. To verify that this cDNA clone represents the *par-2* mRNA, we identified two additional lesions in this transcribed region that are associated with *par-2* alleles. A polymorphism was found in the *par-2* allele *lw32* by SSCP analysis, and a polymorphism was found in the *par-2* allele, *zu191*, by analysis of PCR-amplified DNA from single worms. The location of both these polymorphisms is shown in Fig. 3*A*. Sequence analysis has revealed that the *lw32* lesion is a C → T transition at nucleotide 711 of the cDNA resulting in the formation of a TGA stop codon. The truncated protein is 233 amino acids long and is missing a putative ATP-binding site (see below). The *zu191* polymorphism is a 19-nucleotide insertion/duplication shown in Fig. 3*B*. This insertion into the gene causes a stop codon at the point of insertion, truncating the open reading frame to 197 amino acids.

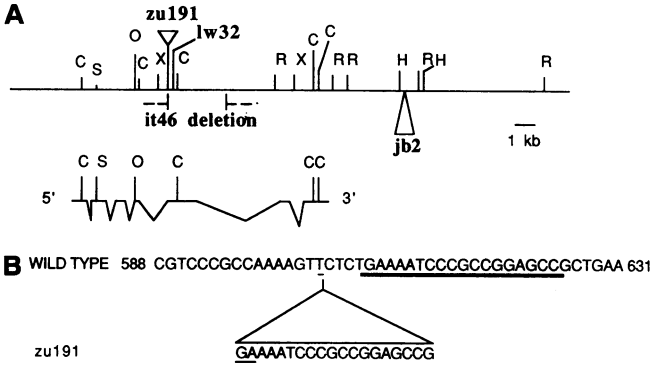

FIG. 3. (*A*) Genomic map of *par-2* region. The sites of the aberrations resulting in allele polymorphisms are indicated by bold print. A map of the *par-2* cDNA is shown below the genomic region. Exons are indicated by horizontal lines; introns are indicated by diagonal lines. The precise intron/exon boundaries are not known. For the purpose of this map, introns were deduced either by the identification of restriction enzyme sites that were present in genomic DNA but absent from the cDNA or from size differences between PCR amplification products of specific exons from genomic DNA and the cDNA clone (data not shown). Restriction sites present in both the genomic sequence and the cDNA sequence are indicated by lowercase letters above the cDNA map: X, *Xba* I; C, *Cla* I; R, *Eco*RI; *H*, *Hin*dIII; O, *Xho* I; S, *Sac* I. (*B*) Lesion associated with the *zu191* polymorphism. DNA corresponding to the putative fifth exon was amplified by PCR from single *par-2(zu191)* animals and sequenced. The corresponding sequence from wild type is shown, and the point of insertion in *par-2(zu191)* is indicated by the triangle below the sequence. The 19-bp insertion is identical to the sequence (bold underline) found 3′ to the insertion site. The insertion event generates a TGA stop codon (plain underline).

Sequence Analysis of the *par-2* cDNA. The *par-2* cDNA encodes a single large open reading frame of 628 amino acids. When the putative protein product derived from the *par-2* cDNA was compared with known protein sequences in the GenBank, Swiss-Prot, and PIR databases (October, 1993), two distinct motifs were observed. The first motif was a cysteine-rich region belonging to a recently recognized, rapidly growing class of proteins. This motif is present in proteins derived from viruses, yeast, flies, mice, frogs, and humans (22–25), and a peptide containing this motif has been shown to bind zinc (22). The Par-2 version of this motif is shown in Fig. 4*A*, compared with the domain present in other members of the family. As in other family members, the putative zinc-binding motif of Par-2 is found in the amino-terminal end of the protein. While many of the proteins in this family harbor nuclear-localization signals, no such signal has been identified in the predicted Par-2 protein.

The predicted Par-2 also possesses a putative ATP-binding site of the myosin class (type A; see ref. 28). Fig. 4*B* shows the type A ATP-binding site consensus sequence, the Par-2 putative ATP-binding site, and the sequence of selected proteins containing this motif for comparison. Par-2, however, lacks other hallmarks of myosin proteins such as actin-binding domains or an α-helical coiled-coil structure.

DISCUSSION

We report the isolation and sequence analysis of the maternal-effect lethal gene *par-2*. *par-2* plays an essential role in partitioning of cytoplasmic factors; the isolation of the gene is a step toward understanding this process at the molecular level. We present evidence that DNA sequences adjacent to and including the site of a transposon-associated mutation in *par-2* rescue the mutant *par-2* phenotype. These sequences identify a cDNA clone that is likely to be derived from the *par-2* mRNA. The transcription unit represented by this cDNA lies on an 80-kb YAC that rescues the *par-2* mutant phenotype; the transcript is germ-line enriched, as one would expect for the *par-2* transcript, and there are lesions in exons of this transcript associated with three *par-2* alleles. We feel that these data provide compelling evidence that we have isolated the *par-2* gene. The Tc*1* insertion site in *par-2(jb2)* does not appear to be in coding sequences and thus may disrupt a 3′ regulatory region. Alternatively, insertion of the transposon may not cause the mutant phenotype and merely may be linked to *par-2*. Sequencing of this mutant allele will verify whether other sequence alterations are present.

The presence of a possible zinc-binding motif in the predicted Par-2 protein is intriguing. Many proteins containing similar motifs have been shown to bind specifically to nucleic acids via a structure termed the zinc finger (30). Presently, it is unclear whether Par-2 and other proteins with the conserved cysteine-rich motif bind to nucleic acids. Evidence on individual members of this class has led to predictions of DNA binding (23, 25), RNA binding (24, 31), and protein–protein interactions (30, 32). If Par-2 functions as a DNA-binding protein, it may activate downstream genes responsible for establishing or maintaining asymmetry in the early

A **Zinc-binding motif**

Consensus	CX	(I/V)	CX11-30	CXHX	(F/I/L)	CX2C	(I/L/M)	X10-18	CPXC
par-2	CP	L	CX11	CGHS	Y	CEPC	I	X9	CVIC
xnf7(Xenopus)	CP	L	CX11	CGHN	F	CRSC	I	X11	CPEC
ring1 (Human)	CP	I	CX12	CLHR	F	CXDC	I	X10	CPTQ
neu(Drosophila)	CT	I	CX11	CGHM	C	CYDC	I	X10	CPLC
RAD-18(Yeast)	CH	I	CX11	CGHT	F	CSLC	I	X9	CPLC

B **ATP-binding motif**

ATPase Consensus	G	X	X	G	X	G	K	T	X	X	X	X	X	I
par-2	G	H	S	G	A	G	K	T	T	F	V	R	A	-	I
C. elegans myosin	G	E	S	G	A	G	K	T	E	N	T	K	K	V	I
Yeast MYO1	G	E	S	G	A	G	K	T	E	N	T	K	K	-	I
Bovine ATPase B	G	G	A	G	V	G	K	T	V	F	I	M	E	L	I

FIG. 4. (*A*) Zinc-binding motif. The consensus sequence for the zinc-binding region is shown along with this motif from Par-2 and from several other members of this class. The references for the sequences listed here are as follows: xnf7 (26); ring1 (22); neu (27); RAD18 (25); consensus sequence (27). (*B*) ATP-binding motif. The consensus sequence for the type A ATP-binding site is shown along with several members of this class of proteins and the putative *par-2* ATP-binding site. *C. elegans* myosin and bovine ATPase B sequences are from ref. 28. Yeast MYO1 sequence is from ref. 29.

embryo. If Par-2 binds to RNA, it could participate in generating asymmetry by translocating RNA molecules or by regulating the activity of specific RNAs, as is seen in the negative regulation of hunchback mRNA by the zinc-finger-containing protein encoded by the nanos gene (33). If Par-2 is not involved in nucleic acid binding, it may instead interact with other proteins such as components of the microfilament structure, the transport machinery, or partitioned determinants.

The predicted Par-2 protein also contains a putative ATP-binding site of the myosin class. The presence of this motif in Par-2 is consistent with an active, energy-consuming role in cytoplasmic sorting. However, no requirement can be ascribed to either the ATP-binding site or the cysteine-rich domain until specific mutations in these motifs are shown to affect function.

Studies of early *C. elegans* embryogenesis cited above imply the existence of cytoskeletal architecture dedicated to the process of moving or preventing movement of molecules involved in the process of determining cell fate. Because all five *par* genes affect the distribution of cytoplasmic components in gene-specific ways (6, 34), it is likely that they function in a common process required for the segregation of cytoplasmic determinants. Additional molecular analysis of *par-2* and the other *par* genes should elucidate their precise role in this process.

We thank Niansheng Cheng for providing the *it46* allele and Jocelyn Shaw for providing the *lw32* allele. We thank Victor Ambros, who provided laboratory space and encouragement for much of this work. We also thank Iva Greenwald for her patience, since writing this manuscript detracted from current projects. We thank Gautam Kao for reading the manuscript and for many thoughtful discussions. This project was supported by a predoctoral training grant to the Department of Cellular and Developmental Biology at Harvard University (D.J.L. and C.C.M.), a postdoctoral grant from the American Cancer Society (L.B.), grants from the National Institutes of Health to K.J.K. (HD27689) and D.T.S. (HD19039), and a grant from the American Cancer Society to D.T.S. (NP-647).

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