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Genetics

Isolation and characterization of *Caenorhabditis elegans* DNA sequences homologous to the v-*abl* oncogene

(Abelson murine leukemia virus/proto-oncogene/tyrosine protein kinase)

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ABSTRACT **DNA sequences homologous to the v-*abl* oncogene were isolated from a *Caenorhabditis elegans* genomic library by their ability to hybridize with a v-*src* probe. The DNA sequence of 2465 nucleotides of one clone was determined. This region corresponds to the 5′ protein kinase domain of v-*abl* plus ≈375 base pairs toward the 3′ end. Four potential introns were identified. The homology between the deduced amino acid sequence of the *C. elegans* clone and that of the 1.2-kilobase-pair protein kinase region of v-*abl* is 62%. The tyrosine residue corresponding to the tyrosine that is phosphorylated in the v-src protein is conserved in the *C. elegans* sequence. When 95 amino acids around this tyrosine were compared with the corresponding sequences of *Drosophila* c-abl, v-abl, and v-src, the identities were 83%, 79%, and 56%, respectively. Hybridization of the cloned DNA with *C. elegans* poly(A)$^+$ RNA revealed a major transcript of 4.4 kilobases.**

It is now well-established that the oncogenes of RNA tumor viruses are derived from sequences in the genomes of host cells (for review, see ref. 1). The corresponding cellular sequences, termed "proto-oncogenes," have been conserved across long evolutionary distances, appearing in yeast (2) and *Drosophila* (3–6) as well as in vertebrate organisms. Early predictions that proto-oncogenes would play important roles in control of cellular growth and development are proving to be correct. It is now known that one of the viral oncogenes (v-*sis*) is related to a cellular growth factor (7, 8), two (v-*erbB* and v-*fms*) are related to growth factor receptors (9, 10), and one (v-Ha-*ras*) is related to proteins that are required for resumption of growth by haploid spores of yeast (11, 12).

The study of proto-oncogenes would appear to be especially favorable in the yeast system where one can combine biochemical analyses with a powerful genetic system. Using this combined approach, it recently has been demonstrated that the two yeast proto-oncogenes, *RAS1* and *RAS2*, encode proteins that regulate adenylate cyclase activity (13). The strong amino acid sequence homologies (14–17) and functional homologies (i.e., GTP binding ability) (13, 18–21) among the mammalian ras proteins, the yeast RAS proteins, and the family of mammalian G proteins suggest that all three systems will participate in transduction of extracellular signals across cellular membranes. The power of the yeast system for study of other proto-oncogenes, however, is limited by the failure to identify any but the *ras*-related sequences (J. M. Bishop, personal communication). The greater complexity of *Drosophila*, as compared to yeast, predicts that there may be more proto-oncogenes present in this organism, and in fact at least six such sequences have so far been identified (3–6).

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Caenorhabditis elegans provides an organism of intermediate complexity between yeast and *Drosophila* and is characterized by a number of features that appear to be ideally suited for the study of development. The complete cell lineage of the organism, from fertilized egg to adult, has been described and can be visualized by Nomarski optics (22–24). Since populations are predominantly self-fertilizing hermaphrodites, homozygosity can be achieved in the F_2 generation, thus simplifying genetic analysis (25). Many *C. elegans* mutants have already been described with altered cell lineages (26–29). It seems reasonable to expect that proto-oncogenes will play critical demonstrable roles in the growth and development of this organism.

Sequences homologous to several of the viral oncogenes have been detected in *C. elegans* by using viral-specific probes (30). As a first step toward understanding the function of proto-oncogenes in *C. elegans*, we have isolated a number of these sequences. We report here the cloning and characterization of sequences homologous to the *abl* oncogene of the Abelson murine leukemia virus.

Interest in the c-*abl* locus has been heightened by recent findings that implicate an abnormal c-abl protein product in the pathogenesis of human chronic myelogenous leukemia (31, 32). Such a correlation between an abnormal proto-oncogene protein and the process of malignancy makes understanding of normal proto-oncogene function especially important. We believe that a genetic, cytological, and biochemical analysis of c-*abl* expression in *C. elegans* will contribute to this understanding.

MATERIALS AND METHODS

Preparation of Nematode DNA. Nematodes were grown and DNA was prepared according to the procedure of Sulston and Brenner (33) with the following modifications. DNA for library construction was prepared from animals grown on a bacterium (*Klebsiella aerogenes*) containing (G+C)-rich DNA [57% (34)]. *C. elegans* DNA [36% G + C (33)] was then separated from any residual bacterial DNA by CsCl density equilibrium centrifugation.

Library Construction and Screening. A library of *C. elegans* DNA was constructed in the λ DNA cloning vector L47.1 (35) by using methodologies described by Maniatis *et al.* (36). Fragments of nematode DNA [12–18 kilobase pairs (kbp)] obtained from partial digestions with *Sau*3A were ligated to λ DNA arms purified from a *Bam*HI digestion of L47.1 DNA. After *in vitro* packaging, plaques were transferred to nitrocellulose filters and hybridized under the following low-stringency conditions. Filters were prehybridized overnight at 50°C in 4× STE (0.6 M NaCl/0.12 M Tris, pH 8.0/0.008 M EDTA)/0.1% sodium pyrophosphate/0.1% $NaDodSO_4$/tRNA (20 μg/ml)/10× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02%

Abbreviation: kbp, kilobase pair(s).

polyvinylpyrrolidone). Hybridizations were performed at 50°C in a similar solution containing nick-translated DNA probe (specific activity, ≈4 × 10^8 cpm per μg of DNA). Filters were washed at 50°C in 0.5× STE/0.1% $NaDodSO_4$/0.1% sodium pyrophosphate, using three changes of buffer over a period of 3 hr.

v-*src*-Specific Probe. The *Pvu* II E fragment (800 bp) of the Rous sarcoma virus, Schmidt–Ruppin strain, was used as the probe for v-*src*-related sequences. The cloned fragment (p*src* *Pvu* II E) was kindly provided by J. M. Bishop.

RESULTS

Detection and Isolation of Sequences with Homology to v-*src*. *C. elegans* DNA was first analyzed by Southern analysis to see if sequences homologous to the v-*src* probe could be identified. When hybridizations and washes were performed under low stringency conditions (50°C rather than 65°C), a prominent *Hin*dIII band of 6.3 kbp was detected as well as two faint bands of 10.0 and 7.6 kbp (data not shown). Since these conditions permitted detection of discrete hybridizing bands, the *C. elegans* library was screened under the same conditions (50°C) using the v-*src* sequences as probe. Two clones were isolated that appeared to be identical by restriction enzyme analysis, and one of these (λS-6) was analyzed further.

Restriction enzyme analysis of λS-6 revealed that the homology to v-*src* was contained in a 3.3-kbp DNA fragment obtained by digestion with *Eco*RI and *Sph* I. To ensure that this fragment was an accurate representation of sequences in the nematode genome, *C. elegans* DNA was analyzed by Southern transfer using the 3.3-kbp fragment as probe. The results are shown in Fig. 1. Nematode DNA digested with *Eco*RI/*Sph* I (lane a) shows only one band with homology to the 3.3-kbp probe. The fact that this band comigrates with the corresponding band of the cloned 3.3-kbp fragment (lane b) suggests that the clone contains no extraneous sequences that may have arisen during construction of the library. Additional evidence for this is seen in the *Hin*dIII/*Sph* I and *Eco*RI/*Hin*dIII digests (lanes c and e, respectively) where the sizes of two of the *C. elegans* hybridizing bands are the same (1.8 and 1.5 kbp, respectively) as those determined by restriction enzyme mapping of the cloned 3.3-kbp fragment (data not shown).

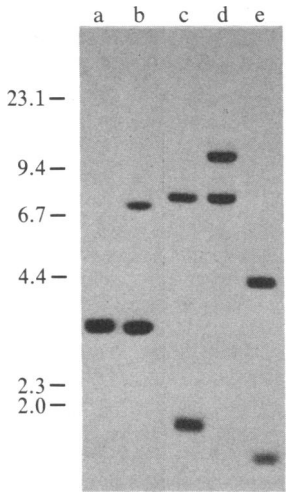

FIG. 1. Analysis of *C. elegans* DNA by hybridization with the λS-6 probe. One microgram of *C. elegans* DNA was digested with *Eco*RI/*Sph* I (lane a), *Sph* I/*Hin*dIII (lane c), *Hin*dIII (lane d), and *Hin*dIII/*Eco*RI (lane e), and analyzed by Southern transfer. Lane b contains 40 pg of an *Eco*RI/*Sph* I digestion of the mp18 recombinant clone containing the 3.3-kbp *Eco*RI/*Sph* I fragment. The probe was the nick-translated 3.3-kbp *Eco*RI/*Sph* I fragment. Hybridizations and washes were at 65°C. Molecular weight markers (kbp) are from phage λ DNA digested with *Hin*dIII. The upper band in lane b results from cross-hybridization between the λS-6 probe and mp18 vector DNA.

Interestingly, the genomic DNA which hybridized after *Hin*dIII digestion did not correspond to the prominent 6.3-kbp band that had hybridized with the v-*src* probe. Rather, two bands of 10.5 and 7.5 kbp (lane d) were detected. These appear to correspond to the two faint bands observed after hybridization of nematode DNA with the v-*src* sequences.

DNA Sequence Analysis: Homology to v-*src* and v-*abl*. For DNA sequence analysis, the 3.3-kbp *Eco*RI/*Sph* I fragment of λS-6 was subcloned into the M13 phage vectors mp18 and mp19 (37). Deletion clones (38) were then obtained (Fig. 2) and the DNA sequence of 2465 nucleotides determined (39) (Fig. 3). When the predicted amino acid sequence was compared with that of the v-src protein kinase domain (41), it was apparent that there was considerable homology in one of the six reading frames. An even greater degree of homology was detected when the same region was compared with that of v-abl (42, 43). Excluding four interruptions in sequence that have been tentatively interpreted as introns (see below), there is 62% amino acid homology between the *C. elegans* and v-abl sequences from position 97 (where v-abl begins) to position 2090. This region covers the 1.2 kbp that is necessary and sufficient for the tyrosine kinase activity of v-abl (44). Since this homology with v-abl is greater than that for other members of the tyrosine protein kinase family of oncogenes (data not shown), we have designated the λS-6 sequences as corresponding to the *C. elegans* c-*abl* genetic locus.

Introns. Introns in genes isolated from *C. elegans* are generally smaller and fewer than those of vertebrate organisms; they are rich in adenine and thymine base pairs; and they contain boundary consensus sequences that are similar to those of other organisms (T. Blumenthal, personal communication). Four introns have tentatively been assigned to sequences in the c-*abl* gene. These span nucleotides 304–411 (108 bp), 756–1331 (576 bp), 1428–1478 (51 bp), and 1920–1978 (59 bp), and their A+T contents are 66%, 75%, 69%, and 80%, respectively. All four are associated with a relatively large interruption in the amino acid homology with v-*abl*. Introns 1, 2, and 4 contain termination codons in all three reading frames and are bounded by sequences that

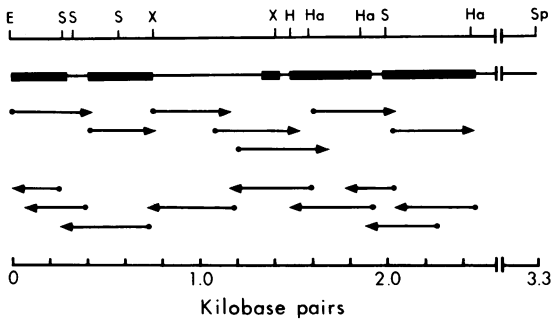

FIG. 2. Restriction map and proposed exon/intron map of the sequenced DNA, and strategy for DNA sequence analysis. The 3.3-kbp *Eco*RI/*Sph* I λS-6 fragment was cloned into mp18 and mp19. A series of deletion clones (38) was obtained in each case such that the deletion extended into the cloned DNA from either the *Eco*RI site (mp19, upper arrows) or the *Sph* I site (mp18, lower arrows). Closed circles represent sequences that are adjacent to the sequencing primer site in each clone. The direction and extent of DNA sequence analysis are indicated by arrows. The alternating solid bars and lines within the sequenced region correspond to proposed exons and introns, respectively (see Fig. 3). E, *Eco*RI; S, *Sau*3A; X, *Xho* I; H, *Hin*dIII; Ha, *Hae* III; Sp, *Sph* I.

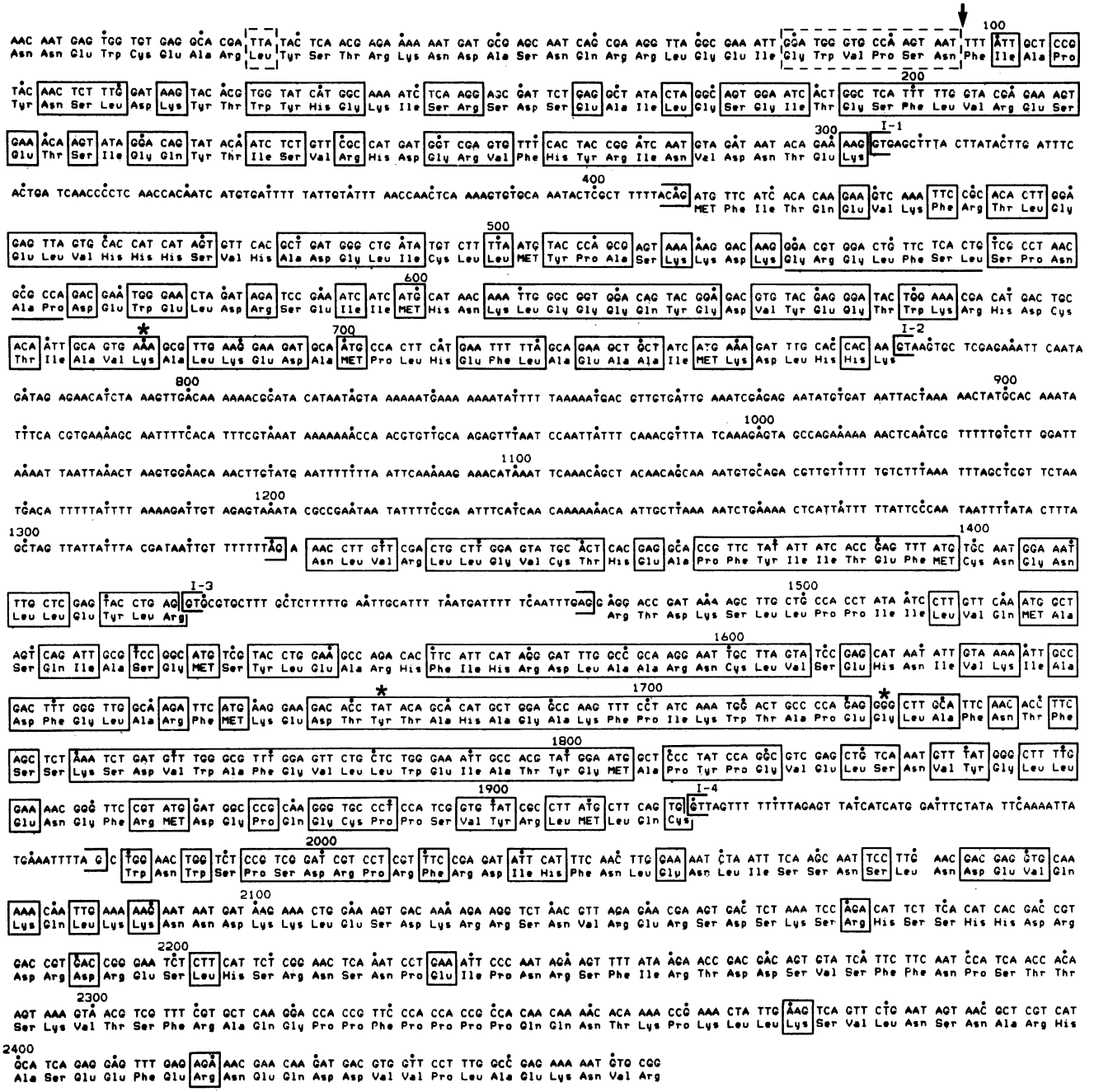

FIG. 3. Nucleotide and deduced amino acid sequence of λS-6. DNA sequencing was performed (39) by using dATP[α-^{35}S] (400 Ci/mmol; 1 Ci = 37 GBq; Amersham). Four putative introns (I) are indicated by brackets. The beginning of the corresponding v-abl sequence is marked by an arrow at position 97. Amino acids identical to those of v-abl are enclosed in solid boxes. The dashed boxes 5′ to the beginning of the v-abl sequence indicate amino acid identities with the mouse c-abl sequence (40). Amino acids marked by asterisks indicate sequences described in the text corresponding to the conserved v-src tyrosine, lysine, and alanine (glycine in *C. elegans*) at positions 1672, 679, and 1723, respectively. Sequences in *C. elegans* that contain one extra amino acid as compared with v-abl are underlined.

agree well with the *C. elegans* consensus sequences. Intron 3 contains termination codons in two reading frames and boundary sequences that agree less well with the *C. elegans* consensus. However, the presence of two adjacent stop codons in the first reading frame along with the size, high A+T content, and general correspondence with the boundary consensus sequences, argue for the designation of this region as a true intron. Confirmation of these intron designations will require sequencing of cDNA clones.

One of the c-*abl* introns (I-1) occurs at a position identical to one of the introns in the mouse c-*abl* gene (40). However, the locations of the other three appear to have no strong correlation with those of either a second mouse c-*abl* gene intron (40), a putative splice site in the human c-*abl* locus (45), or the 85-bp intron in *Drosophila* c-*abl* (3). (The *Drosophila* c-*abl* locus is referred to as *Dash* by some investigators.)

Conservation of Sequences in the Protein Kinase Domain. v-*src* has been the prototype to which members of the tyrosine kinase family of oncogenes are compared. The p60$^{v\text{-}src}$ protein contains two amino acids in its protein kinase domain that are highly conserved among members of this family. These are the tyrosine, which is phosphorylated in the v-src protein product (46), and the lysine, which is associated with the ATP-binding site of pp60$^{v\text{-}src}$ (47). *C. elegans* c-*abl* contains amino acids that correspond to both of these residues, at positions 1672 and 679, respectively. A second

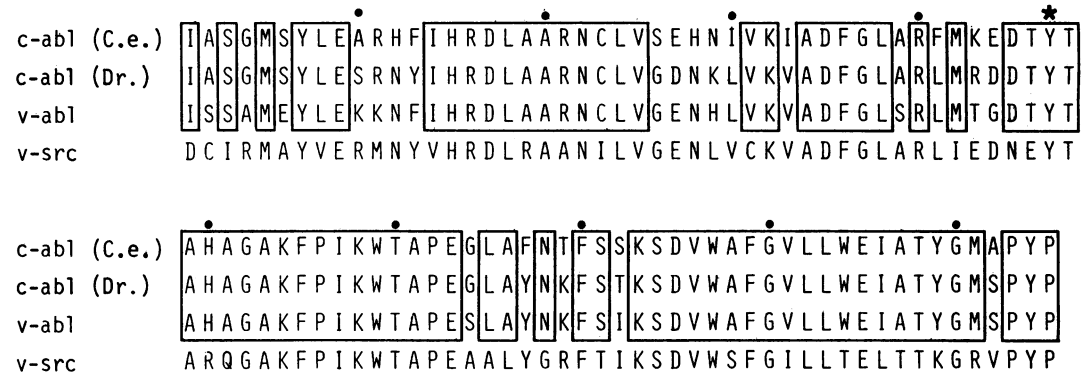

FIG. 4. Comparison of the deduced amino acid sequence in the conserved tyrosine kinase region of *C. elegans* (C.e.) c-abl with the corresponding sequences of *Drosophila* (Dr.) c-abl, v-abl, and v-src. The amino acids correspond to nucleotides 1534–1818 in Fig. 3. The tyrosine that is phosphorylated in $pp60^{v\text{-}src}$ is indicated by an asterisk. Amino acids that are common to the *C. elegans*, *Drosophila*, and v-abl sequences are shown by solid boxes. Closed circles appear above every 10th amino acid. Amino acids are identified by the single-letter code.

tyrosine, which is phosphorylated in the v-abl protein (48), is not conserved in either $pp60^{v\text{-}src}$ or the *C. elegans* c-*abl* sequence. This tyrosine would occur at position 658 in Fig. 3. Fig. 4 shows a comparison of 95 amino acids in the corresponding protein kinase regions of *C. elegans* c-abl, *Drosophila* c-abl, v-abl, and v-src. This region, which includes nucleotides 1534–1818, contains the v-src conserved tyrosine (position 1672) and the 100 base pairs that are most conserved among the v-abl, v-src, *Drosophila* c-abl and *Drosophila* c-src sequences (3) (nucleotides 1672–1771). Of the 95 amino acids compared, 56% are identical in *C. elegans* c-abl and v-src, 79% are identical in *C. elegans* c-abl and v-abl, and 83% are identical in *C. elegans* c-abl and *Drosophila* c-abl. At the nucleotide level, the order of relatedness is similar: 56% identity with v-*src*, 68% identity with v-*abl*, and 73% identity with *Drosophila* c-*abl*.

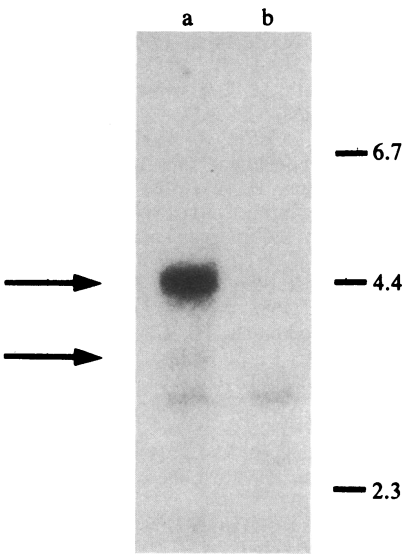

FIG. 5. Analysis of *C. elegans* RNA by hybridization with *C. elegans* c-abl. Nematodes and eggs were ruptured in a French press (11,000 psi; 1 psi = 6.89 kPa) and RNA was prepared by using the guanidinium/CsCl procedure of Chirgwin *et al.* (50) and one cycle of chromatography on oligo(dT)-cellulose. Ten micrograms of poly(A)$^+$ RNA (lane a) and 20 μg of poly(A)$^-$ RNA (lane b) were denatured, electrophoresed through a 6% formaldehyde/1.2% agarose gel, and transferred to nitrocellulose paper. The probe was the nick-translated 3.3-kbp *Eco*RI/*Sph* I fragment from λS-6. Hybridizations and washes were at 65°C. Molecular weight markers (kilobases) are *Hin*dIII fragments of phage λ DNA. Arrows indicate the 4.4- and 3.5-kilobase bands specific for poly(A)$^+$ RNA.

The glycine at position 1723 in the *C. elegans* c-abl sequence is also of interest. The corresponding amino acid in v-src is an alanine, which appears to be necessary for the tyrosine kinase activity and transforming ability of $pp60^{v\text{-}src}$, since both activities are lost when this amino acid is replaced by threonine (49). Alanine at this position is conserved in *Drosophila* c-src but is a serine in v-abl and glycine in both *Drosophila* c-abl and *C. elegans* c-abl.

Transcription of c-*abl*. To detect RNA transcripts of the c-*abl* gene in *C. elegans*, poly(A)$^+$ RNA was prepared (50) from a nematode population that included all developmental stages, from egg to adult. Both poly(A)$^+$ and poly(A)$^-$ RNA were analyzed by hybridization with the ^{32}P-labeled 3.3-kbp *Eco*RI/*Sph* I λS-6 fragment, and the results are shown in Fig. 5. One band of 3.0 kb is seen in both the poly(A)$^+$ and the poly(A)$^-$ RNA. In the poly(A)$^+$ RNA, there is an additional major transcript of 4.4 kb and a minor transcript of 3.5 kb that hybridize with the *C. elegans* probe. It is not known whether the 3.5-kb band represents a second, minor c-*abl* transcript or arises from another related gene (such as c-*src*).

DISCUSSION

Each cell division in *C. elegans* from the fertilized embryo to adulthood is visible and precisely regulated (22–24). The genetic tools required to dissect this developmental program have been established, and >50 genetic loci that alter the cell lineage pattern have been described (29). Proto-oncogenes may play pivotal roles within this cell lineage program. As a first step toward understanding proto-oncogene function in *C. elegans*, we have isolated and determined the DNA sequence of 2465 base pairs of the c-*abl* locus.

The finding of DNA sequences in *C. elegans* that are homologous to the v-*abl* oncogene provides another link in the known evolutionary conservation of this sequence. Although c-*abl* sequences have not been isolated from yeast, the corresponding gene is present in *Drosophila*. The *Drosophila* c-*abl* sequence is remarkedly similar (83% amino acid homology) to that of *C. elegans* in the region compared in Fig. 4. However, when the two amino acid sequences are compared over the entire length of known *Drosophila* c-abl sequence (3) (nucleotides 547–2060 in Fig. 3), the homology drops to 64%. *Drosophila* c-abl and *C. elegans* c-abl also are similar in that they both have a glycine residue (serine in v-abl) that corresponds to the src-specific alanine conserved in v-src and *Drosophila* c-src (3).

One major RNA transcript of 4.4 kb was found in poly(A)$^+$ RNA from *C. elegans* by using the 3.3-kbp λS-6 probe. This is considerably shorter than corresponding c-*abl* transcripts

in *Drosophila* (6.2 kb) (51), mouse (6.5 and 5.5 kb) (52), and human cells (7.0 and 6.0 kb) (52). The mouse transcripts contain the entire 3.9-kbp v-*abl* region plus additional sequences at the 5′ and 3′ ends (52). In *C. elegans*, the c-*abl* transcript may correspond to this pattern with shorter sequences at the two ends, or it may be missing some of the v-*abl* sequences present in other organisms.

The fact that only a single band was observed when the c-*abl* clone was hybridized to *C. elegans* DNA (Fig. 1, lane a) suggests that there is a single genetic locus for the c-*abl* gene. This is supported by results of other Southern transfer experiments using a variety of restriction enzymes and hybridization conditions of both high and low stringency (data not shown). The existence of an RNA transcript homologous to the c-*abl* probe further suggests that this genetic locus represents a functional *C. elegans* gene.

In *Drosophila*, c-abl RNA is detected in unfertilized eggs and embryos up to 4 hr after fertilization (51); patterns of expression of c-*abl* in mouse tissues suggest that the locus is also developmentally regulated in mammals (52, 53). In *C. elegans*, it is not known whether c-*abl* is active at all stages of the nematode life cycle or whether it is preferentially expressed in specific periods of development. The known cell lineage of *C. elegans* affords an excellent opportunity for determining precisely when and where the c-*abl* locus is active in the development of the organism.

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