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Expression of the *src* and *abl* Cellular Oncogenes During Development of *Drosophila melanogaster*

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Transcription of the *Drosophila* homologs of the *abl* and *src* cellular oncogenes, termed *Dash* and *Dsrc*, respectively, was studied. Despite the fact that *Dash* and *Dsrc* share sequence homology, they have distinct patterns of transcription during development. The *Dash* transcript, 6.2 kilobases long, is found in maternal RNA stored in unfertilized eggs and in embryos up to 4 h after egg laying. In contrast, *Dsrc* transcripts are 3.2, 4.8, and 5.2 kilobases long. They are detected in eggs and embryos and to a lesser extent in larvae and adult flies. The relative amount of each of the three *Dsrc* transcripts changes during the different stages of development.

DNA sequences homologous to v-*src* and v-*abl* can be detected in the genome of *Drosophila* sp. (14). Sequence analysis has demonstrated that the *Drosophila abl* and *src* genes (termed *Dash* and *Dsrc*, respectively) have conserved multiple amino acids in the region corresponding to the carboxy terminus of $pp60^{src}$ (4), which was also shown to be conserved in all members of the *src* gene family in vertebrates (for a review, see reference 13). This region is essential for transformation and kinase activity of $pp60^{src}$(6). In addition to the consensus sequences, common to all members of the *src* gene family, over 110 residues in which v-*abl* and v-*src* vary have been conserved in the closest *Drosophila* homolog (4). It is thus likely that *Dash* and *Dsrc* fulfill the distinct roles of their closest vertebrate counterparts. In this paper, we studied the expression pattern of the two genes.

To identify transcripts of the *Dash* gene, a *Bam*HI-*Ava*II fragment of 800 base pairs from the genomic *Dash* clone was used as a probe. This fragment contains the major region of homology to v-*abl* (3). Polyadenylated [poly(A)$^+$] egg RNA was denatured, electrophoresed through a formaldehyde-agarose gel, transferred to nitrocellulose, and hybridized with the genomic probe. A single transcript, 6.2 kilobases (kb) long, reacted with the probe (Fig. 1A). When flanking fragments from the genomic clone were used as a probe, the same transcript was detected. The 6.2-kb transcript therefore corresponds to the authentic *Dash* transcript and is not related to a flanking gene.

To determine whether the expression of *Dash* is developmentally regulated, poly(A)$^+$ RNA from unfertilized eggs, embryos, larvae, and adult flies was fractionated and hybridized with the *Dash* probe. The results show that transcription is extremely specific to a defined developmental stage. The 6.2-kb transcript was detected in RNA from unfertilized eggs but not in embryonal, larval, or adult RNA. Only after very long exposures could faint 6.2-kb bands be detected in embryonic and adult RNA blots probed with pDash DNA (Fig. 1B). It should be emphasized that careful quantitation was done to verify that equal amounts of undegraded RNA were loaded on each lane. In addition, the same filter was hybridized with a *Drosophila* α-tubulin probe which detected distinct tubulin transcripts at about the same concentration in all lanes (data not shown).

It has been reported that a large fraction of *D. melanogaster* mRNA is non-poly(A)$^+$ (15). Therefore, it is possible that there are additional *Dash* transcripts which are non-poly(A)$^+$ and thus were lost during isolation of the poly(A)$^+$-containing RNA fraction. To approach this question, we fractionated total RNA from different developmental stages on a benzoylated cellulose (BC) column. Under appropriate conditions (as described in the legend to Fig. 1), rRNA and tRNA will not bind to the BC column, whereas mRNA will bind regardless of whether it is poly(A)$^+$ or not (11, 12). Total RNA from eggs, embryos, or adult flies was loaded on a BC column, and the bound RNA, termed BC-RNA, was eluted, gel fractionated, transferred to nitrocellulose, and hybridized with *Dash* probe. The results (Fig. 1C) were similar to those obtained with poly(A)$^+$ RNA; the same 6.2-kb transcript was detected only in egg RNA. We conclude that if there are *Dash* transcripts which are non-poly(A)$^+$, they are not different from the poly(A)$^+$-containing *Dash* transcripts.

The maternal *Dash* transcripts stored in the egg are no longer found in the RNA of 0- to 16-h-old embryos that were used in the experiment described above. The period during embryogenesis in which these transcripts are still found may be important in elucidating the role of *Dash* during embryogenesis. Synchronized embryos were collected and aged for different periods after egg laying. Poly(A)$^+$ RNA was isolated and blotted onto nitrocellulose paper. Probing these RNA blots with *Dash* RNA revealed that although *Dash* transcripts were present in 4-h-old embryos, they were barely detected in 4- to 8-h-old embryos (Fig. 2). Accordingly, they were absent in 0- to 16-h-old embryos when the proportion of older embryos (>4 h) was greater than that of the younger ones. Thus, most *Dash* transcripts are present in the developing embryo not longer than 4 h after egg laying. *Dash* transcripts are therefore part of the maternal RNA of the fruit fly which is synthesized during oogenesis, stored in the eggs, and utilized during early embryonic development.

To study the expression of the *Dsrc* gene, poly(A)$^+$ RNA preparations of unfertilized eggs, embryos, larvae, and adult flies were blotted and hybridized to a pDsrc probe (3). Three transcripts of 3.1, 4.8, and 5.2 kb were detected (Fig. 3A).

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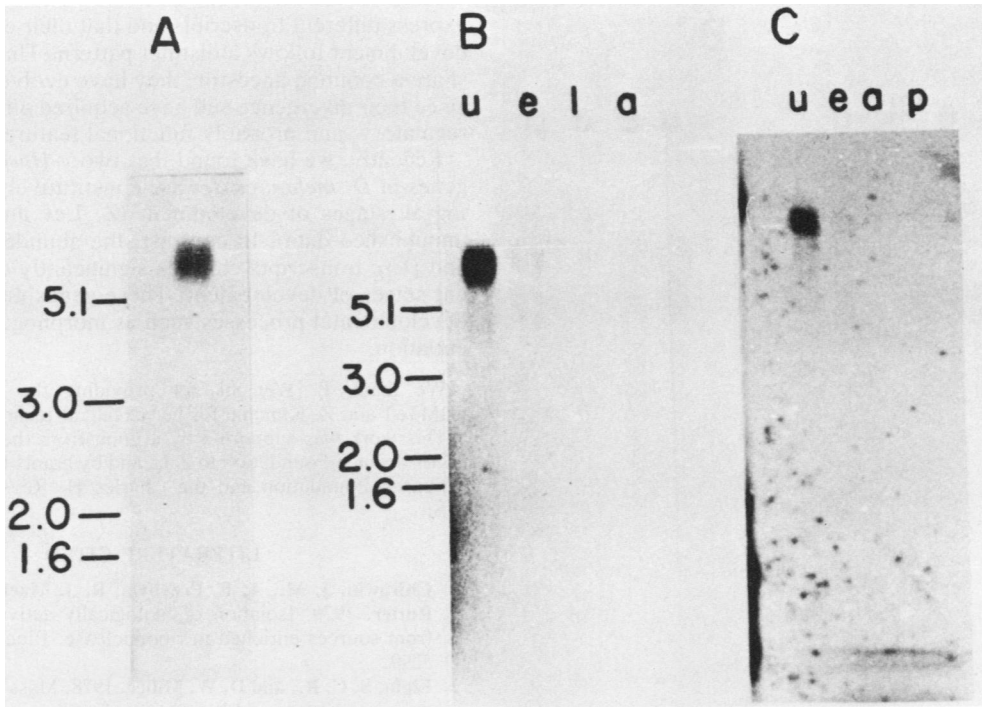
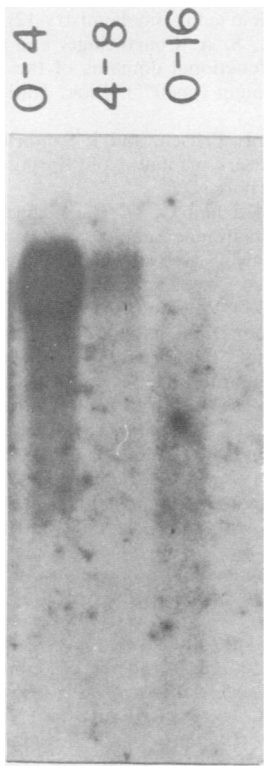


FIG. 1. Developmental expression of *Dash*. (A) RNA blot analysis of egg poly(A)$^+$ RNA with a *Bam*HI-*Ava*II fragment from the genomic pDash clone (3); (B, C) RNA blot analysis of (B) poly(A)$^+$ RNA and (C) BC-RNA with pDash DNA probe. u, Unfertilized eggs; e, embryos; l, larvae; a, adult; p, adult poly(A)$^+$ RNA. Size markers were HeLa and *Escherichia coli* rRNAs (from top to bottom): HeLa 28S, *E. coli* 23S, HeLa 18S, *E. coli* 16S (8). Unfertilized eggs (10), 0- to 16-h-old embryos (2), third instar larvae (9), and adult flies were used to obtain total RNA by a combination of the guanidine-hydrochloride (1) and urea methods (5). Poly(A)$^+$ RNA was prepared by chromatography on an oligodeoxy thymidylic acid-cellulose column (T-3; Collaborative Research, Inc., Waltham, Mass.). BC-RNA was prepared by chromatography on a BC column (Syngene, Fort Collins, Colo.). Binding was at 0.3 M NaCl, 5 mM EDTA, 50 mM Tris-hydrochloride, pH 7.4. The bound material, termed BC-RNA, was eluted with 50% ethanol–5 mM EDTA–50 mM Tris-hydrochloride, pH 7.4. A 3-μg amount of RNA was loaded in each lane. Electrophoresis of RNA in agarose gels containing formaldehyde, transfer of RNA to nitrocellulose, labeling of DNA by nick translation, hybridization, and exposure to X-ray film were carried out essentially as described previously (7). Exposure was for 10 days.



The same transcripts were also detected by a *Bgl*II-*Hin*dIII fragment of 900 base pairs which contains the coding region corresponding to the carboxy terminus of the protein (4; data not shown). The level of each of the *Dsrc* transcripts did not change coordinately during development. The 3.1-kb transcript was present in egg RNA at fairly high levels. Significantly reduced amounts were detected in embryonic, larval, and adult RNA. The larger 4.8-kb transcript was represented in egg RNA at a high level and at a substantially reduced amount in embryonic RNA. In contrast, the 5.2-kb transcript was almost unseen in egg RNA but appeared at a high level in the embryonic RNA. Both 4.8- and 5.2-kb transcripts were present at very low levels in larval RNA and at moderate levels in adult RNA.

The accumulation of the 5.2-kb transcript during embryogenesis is demonstrated in Fig. 4. Analysis of poly(A)$^+$ RNA prepared from synchronized embryos showed clearly that the 5.2-kb transcript was undetectable in eggs and in 2-h-old embryos, but was abundant in 4- to 8-hr-old embryos.

Using BC-RNA rather than poly(A)$^+$ RNA, no new transcripts were discovered, nor were changes in the relative concentration of the different transcripts observed (Fig. 3B).

In conclusion, this work demonstrates that *Dash* and *Dsrc*

FIG. 2. *Dash* expression during embryogenesis. RNA blots of poly(A)$^+$ RNA were prepared from embryos collected and aged to the indicated periods of time after egg laying. The probe was pDash DNA (8). Exposure was for 7 days.

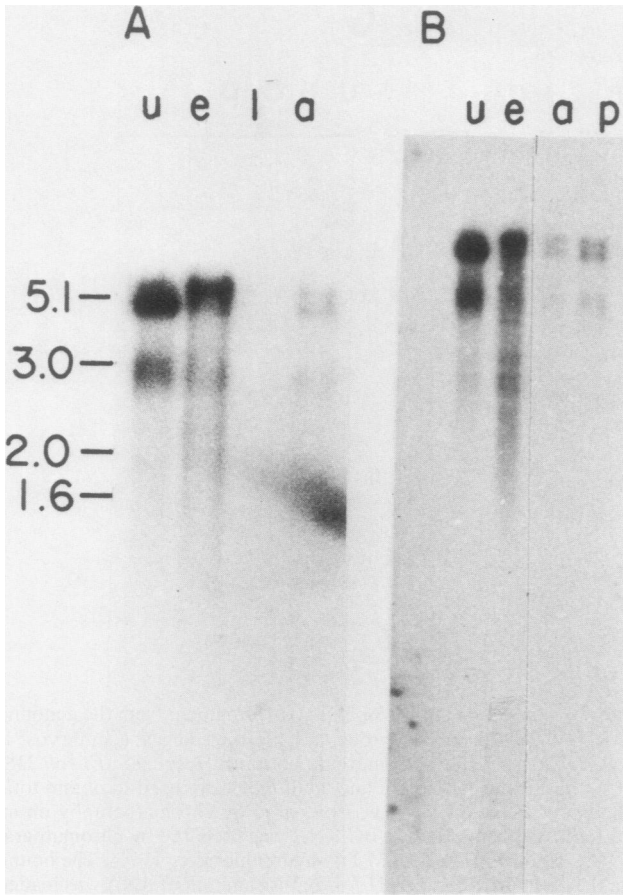


FIG. 3. Developmental expression of *Dsrc*. (A) RNA blot analysis of poly(A)$^+$; (B) BC-RNA with pDsrc DNA probe. u, Unfertilized eggs; e, embryos; l, larvae; a, adults; p, adult poly(A)$^+$ RNA. For explanation of size markers, see the legend to Fig. 1. Exposure was for 10 days.

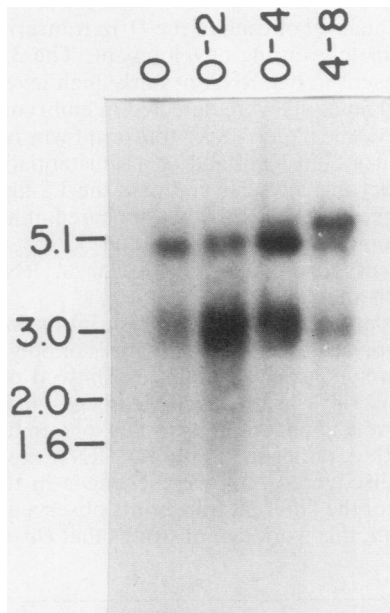


FIG. 4. *Dsrc* expression during embryogenesis. RNA blots of poly(A)$^+$ RNA were prepared from embryos collected and aged to the indicated periods of time after egg laying. The probe was pDsrc (8). Exposure was for 7 days.

express different transcripts and that their expression during development follows a distinct pattern. Thus, although they share a common ancestor, they have evolved independently since their divergence and have acquired different structural, regulatory, and probably functional features.

Recently, we have found that two v-*Ha-ras*-related oncogenes in *D. melanogaster* are constitutively expressed during all stages of development (Z. Lev and N. Leibovitz, unpublished data). In contrast, the abundance of the *Dash* and *Dsrc* transcripts changes significantly during the different stages of development. These genes may play a role in developmental processes such as morphogenesis and differentiation.

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