The EMBO Journal vol.4 no.10 pp.2609-2615, 1985

Alternative processing and developmental control of the transcripts of the *Drosophila abl* oncogene homologue

John Telford[1], Jean Burckhardt, Barbara Butler[2] and Vincenzo Pirrotta[2]

European Molecular Biology Laboratory, Postfach 102209, D6900 Heidelberg, FRG

[1]Present address: SCLAVO, Centro di Ricerca, via Fiorentina 1, 53100 Siena, Italy

[2]Present address: Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Communicated by V.Pirrotta

***Drosophila* sequences homologous to the *abl* oncogene are located near the 5′ end of a gene (*Dash*). The *Dash* gene is transcribed to give long RNAs (5–6 kb) and short RNAs (3.0 kb) that lack some of the internal exons of the gene including some of the sequences coding for the protein kinase domain. The gene is composed of at least five short exons and a long 3′ exon. The 3′ exon is processed in several alternative ways. It contains an intronic sequence which is spliced out in ~50% of the transcripts. S1 mapping shows the existence of five different 3′ ends, presumed polyadenylation sites, differing by up to 1 kb. Three of these are maternal-specific while the other two are utilised during development. *Dash* RNA is most abundant in eggs and early embryos, becomes very rare during larval development and returns in a burst of activity in early pupae.**

Introduction

A large subset of vertebrate cellular oncogenes is constituted by the *src* family which includes *src, yes, abl, fes*, etc. (see recent reviews by Bishop, 1983; Müller and Verma, 1984; Heldin and Westermark, 1984). The products of these genes have in common the ability (under appropriate conditions) to act as protein kinases, phosphorylating tyrosine residues on target proteins and also frequently autophosphorylating. The members of the *src* family also have related sequences. In some cases the sequence homology is extensive (80% between the amino-acid sequences of *yes* and *src*) but generally not sufficient to result in cross-hybridisation between the genes. The homology is most pronounced in the part of the genes coding for the protein kinase domain. Homology has also been found recently between these oncogenes and growth factor receptors to which they are functionally related by virtue of their tyrosine-kinase activity.

The *src*-related oncogenes are highly conserved in vertebrates and in many cases in organisms phylogenetically much more distant. *Src*-related sequences have been found in the genomes of nematodes and of *Drosophila* (Shilo and Weinberg, 1981). Screening *Drosophila* genomic libraries for sequences homologous to oncogene probes, we have isolated a number of clones which hybridise to v-*src*. One set of these clones corresponds to a gene whose structure and expression we report here.

Hoffman-Falk *et al.* (1983) and Simon *et al.* (1983) have shown that the *Drosophila* genome contains three sites with homology to the tyrosine kinase domain of *src*. One of these sites, located at 73B on the third chromosome, is more related to *abl* than to *src* (Hoffman-Falk *et al.*, 1983). This site, called *Dash*, has homology to v-*abl* over a region of 700 bp which can be translated to 74% homology in the amino-acid sequence (Hoffmann *et al.*, 1984).

In this paper we show that the *abl* homologous sequences at the *Dash* site are part of a gene which bears striking similarity to the human and murine c-*abl*. The parallel between the *Drosophila* and the mammalian genes extends not only to their structure but also to the way they are expressed. The molecular and, eventually, the genetic analysis of the *Drosophila* gene may help to determine the normal developmental and cellular function of *abl* and related cellular oncogenes.

Results

Isolation of the Drosophila abl sequences

We originally used a 3.1-kb *Eco*RI fragment of the v-*src* gene that includes the sequences coding for the tyrosine kinase domain to screen a *Drosophila* genomic clone library. Hybridising under low-stringency conditions (6 × SSC, 65°C) we isolated a number of clones with varying degrees of homology to the probe. One of these clones, λS3, also hybridised to a v-*abl* probe and forms the starting point of this study.

Clone λS3 hybridised *in situ* to salivary gland chromosomes at position 73 B on the left arm of the third chromosome. Other clones from this region had been isolated during a chromosomal walk initiated from chromosome fragments microdissected from region 73A (B.Butler and V.Pirrotta, unpublished). In particular, a cosmid clone isolated in that walk contained the λS3 sequences and its orientation in the walk allowed us to establish the orientation of the *src* and *abl* homologous sequences in the chromosome.

Although the v-*src* probe detected several other clones that hybridised to at least two other chromosomal sites, we could not detect cross-hybridisation between λS3 and these clones. Similar results have since been published by Hoffmann-Falk *et al.* (1983) and by Simon *et al.* (1983). Hoffmann *et al.* (1984) have sequenced the region of homology at 73B and found that it is related to *src* but even more closely related to *abl*. Genomic Southern blots hybridised with the *abl* homologous region of λS3 under non-stringent conditions (2 × SSC, 65°C) fail to detect any sequences other than those corresponding to the 73B locus (data not shown).

The homology to *src* or *abl* at the 73B locus is limited to the region coding for the tyrosine kinase domain. When clones corresponding to this locus were hybridisd under relaxed conditions to other probes representing the v-*src* or v-*abl* genes, we failed to detect any other significant cross-hybridisation. If homology to other parts of these oncogenes exists, it is too low to be detected by cross-hybridisation.

The Dash locus is transcribed

To determine whether the region of homology at the 73B locus (henceforth called *Dash*, according to Hoffmann-Falk *et al.*,

1983) corresponds to an active gene, we searched for RNA transcripts. Drosophila poly A^+ RNA from different developmental stages was separated by formaldehyde-agarose gel electrophoresis and a Northern blot of the gel was hybridised to a 1.7-kb *Bam*HI fragment which included the *src/abl* homology region (probe 2, Figure 2). Under non-stringent conditions this probe reveals at least three different RNA species which are represented differently in the four major developmental stages. Figure 1 shows a rather diffused major band corresponding to an RNA of 5.5 – 6 kb which is most abundant in adults, a band around 3.0 kb which is considerably weaker but approximately constant throughout development and a band around 1.7 kb which is strongest in embryos. After a more stringent wash (0.1 × SSC, 65°C), the 1.7-kb species is no longer detectable while the other species are not appreciably affected. The same probe, hybridised to *Drosophila* genomic DNA displays only the bands corresponding to the *Dash* locus under stringent or relaxed conditions. We suppose, therefore, that the upper two bands represent RNA transcripts of the *Dash* locus and that the 1.7-kb species may be due to cross-hybridisation with another locus whose transcripts are much more abundant than those from the *Dash* locus, and hence become detectable at low stringency. The *Dash* transcripts themselves are rather rare, estimated by comparison with *white* RNA (Pirrotta and Bröckl, 1984) and with actin RNA (Anderson and Lengyel, 1984) as 0.001% of the total poly A^+ RNA in adult flies.

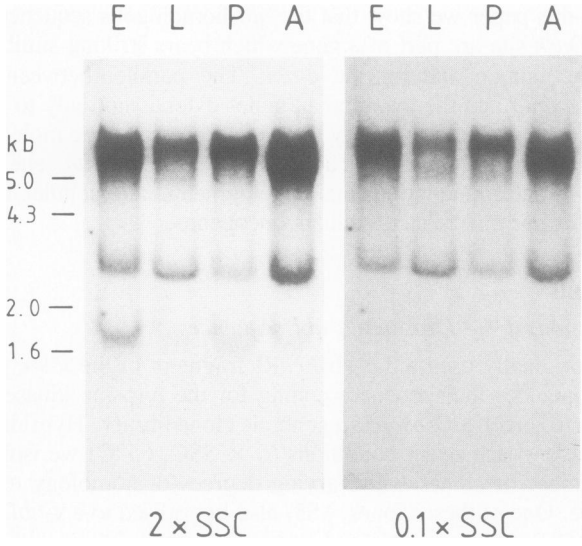

Fig. 1. Transcription of the *Dash* locus. Poly A^+ RNA (30 μg) from *Drosophila* embryos **(E)**, larvae **(L)**, pupae **(P)** and adult flies **(A)** was separated by formaldehyde/agarose electrophoresis, transferred to a nitrocellulose filter and hybridized with a 1.7-kb *Bam*HI fragment of the *Dash* locus containing the *src/abl* homology. The **left panel** shows an autoradiogram of the filter after washing at low stringency (2 × SSC, 65°C). The **right panel** shows the same filter after washing at high stringency (0.1 × SSC, 65°C).

Extent of the Dash gene

That the 3.0- and 5.5 – 6-kb size classes are genomic *Dash* transcripts is confirmed by the fact that they are revealed when flanking fragments are used as probes to delimit the extent of the transcription unit. The 5.5 – 6-kb size class is detected by probes 1 to 6 shown in Figure 2, covering a region of 9 kb. Fragments to the left of this region (not shown) do not hybridise to any RNA. The rightmost probe, number 7, no longer reveals the 5.5 – 6-kb RNA but hybridises instead to two abundant RNA species of 1.9- and 2.8-kb. We suppose therefore, that the *Dash* gene is bracketed by the *Hind*III site at the left end of the map in Figure 2 and the *Eco*RI site 9 kb to the right of it. Fragments from both ends of this region hybridise to the 3.0-kb RNA but some of the internal fragments do not, indicating that some sequences included in the large RNA are absent in the short RNA species. In particular, although probe 3 hybridises strongly to this RNA, a 0.6-kb subfragment of this probe (probe 2) reveals the 5.5 – 6-kb species but not the 3.0-kb species, or only very weakly. This is remarkable since probe 2 contains the sequences homologous to the region of v-*src* essential for its transforming activity. These results indicate that the *Dash* gene produces two types of RNA transcripts: one that includes the sequences homologous to v-*src* and one that does not. Another puzzling result is that obtained with probe 5. This, an internal probe, reveals the 5.5 – 6-kb RNA and in addition two small RNAs of

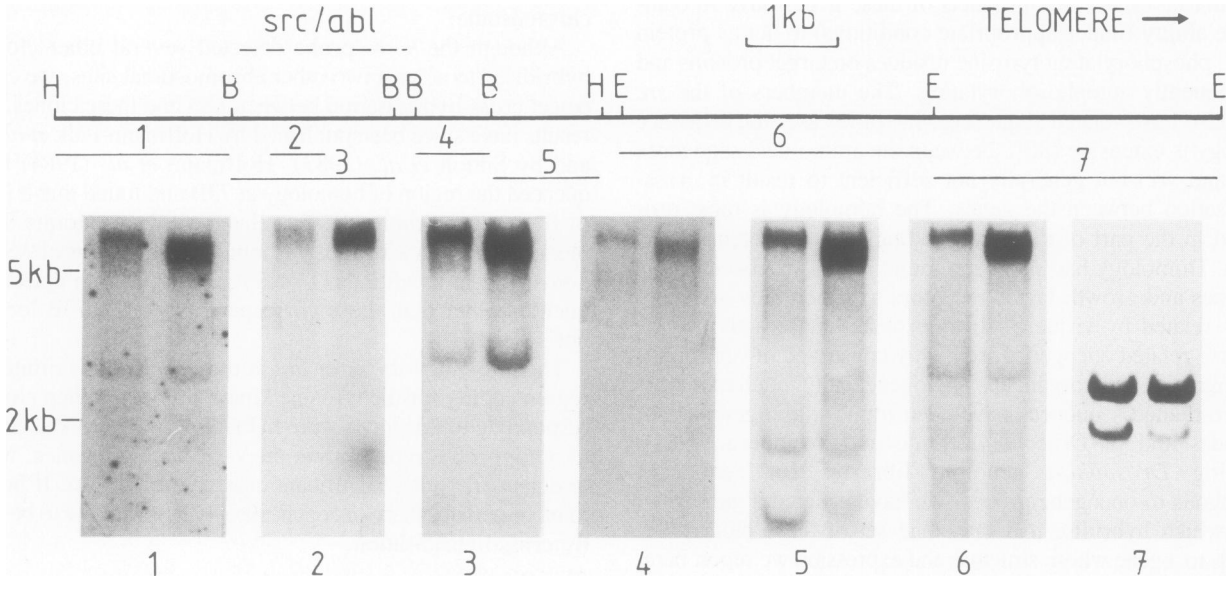

Fig. 2. Extent of the *Dash* gene. A restriction map of the *Dash* locus is shown. The underlined fragments were hybridised to Northern blots of poly A^+ RNA from pupae and adult flies to determine the limits of the transcription unit.

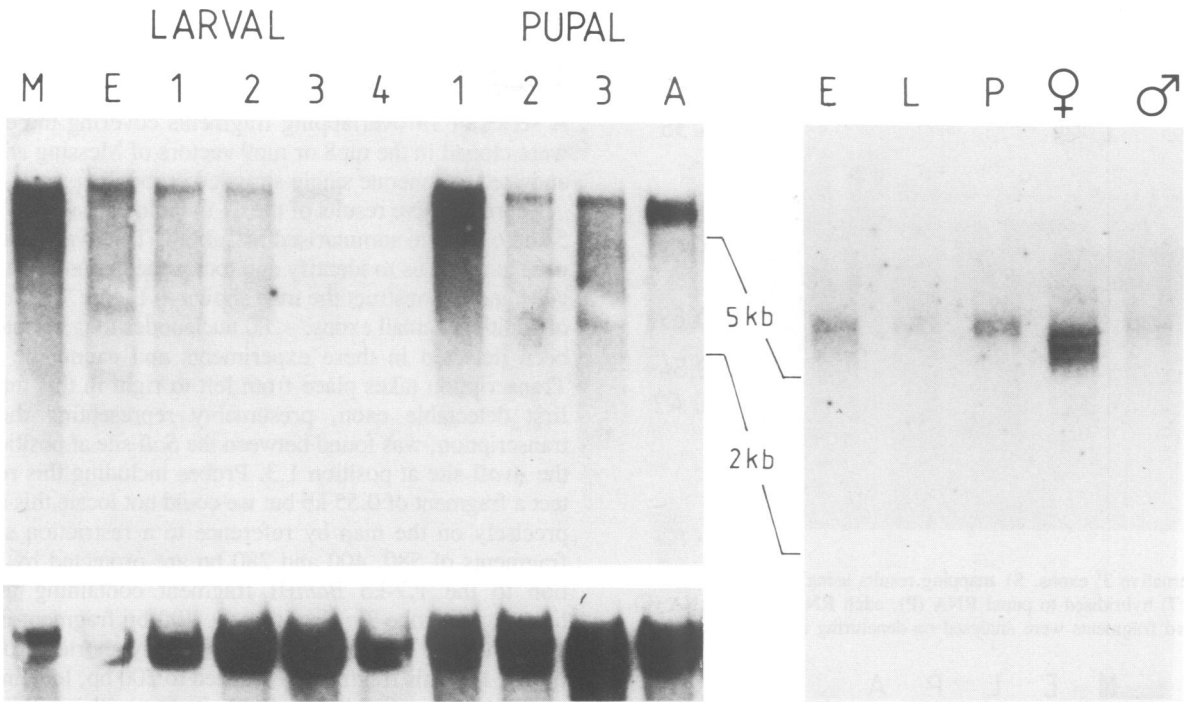

Fig. 3. Developmental expression of the *Dash* locus. The **left panel** shows poly A^+ RNA from unfertilized eggs **(M)**, embryos **(E)**, larval and pupal stages at 24-h intervals **(larval 1–4, pupal 1–4)** and adult flies **(A)** were electrophoresed, blotted and hybridised with probe 3 (see Figure 7). The same filter was subsequently hybridised to an actin probe **(lower panel)** as a control. The **right hand panel** shows a similar gel after longer electrophoresis to increase resolution. **(E)** embryonic, **(L)** larval, **(P)** pupal, (♀) adult flies, (♂) adult male RNA.

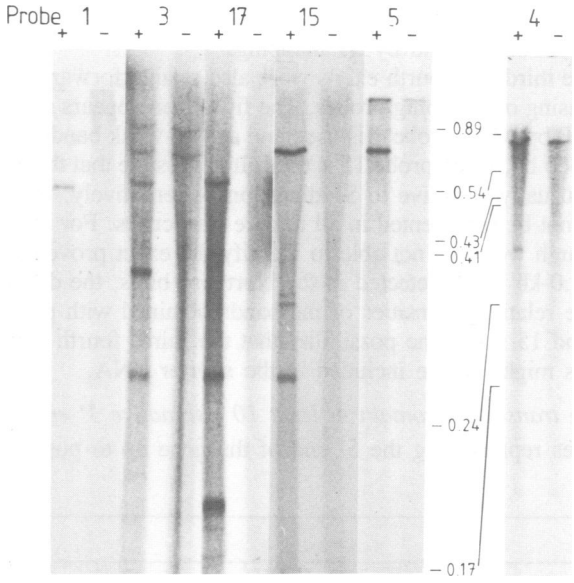

Fig. 4. S1 mapping of mature transcripts. Pupal poly A^+ RNA was hybridised to labelled single-stranded probes from the *Dash* gene, digested with S1 nuclease and the products analysed on denaturing acrylamide gels. The probe numbers refer to the regions underlined in Figure 7. Each probe was hybridised to poly A^+RNA **(+lane)** and to tRNA, **(−lane)**, as control. Mol. wt. markers are indicated.

1.2- and 1.7-kb. One possible explanation for these bands is that this part of the *Dash* gene bears homology to some other *Drosophila* locus which produces the two small RNAs.

Dash transcripts change during development

Both Figures 1 and 2 show that the 5.5–6-kb band from the different developmental stages changes in appearance. The band seen in embryos and adults is distinctly broader and of slightly lower mol. wt. than that found in larval or pupal RNA. To investigate this difference, we isolated RNA from different developmental stages at 24-h intervals as well as from young adult flies and from unfertilised eggs. Since no RNA is synthesised in the eggs, this last preparation corresponds to maternal RNA. Figure 3 shows a Norther blot of these RNAs, hybridised with probe 3. Transcription of the gene is clearly modulated during development, with the greatest accumulation of *Dash* RNA occurring in the eggs, in early pupae and in adults while little or no *Dash* RNA is found in larvae. The lower left panel in Figure 3 shows a section of the same filter rehybridised with an actin probe. Although expression of the actin genes is also known to vary during development (Zulaf *et al.*, 1981) this serves as an approximate control for the developmental profile.

The right-hand panel shows a Northern blot of a gel after much longer electrophoresis to increase resolution. This blot, hybridised with the same probe, shows that the diffuse appearance of the 5.5–6-kb band seen in adults is due to a real qualitative difference in the RNA. Maternal RNA and adult females, but not males, contain multiple bands of a mol. wt. ranging from 5.4 to 6.0 kb, while embryos, larvae, pupae and adult males contain RNA species of 5.8–6.0 kb. This difference, confirmed by S1 mapping analysis (see below), indicates that there are three different developmental phases in the transcription of *Dash*. One occurs in females, probably confined to the ovaries and developing oocytes, and produces RNA of 5.4–5.8 kb which is accumulated in the maturing oocytes and is found in unfertilised eggs and early embryos. This type of RNA disappears in late embryos and RNA species of 5.8–6 kb accumulate instead, though to a much lesser extent. This activity tapers off in larvae until no *Dash* RNA could be detected in the late third instar stage. A new, strong burst of synthesis in early pupae accumulates the 5.8–6.0-kb RNA. Late pupae and adults, both male and female, contain low levels of the 5.8–6.0-kb species.

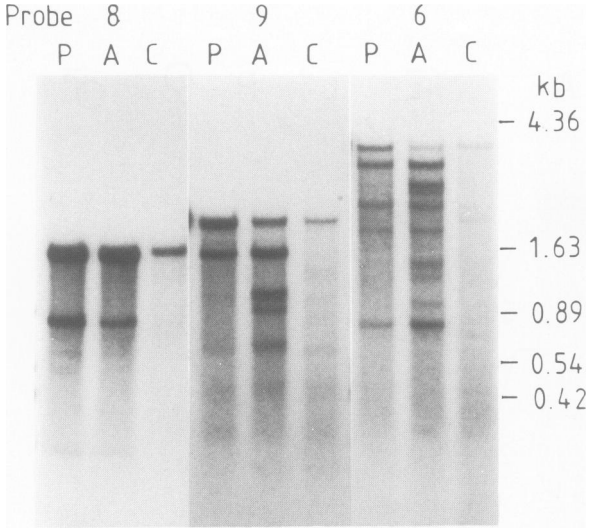

Fig. 5. Alternative 3′ exons. S1 mapping results using probes 8, 9 and 6 (see Figure 7) hybridised to pupal RNA **(P)**, adult RNA **(A)** or tRNA **(C)**. The protected fragments were analysed on denaturing agarose gels.

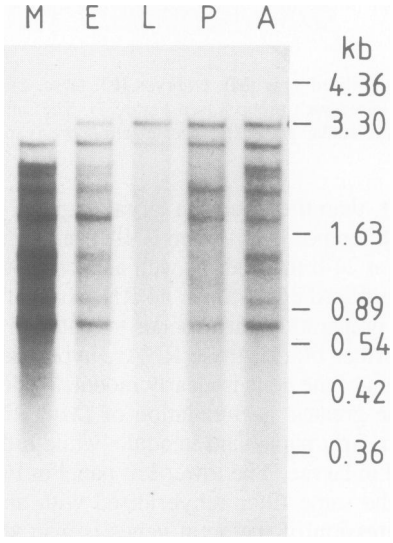

Fig. 6. Developmental variation in the structure of the 3′ exon. S1 protection results using probe 6, hybridised to maternal **(M)**, embryonic **(E)**, larval **(L)**, pupal **(P)** or adult **(A)** RNA.

The mature RNAs are spliced from a large transcript

To determine the anatomy of the *Dash* gene and the nature of the differences in the transcripts, we used S1 nuclease mapping. A series of 19 overlapping fragments covering the entire gene were cloned in the mp8 or mp9 vectors of Messing *et al.* (1981) and used to generate single-stranded probes by primer extension.

Representative results of the S1 mapping are shown in Figures 5 and 6 and are summarised in Table I. The overlapping probes used allowed us to identify and locate the exons constituting the gene and to construct the map shown in Figure 7. The existence of additional small exons, <80 nucleotides long, would not have been detected in these experiments and cannot be excluded. Transcription takes place from left to right in this map and the first detectable exon, presumably representing the start of transcription, was found between the *Sal*I site at position 0.4 and the *Ava*II site at position 1.3. Probes including this region protect a fragment of 0.55 kb but we could not locate this exon more precisely on the map by reference to a restriction site. Three fragments of 580, 400 and 280 bp are protected by hybridisation to the 1.7-kb *Bam*HI fragment containing the *src/abl* homology (probe 3). Note that the 400-bp fragment is more intense than the other two. When a slightly shorter probe is used (probe 17), this fragment is reduced to 200 bp, locating this, the second exon, astraddle the *Bgl*II site at position 2.0 in the map. The second intron revealed by these results was predicted by the partial nucleotide sequence of *Dash* determined by Hoffmann *et al.* (1984). They found that the homology between *Dash* and *abl* was interrupted by a gap of 85 nucleotides which also contained termination codons in all three reading frames. The position of this gap coincides exactly with the position and extent of the second intron we find by S1 mapping.

The third and fourth exons were also straightforwardly mapped using overlapping probes. The fifth exon appears as a band of 350 bp when probe 4 is used and as two weak bands, of 350 and 365 bp, when probe 15 is used. It is possible that this region is unusually sensitive to S1 digestion. Alternatively, this exon may not be represented in all mature transcripts. For example, although we were not able to identify the exact provenance of the 3.0-kb RNA detected in the Northern blots, the difference in the relative intensities of the bands obtained with probes 3, 17 and 15 raises the possibility that the third, fourth and fifth exons might not be included in the shorter RNA.

Dash transcripts contain at least 10 alternative 3′ ends

Probes representing the 5′ end of the gene up to position 6.0

Table I. Summary of S1 mapping results

	Probe																			
	1	2	3	4	5	6 Pupal	6 Adult specific	8	9 Pupal	9 Adult specific	10	11	12	13 Pupal	13 Adult specific	14	15	16	17	18	19
Fragments	0.54	0.30	0.58	0.35	0.78	(3.35)	2.60	(1.35)	(1.90)	1.05	0.54	0.29	1.05	(2.0)	1.15	0.54	0.83	1.2	0.58	0.58	0.19
		0.20	0.40			2.9	2.45	0.82	1.45	0.91	0.19	0.28		1.55	1.00	0.19	0.36	0.56	0.27	0.39	
			0.28			2.20	1.35	0.19		0.67					0.78		0.28	0.28	0.20		
						1.80	1.20														
						0.80	0.96														

Fragment sizes are given in kb. The fragments given in parentheses were the same site as the probe but clearly protected above background. All the probes except 6, 9 and 13 gave the same results with pupal and adult RNA. Probes 6, 9 and 13 hybridised to adult RNA gave the pupal fragments plus a set of new fragments listed under the adult specific column.

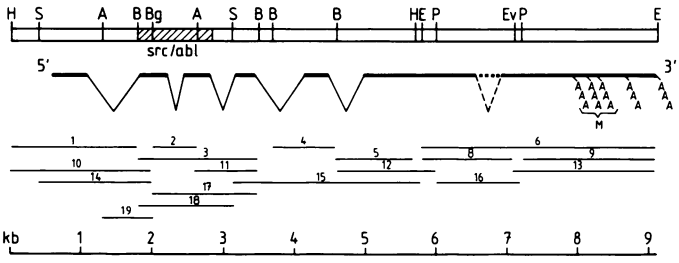

Fig. 7. Transcription map of the *Dash* locus. The hatched area in the restriction map shows the region of homology to *src/abl*. Solid lines indicate exons and summarize the results of the S1 mapping experiments. Small exons <80 nucleotides would not have been detected in our S1 mapping experiments. Dotted lines indicate the alternative splicing pattern. Presumed polyadenylation sites are indicated and the three maternal-specific sites are bracketed. The numbered overlapping fragments shown below the map represent the probes used for Northern blot hybridisation and for S1 mapping.

in the map (probe 12) give essentially the same S1 mapping results regardless of the developmental stage of the RNA. Except for some discrepancies in the intensities of the bands obtained, the results could be easily interpreted to give an exon map.

However, probes more distal than position 6 gave complex patterns of protected fragments indicating that a number of alternative arrangements are represented in the RNA. Probes 8 and 16 show that two alternative splicing patterns are present in both pupal and adult RNA (Figure 6). Probes 9 and 13 show that there are five possible 3′ termini of which three are specifically found in adult RNA and are absent in pupal RNA. Probe 6, which combines together the regions covered by probes 8 and 13, yields ten protected fragments with adult RNA, five of which are protected also by pupal RNA. The shortest of these protected fragments corresponds to the end of the sixth exon. The nine remaining fragments (four in pupal RNA) represent different 3′ ends. If all possible permutations of the five 3′ ends indicated by probes 9 and 13 and the two splicing patterns indicated by probes 8 and 16 were realised, we would expect ten 3′ end fragments protected by adult RNA. However, a simple calculation shows that all ten 3′ ends most probably do occur but that two of them coincide in size. A 2.2-kb fragment coming from the adult-specific RNA containing the unspliced last intron and the first 3′ end, would in fact co-migrate with a fragment of similar size produced by the shortest pupal RNA, with the last intron spliced off.

The adult-specific RNA species are found in maternal RNA

S1 mapping using probe 6 and RNA from the major developmental stages confirms the results obtained with Northern blots (Figure 3) and shows that the adult-specific RNA species, utilising the three more proximal termination sites are also found in maternal RNA. Figure 6 shows that while adult RNA contains both the pupal-type 3′ ends and the shorter 3′ ends in roughly equal proportions, the shorter ends predominate in maternal RNA while the longest 3′ end, containing the unspliced last intron (top band) is almost completely absent. In embryonic RNA, representing 0 – 16 h-old embryos, the maternal RNAs are still visible, though greatly reduced in intensity, while longer 3′ ends predominate. RNA from all three larval instar stages combined contains very little *Dash* RNA but what can be seen corresponds to the two longer 3′ ends. *Dash* sequences are well represented again in pupal RNA and correspond to the two longer 3′ ends. Adult (male plus female) RNA shows all the species but, as Figure 3 indicates, the shorter 3′ ends are in fact contributed only by the females and are most likely specific for the ovaries.

Discussion

Structural correspondence between Dash and c-abl

Our results show that the *Dash* locus is part of a *Drosophila* gene which is not only partially homologous to the mammalian *abl* gene but resembles it in several structural and functional respects.

The c-*abl* gene has now been cloned from the mouse genome (Wang *et al.*, 1984) and from the human genome (Heisterkamp *et al.*, 1983). In both cases, the gene is large, distributed over 30 kb, and contains ten or more short exons of a few hundred nucleotides each, while the 3′ exon, at least in mouse, is much longer (>3 kb). Although *Drosophila* genes tend to have fewer and shorter exons than their vertebrate counterparts, the *Dash* gene still covers about 8.5 kb and includes at least six short exons and a long 3′ exon.

Unlike other members of the *src* family, which have the protein kinase domain near the 3′ end, the *abl* gene in both man and mouse has it near the 5′ end. The part of the *Dash* gene that is homologous to the tyrosine kinase domain of v-*abl* is also located near the 5′ end of the gene. The sequence homology reported by Hoffmann *et al.* (1984) is distributed over the second and third *Dash* exons and aligns the *Bgl*II site at position 2.0 in the restriction map shown in Figure 4 with nucleotide 362 of the v-*abl* sequence (Reddy *et al.*, 1983). Since v-*abl* lacks ~350 nucleotides from the 5′ end of the c-*abl* gene (Wang and Baltimore, 1983) this corresponds roughly to nucleotide 712 in c-*abl* RNA while the *Bgl*II site in *Dash* corresponds to nucleotide 750 in the *Dash* RNA, according to the exon map in Figure 7.

Dash transcription

Transcripts of c-*abl* have been found in all tissues and developmental stages analysed in the form of two major RNA species of ~5 and 6 kb in mouse and 6.0 and 7.0 kb in man (Müller *et al.*, 1982; Wang and Baltimore, 1983). Levels are higher in the early mouse embryo than at later stages but they are highest in the testes of the adult mouse (Müller *et al.*, 1982) where a third species of 3.7 kb was found in addition to the other two, and in greater abundance.

In *Drosophila* we also find superficially long and short transcripts. A short RNA species of 3.0 kb may perhaps parallel the 3.7-kb species found in mouse testes, although we have no evidence for tissue specificity in our case. The larger *Dash* RNA species break down, upon S1 mapping analysis, into 10 different transcripts rather than just two. It is tempting to suggest an analogy between the two alternative usages of the sixth intron and the two size classes found in mouse and man. These two alternative splicing patterns appear to be present at all developmental stages although we would not have detected possible tissue specifities.

Developmental controls

Superimposed on the alternative splicing of the last intron, we find in *Drosophila* five possible 3′ ends, presumably polyadenylation sites. Three are found only in adult females and maternal RNA, two are used in embryos, pupae and adults. Our results strongly suggest that the female and maternal specific RNA species in fact originate in the ovaries.

Although we cannot exclude that the shorter RNAs are produced by stepwise processing of the longer ones, we think this unlikely since the developmental profile shows no precursor-product relationship between longer and shorter 3′ ends. We do

not see the shorter ends accumulating after the early pupal burst of *Dash* transcription or during the gradual disappearance of the longer transcripts in the course of larval development. More probably the different 3′ ends are produced directly by processing of a primary transcript. The three earlier polyadenylation sites are preferentially utilised in the ovaries. It is not impossible that terminal heterogeneity occurs also in the mammalian c-*abl* RNA. Such heterogeneity, if it exists, could easily have gone undetected in the experiments reported in the literature, given the large size of the *abl* RNA.

The developmental profile shows that the levels, as well as the 3′ ends of the *Dash* RNA change during development. We have no direct evidence to prove that these changes in abundance reflect changes in transcriptional activity rather than in the rate of degradation. In either case, the changing levels indicate developmentally controlled changes in *Dash* activity. Our results differ sharply from those of Lev *et al.* (1984) who detected *Dash* RNA only in unfertilized eggs and early embryos. Part of the discrepancy may perhaps be attributed to the generally low abundance of *Dash* RNA and its relatively high mol. wt. which renders it more susceptible to degradation.

Correlation with cell proliferation

In view of the suspected involvement of c-*abl* in the control of cell proliferation, it is interesting, but entirely speculative, to try to attribute a significance to the bursts of *Dash* activity in the course of development.

If we look for a correlation between cell proliferation and *Dash* RNA levels, we find that the maximum accumulation of transcripts occurs in the maternal RNA present in the egg. Embryonic cells, of course, divide rapidly, and in particular preblastoderm nuclei undergo a series of twelve extremely rapid divisions leading to the cellular blastoderm stage. The lowest levels of *Dash* RNA are found in the larval stages when cell division has ceased in most larval tissues but substantial cell division occurs in imaginal discs. Around puparium formation a burst of rapid cell proliferation with a doubling time of 2.8 h occurs in the histoblasts which give rise to the abdominal structures (Garcia-Bellido and Merriam, 1971; Guerra *et al.*, 1973). This burst, which is over by the second day of pupal development, coincides with the high levels of *Dash* transcripts that appear in the early pupae and subside in the course of pupal development. We have no evidence to determine whether the correlation has any significance or whether the *Dash* function is at all related to the function of the vertebrate *abl* gene.

Alternative RNA processing and gene regulation

A number of cellular genes in vertebrates as well as in *Drosophila* are now known whose transcripts can be processed by alternative pathways. In some cases the alternative RNA species produced encode different proteins (Rosenfeld *et al.*, 1984) or different forms of a protein (Rogers *et al.*, 1980; Cheng *et al.*, 1982). In other cases, although multiple RNA species differing in the length of their 3′ untranslated region are produced from a single gene, they all give the same translation product (Setzer *et al.*, 1980). We do not know in our case whether the last intron or the earlier polyadenylation sites affect the coding region but the 3.0-kb species almost certainly encodes a shorter polypeptide. It is interesting to note that a large tract at the 3′ end of the mouse c-*abl* RNA is not translated. Wang *et al.* (1984) find that a 1.2-kb cDNA clone of c-*abl* RNA contains no extensive open reading frame. Furthermore, they conclude that this cDNA clone represents the 5.5-kb c-*abl* RNA, implying that the 6.5-kb RNA species contains an additional kilobase of untranslated sequence.

Different untranslated 3′ sequences might serve to regulate the expression of the mRNA by altering its conformation, its ability to bind proteins and its translational potential. Miller *et al.* (1984) have found that the untranslated 3′ region of the c-*fos* oncogene contains a sequence that interacts with part of the coding region to inhibit it from transforming cultured cells. It is possible that the different 3′ ends found in the *Dash* transcripts might similarly regulate the translational potential or the cellular localisation of the product.

Materials and methods

Cloning of v-src related genes

A clone library of *Drosophila* DNA constructed in λEMBL 4 (Pirrotta *et al.*, 1983) was hybridized with a ^{32}P-labelled 3.1-kb *Eco*RI fragment from the Rous sarcoma virus at 65°C in 6 × SSC. Positive signals were picked and rehybridised with a 0.6-kb *Pst*I fragment of the v-*src* gene. Restriction fragments were subcloned in plasmid or M13 vectors by standard procedures.

Preparation of poly A$^+$ RNA

Mass collections of eggs were usually carried out for 15 h. After development at 25°C to the appropriate stage, eggs, embryos, pupae or adult flies were frozen by dropping into liquid nitrogen. Frozen material was added to an ice cold 1:1 mixture of RNA buffer (0.1 M sodium cacodylate, 0.5 M NaCl, 0.01 M EDTA, 0.1% diethyl pyrocarbonate pH 7.0) and water-saturated phenol and homogenized with a Polytron blender. SDS was added to 1% final concentration and the nucleic acids were purified by repeated extractions with phenol and chloroform. Purified nucleic acids were treated with RNase-free DNase I (10 μg/ml) in 50 mM Tris, 10 mM $MgCl_2$, 10 mM $CaCl_2$ pH 7.5 for 30 min at 37°C, centrifuged at 100 000 g for one hour to pellet polysaccharides and re-extracted with phenol and chloroform. The poly A$^+$ fraction was isolated by poly U Sepharose chromatography as described by Pirrotta and Bröckl (1984).

Northern blot hybridization

Poly A$^+$ RNA was denatured in 50% formamide, 2 M formaldehyde at 65°C before separation on 1% agarose gels containing formaldehyde (Maniatis *et al.*, 1982). After electrophoresis, the gel was soaked for 30 min in 20 × SSC and the RNA was transferred to nitrocellulose filters by the method of Southern (1975). Hybridization was carried out at 43°C in 50% formamide, 5 × SSC, 1 × Denhardt's solution and 100 μg/ml denatured salmon sperm DNA.

S1 mapping

^{32}P-labelled complementary strand was synthesized from M13 subclones by primer extension using Klenow polymerase. After digestion with appropriate restriction enzymes and denaturation in 80% formamide at 100°C, fragments were separated by electrophoresis on low melting-temperature agarose gels. Radioactive bands corresponding to the required probes were excised and heated to 65°C. After phenol extraction the DNA was precipitated with an equal volume of isopropanol.

Approximately 10 000 c.p.m. of labelled probe was hybridized overnight at 54°C with 10 μg poly(A)$^+$ RNA in 20 μl 80% formamide, 40 mM piperazine-N, N-bis (2 ethane sulphonic acid) pH 6.4, 1 mM EDTA, 0.4 M NaCl. After hybridization, samples were digested with 1000–2000 units of S1 nuclease (Boehringer) in 300 μl S1 buffer (0.3 M NaCl, 0.05 M Na-acetate pH 4.6, 4 mM $ZnSO_4$, 15 μg/ml denatured salmon sperm DNA). Reactions were stopped by addition of 50 μl 4 M ammonium acetate plus 5 μg tRNA, extracted with phenol:chloroform:isoamyl alcohol (25:25:1) and precipitated with an equal volume of isopropanol. Samples were resuspended in 80% formamide, heated to 100°C for 2 min and analyzed on either 3.5% polyacrylamide gels containing 7 M urea or 1% formaldehyde-agarose gels. Acrylamide gels were dried and agarose gels were blotted to nitrocellulose filters for autoradiography.

Acknowledgements

We thank D.Stehelin for encouragement and helpful discussions, J.M.Bishop for v-*src* DNA and R.Muller for v-*abl* DNA. We are grateful to C.Christensen for photographic service and Evelyn Stuart for typing the manuscript. B.B. was supported by a grant from the Swiss National Science Foundation to R.Nothiger.

References

Anderson,K.V. and Lengyel,J.A. (1984) in Stein,G. and Stein,J. (eds.), *Histone Genes*, Wiley, NY, pp. 135-161.

Bishop,J.M. (1983) *Ann. Rev. Biochem.*, **52**, 301-354.

Cheng,H.L., Blatner,F.R., Fitzmaurice,L., Mushinski,J.F. and Tucker,P.W. (1982), *Nature*, **296**, 410-415.

Garcia-Bellido,A. and Merriam,J.R. (1971) *Dev. Biol.*, **26**, 264-276.

Guerra,M., Postlethwait,J.H. and Schneiderman,H.A. (1973) *Dev. Biol.*, **32**, 361-372.
Heisterkamp,N., Crofen,J. and Stephenson,J.R. (1983) *J. Mol. Appl. Genet.*, **2**, 57-68.
Heldin,C.H. and Westermark,B. (1984) *Cell*, **37**, 9-20.
Hoffman-Falk,H., Einat,P., Shilo,B.Z. and Hoffmann,F.M. (1983) *Cell*, **32**, 589-598.
Hoffmann,F.M., Fresco,L.D., Hoffman-Falk,H. and Shilo,B.Z. (1984) *Cell*, **35**, 393-401.
Lev,Z., Leibowitz,N., Segev,O. and Shilo,B.Z. (1984) *Mol. Cell Biol.*, **4**, 982-984.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY.
Messing,J., Crea,R. and Seeburg,P.H. (1981) *Nucleic Acids Res.*, **9**, 309-321.
Miller,A.D., Curran,T. and Verma,I.D. (1984) *Cell*, **36**, 51-60.
Müller,R., Slamon,D.J., Tremblay,J.M., Cline,M.J. and Verma,I.M. (1982) *Nature*, **299**, 640-646.
Müller,R. and Verma,I.M. (1984) *Curr. Top. Microbiol. Immunol.*, **112**, 73-115.
Pirrotta,V., Hadfield,C. and Pretorius,G.H.J. (1983) *EMBO J.*, **2**, 927-934.
Pirrotta,V. and Bröckl,C. (1984) *EMBO J.*, **3**, 563-568.
Reddy,E.P., Smith,M.J. and Srinivaran,A. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3623-3627.
Rogers,J., Early,P., Carter,C., Calame,K., Bond,M., Hood,L. and Wall,R. (1980) *Cell*, **20**, 303-312.
Rosenfeld,M.G., Amara,S.G. and Evans,R.M. (1984) *Science (Wash.)*, **225**, 1315-1320.
Setzer,D.R., McGrogan,M., Nunberg,J.H. and Schimke,R.T. (1980) *Cell*, **22**, 361-370.
Shilo,B.Z. and Weinberg,R.A. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6789-6792.
Simon,M.A., Kornberg,T.B. and Bishop,J.M. (1983) *Nature*, **302**, 837-839.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
Wang,J.Y.J. and Baltimore,D. (1983) *Mol. Cell Biol.*, **3**, 773-779.
Wang,J.Y.J., Ledley,F., Goff,S., Lee,R., Grover,Y. and Baltimore,D. (1984) *Cell*, **36**, 349-356.
Zulauf,E., Sanchez,F., Tobin,S.L., Rdest,U. and McCarthy,B.J. (1981) *Nature*, **292**, 556-558.

Received on 9 July 1985; revised on 6 August 1985