Embryonic expression of a *Drosophila src* gene: alternate forms of the protein are expressed in segmental stripes and in the nervous system

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Expression of the *Drosophila src*-related gene, *Dsrc28C* has been investigated at the protein level using monoclonal antibodies. This analysis has revealed that the *Dsrc28C* gene encodes two protein forms: a 66-kD doublet predicted from the sequence of a cDNA clone and an additional 55-kD form. The 66-kD protein doublet is observed first at the cellular blastoderm stage and is not detectable in embryos after 12 hr of development. Expression of the 55-kD protein lags behind that of the 66-kD doublet and then persists throughout embryogenesis. Indirect immunofluorescence microscopy reveals that *Dsrc28C* protein is localized to the cell periphery during cellular blastoderm and gastrulation. The cell periphery-associated staining is then resolved into ectodermal stripes along the fully extended germ band. After the stripes fade, cytoplasmic staining of the majority of cells within the central and peripheral nervous system is observed. The 66-kD protein was shown to represent the cell periphery-associated form of the protein through antibody staining of larval salivary glands expressing a heat shock promoter-driven, full-length *Dsrc28C* cDNA. Staining of embryos with a monoclonal antibody specific for the 66-kD protein indicates that the 55-kD protein is the nervous system form. Thus, the 66- and 55-kD proteins are products of the *Dsrc28C* gene, which exhibit different temporal and spatial patterns of expression in the embryo.

[Key Words: *src*; oncogene; *Drosophila*; development; segmentation; nervous system]

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The *src* gene family represents the largest and perhaps the most completely characterized group of oncogenes. The original member of this family, the *v-src* gene, transduced by Rous sarcoma virus, is known to have protein tyrosine kinase activity that is necessary for oncogenic potential. Additional members of this extensive gene family have been revealed through several routes (for review, see Hanks et al. 1988). First, additional *src*-related genes were found using the portion of the gene encoding the catalytic portion of the enzyme, the 'kinase domain', as a hybridization probe to search genomic or cDNA libraries. Comparison of sequences from such related genes has allowed identification of highly conserved and, presumably, universally functional domains of the *src* protein (Van Beveren and Verma 1986; Hanks et al. 1988). Second, numerous genes identified through genetic techniques in invertebrates, when cloned and sequenced, were found to possess amino acid sequence homology with the kinase domain of *src*. Protein tyrosine kinase or protein serine/threonine kinase activity has been demonstrated

directly for proteins encoded by genes isolated through each of the above strategies (Reed et al. 1985; Henkemeyer et al. 1988). It is assumed that proteins encoded by other *src* family members also possess protein kinase activity because specific amino acid positions within the kinase domain are conserved. Similarly, there are examples of both vertebrate and invertebrate *src* family members that perform regulatory functions in cell physiology. In vertebrates, the clearest examples of regulatory members of the *src* family are the peptide growth factor receptors that function in signal transduction in response to extracellular stimuli. Notable examples of invertebrate *src*-related genes identified through their regulatory phenotypes are the cell-division control gene *CDC28* in *Saccharomyces cerevisiae* (Lorincz and Reed 1984) [*cdc2* in *Saccharomyces pombe* (Beach et al. 1982)] and a cellular level homeotic gene, *sevenless*, in *Drosophila melanogaster* (Banerjee et al. 1987; Hafen et al. 1987). The latter gene encodes a protein with a *src*-related kinase domain and a large extracellular domain, a structure reminiscent of vertebrate growth factor receptors. Expression of the *sevenless* gene in developing ommatidia determines whether precursor cells will differentiate into R7 photoreceptor cells or lens-secreting

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cone cells (Tomlinson and Ready 1986). An important recent finding is that site-directed mutagenesis of the kinase domain inactivates *sevenless* function (Basler and Hafen 1988).

We have been studying a *Drosophila src*-related gene, referred to as *Dsrc28C*. This gene was isolated by low stringency hybridization with a probe representing the kinase domain of *v-src* and shown to be related most closely to *v-src* and other tyrosine kinases on the basis of key residues within the kinase domain (Wadsworth et al. 1985; Gregory et al. 1987). Therefore, it is likely that the *Dsrc28C* protein possesses intrinsic protein kinase activity. Although *Dsrc28C* represents a mainstream *src* family member when highly conserved amino acids are considered, it is a unique member of this gene family by other criteria. The overall sequence, as well as the structure of the protein, is significantly different from other *src* family proteins. Most notably, the *Dsrc28C* protein is extremely basic with a pI of ~10. An additional unusual feature of the protein is an amino-terminal domain of 27 amino acids that is 74% glycine (Gregory et al. 1987). Expression of the gene has been characterized at the RNA level. *Dsrc28C* transcripts are inherited maternally and expressed during much of embryogenesis but are not abundant during any other developmental stage, suggesting a largely embryonic function for the gene (Wadsworth et al. 1985; Gregory et al. 1987). Because tissue in situ hybridization studies did not reveal preferential RNA expression during embryogenesis, it has not been possible to implicate *Dsrc28C* in any particular developmental process.

To allow a more direct and higher resolution analysis of embryonic expression of *Dsrc28C*, we have produced monoclonal antibodies to document the temporal and spatial expression of the proteins encoded by the *Dsrc28C* gene. We report here that *Dsrc28C* proteins are expressed first in all ectodermal cells, then in a segmentally striped array in extended germ-band embryos, and later on, in the nervous system. In addition, unique forms of the protein are correlated with either ectodermal or nervous system expression. These results suggest that separate forms of the *Dsrc28C* protein are involved in segmentation and neuronal differentiation.

Results

Generation of monoclonal antibodies specific for the Dsrc28C protein

Monoclonal antibodies (mAbs) were produced against a bacterially expressed form of the *Dsrc28C* protein, referred to as plac28C, encoded by the expression plasmid shown in Figure 1B(1). The protein produced from this vector contains all of the amino acids encoded by a full-length *Dsrc28C* cDNA clone (Gregory et al. 1987), as well as 33 amino-terminal residues derived from the polylinker region of the expression vector. mAbI19, which was used for most of the experiments, reacts on immunoblots with plac28C from IPTG-induced plasmid-bearing cells (Fig. 2, lane 1) but not with IPTG-induced proteins from control cells (Fig. 2, lane 6). Additional cDNA expression plasmids were constructed (Fig. 1B) to define the portion of the *Dsrc28C* protein recognized by mAbI19. The proteins produced by these plasmids are shown also in Figure 2. mAbI19 reacts with the proteins produced from plac28C-S containing *Dsrc28C* amino acids 126–590 (lane 2), pWV404 containing *Dsrc28C* amino acids 42–590 (lane 3), and pWV406 containing *Dsrc28C* amino acids 42–381 (lane 5) but does not react with protein produced from pWV405 containing *Dsrc28C* amino acids 409–590 (lane 4). Therefore, the portion of the *Dsrc28C* protein recognized by mAbI19 is located between amino acids 126 and 381.

mAbI19 reacts with two major forms of the Dsrc28C protein in embryos

Because *Dsrc28C* transcripts are most abundant during embryogenesis, immunoblot experiments were performed on protein prepared from a variety of embryonic stages. Two major protein species were detected with mAbI19: a closely-spaced doublet of ~66-kD (referred to hereafter as p66) and a protein of ~55-kD (referred to hereafter as p55) (Fig. 3). The reason for the appearance of p66 as a doublet is unknown. Whether expressed from the *Dsrc28C* gene or from a heat shock promoter-driven full-length cDNA (Fig. 4A), this protein always appears as a doublet; for convenience, both proteins in this dou-

Figure 1. (See p. 336.) *Dsrc28C* expression vectors. (A) *Dsrc28C* cDNA map. (B) Expression vectors used for production of *Dsrc28C* proteins in bacteria. (1) plac28C expression vector. The ATG1 codon is believed to be the normal initiator codon for the p66 *Dsrc28C* protein. Initiation of translation in bacteria takes place at the upstream *lac* ATG. (2) The plac28C-S expression vector. ATG is the *lac* initiator codon and is used for translational initiation of plac28C-S. ATG3 corresponds to the third methionine codon in the cDNA. It is the second in-frame methionine codon in this construct and is used for translational initiation of p28C-S. Independent initiation occurs at both of these codons in *SupE44*-bearing strains of *E. coli* (see text for details). (3–5) pWV404, pWV405, and pWV406 expression vectors, respectively. For each of these vectors, initiation of translation in bacteria takes place at the upstream *lac* ATG. (C) P-element-based expression vectors used to produce *Dsrc28C* proteins in flies. (1) pCasper-hs28C P-element expression vector. The initiator codon shown (ATG1) is believed to be the normal one for the p66 *Dsrc28C* protein. The consensus poly(A) addition sequence shown is from a *Drosophila hsp70* gene. (2) pCasper-hs28C-S P-element expression vector, which was generated from pCasper-hs28C by deleting a *HindIII* fragment that contained the normal initiator methionine codon for p66. ATG3 serves as the initiator codon. In all cases, the terminator codon shown is the one encoded by the full-length *Dsrc28C* cDNA.

blet will be referred to as p66. A minor protein of ~95-kD is recognized also by mAb119 and by another monoclonal antibody raised against plac28C [mAbB3]. The ap-

pearance of the 95-kD protein always parallels the appearance of p66. It is not known whether this protein represents an aggregate of p66 or another form of protein

A. *Dsrc* 28C cDNA

B. *E. coli* protein expression

C. P element transformation

Figure 1. [See preceding page for legend.]

Figure 2. *Dsrc28C* proteins produced by *E. coli* expression vectors. Insoluble proteins prepared from the expression vectors diagramed in Fig. 1B were electrophoresed through an 8% SDS–polyacrylamide gel, transferred to nitrocellulose, and reacted with mAb119. Lanes 1–5 correspond to the expression vectors numbered within Fig. 1B. (Lane 6) Proteins from the host bacterial strain alone (XL1-Blue, Stratagene) were electrophoresed. Arrows indicate the positions of the primary IPTG-induced translation products from the expression vectors. Note that mAb119 does not react with the pWV405 fusion protein containing the carboxy-terminal portion of *Dsrc28C* (lane 4). Lower molecular weight antibody-reactive proteins are presumably breakdown products. (right) Positions and relative molecular weights of marker proteins electrophoresed in a parallel lane.

Figure 3. Developmental expression of *Dsrc28C* proteins. Whole embryo protein (~100 μ g/lane) from the indicated stages was subjected to SDS–PAGE and immunoblotting, as described in Methods. (p66 and p55) Predicted molecular weights of the alternate forms of *Dsrc28C* proteins discussed in this paper; they were not derived from their migration relative to the proteins of known size. (Right) Positions and relative molecular weights of marker proteins electrophoresed in a parallel lane used to estimate the sizes of the *Dsrc28C* proteins. (Top) Hours since egg deposition.

produced by the *Dsrc28C* gene. The low level of expression of this protein has precluded its further characterization. p66 is not detected in preblastoderm embryos; peak expression is observed between 8 and 10 hr of embryogenesis, followed by an abrupt decline by 14 hr of

embryogenesis. p55 is detected first at ~4–6 hr of embryogenesis, reaching its highest levels at a time when expression of the p66 doublet is declining, and persists throughout the remainder of embryogenesis. As noted above, previously we have characterized expression of

Figure 4. Correspondence between embryonic and recombinant *Dsrc28C* proteins. (A) Comigration of *Dsrc28C* protein produced from cloned cDNAs and that produced in embryos from the genomic *Dsrc28C* locus. mAb119-reactive proteins produced from pCasper-hs28C, either in the absence of heat shock (lane 1) or after heat shock (lane 2). (Lane 3) mAb119-reactive proteins from 12-hr embryos. mAb119-reactive proteins produced from pCasper-hs28C-S, either in the absence of heat shock (lane 5) or after heat shock (lane 4). The positions of p66 and p55 are indicated. (B) Immunoblot of V8 protease digestion products of embryonic p66 (lane 1) and the protein produced from pCasper-hs28C (lane 2). Dashes indicate positions of the major comigrating mAb119-reactive digestion products. The arrowhead indicates the position of undigested p66. (C) Immunoblot of protease digestion products from embryonic p55 (lanes 1,3) and the protein produced from pCasper-hs28C-S (lanes 2,4). (Lanes 1,2) V8 digestion products; (lanes 3,4) chymotrypsin digestion products. Dashes indicate the positions of major comigrating mAb119-reactive digestion products. In the case of V8 protease, additional minor common digestion products were observed on the original immunoblot. The arrowhead indicates the position of undigested p55.

the *Dsrc28C* gene at the RNA level (Gregory et al. 1987). Although *Dsrc28C* transcripts are inherited maternally, the results presented in Figure 3 indicate that significant levels of maternal protein are not present in the preblastoderm embryo. Also, we have shown previously that maternal *Dsrc28C* transcripts are polyribosome associated (Wadsworth et al. 1985). Taken together, these results indicate that *Dsrc28C* transcripts deposited in the oocyte represent classical masked maternal mRNA. *Dsrc28C* transcripts are present throughout much of embryogenesis but decline sharply in abundance between 10 to 19 hr. Thus, the expression of mAbI19-reactive proteins correlates well with our previous characterization of *Dsrc28C* RNA expression.

Both mAbI19-reactive proteins are encoded by the Dsrc28C gene

Several experimental approaches were taken to prove that the embryo proteins recognized by mAbI19 are authentic *Dsrc28C* gene products. A full-length *Dsrc28C* cDNA under the control of an hsp70 promoter [pCasper-hs28C, Fig. 1C(1)] was introduced into the germ line of flies by P-element transformation. pCasper-hs28C directs the synthesis of an mAbI19-reactive protein doublet under heat shock conditions in adult flies, which is electrophoretically indistinguishable from the embryonic p66 (Fig. 4A, lane 2,3). In the absence of heat shock, significant amounts of mAbI19-reactive protein are not observed in pCasper-hs28C-bearing adult male flies (Fig. 4A, lane 1). In addition, embryonic p66 and the proteins encoded by the pCasper-hs28C construct yield identical protease V8 digestion products (Fig. 4B). On the basis of these experiments, we conclude that the proteins in the p66 doublet are products of the *Dsrc28C* gene and are encoded by the mRNA represented by the cDNA clone that we have characterized previously (Gregory et al. 1987).

If p55 is encoded by the *Dsrc28C* gene, it could come from an alternate mRNA species, from alternate translational initiation of the same mRNA, or from cleavage of p66. A clue to the origin of p55 came from the observation that cell-free translation of *in vitro* transcripts from the *Dsrc28C* cDNA yields a second major protein of ~55-kD, which could arise from initiation at the third methionine codon (Gregory et al. 1987). To allow a definitive test of the hypothesis that p55 arises from initiation at the third methionine codon within the *Dsrc28C* sequence, a heat shock promoter-driven expression vector (pCasper-hs28C-S) containing a truncated version of the cDNA was constructed [Fig. 1C(2)]. In this construct, the first two methionine codons have been eliminated, leaving the former third methionine codon as the first in-frame methionine codon. The sequence around this methionine codon represents a characteristic initiator codon, as described by Kozak (1987) and modified for *Drosophila* by Cavener (1987). An additional out-of-frame methionine codon that does not resemble an initiator codon is retained in the truncated cDNA. Beyond the amino-terminal truncation site, the sequences in

pCasper-hs28C-S are identical to the full-length *Dsrc28C* cDNA. The protein encoded by pCasper-hs28C-S is electrophoretically indistinguishable from embryonic p55 (Fig. 4A, lanes 3,4) and is not produced at significant levels in non-heat-shocked pCasper-hs28C-S-bearing adult male flies (Fig. 4A, lane 5).

A further comparison between embryonic p55 and that directed by the truncated cDNA sequences in pCasper-hs28C-S clone was made through mapping of V8 protease and chymotrypsin cleavage sites. pCasper-hs28C-S protein and the late embryo p55 were isolated on SDS-polyacrylamide gels and subjected to cleavage with either V8 protease or chymotrypsin during electrophoresis on a second SDS-acrylamide gel as described by Cleveland et al. (1977). The cleavage products were detected by mAbI19 reactivity after transfer to nitrocellulose. The major V8 protease (Fig. 4C, lanes 1,2) and chymotrypsin (Fig. 4C, lanes 3,4) cleavage products of the embryo p55 and pCasper-hs28C-S comigrate. We conclude from these results that p55 is encoded by the *Dsrc28C* gene, presumably resulting from translational initiation at the third methionine codon in the cDNA sequence. This point will be discussed further below.

Distribution of Dsrc28C protein during early embryogenesis

To define the distribution of the *Dsrc28C* proteins, whole embryos of various stages were stained with mAbI19 after fixation and devitellinization (Thomas et al. 1988). No significant staining of preblastoderm embryos is observed (data not shown), in agreement with the lack of detectable *Dsrc28C* protein in 0- to 2-hr embryos by immunoblot experiments (Fig. 3). At the cellular blastoderm stage, a reticular pattern of mAbI19 staining is observed evenly distributed over the embryo (Fig. 5a). A higher magnification view of this pattern over the central portion of an embryo is shown in the upper portion of Figure 5b. Also shown in the lower portion of Figure 5b is an embryo (outlined with dashes) that was processed in parallel with the embryo in the upper portion and was stained only with the fluorescent second antibody. Thus, the intense reticular staining pattern observed in embryos is dependent on mAbI19. Closer examination of a focal plane near the center of a similar embryo (Fig. 5c) reveals that the staining giving rise to the reticular pattern is distributed uniformly around the periphery of cells. This pattern of peripheral intracellular staining persists through gastrulation (Fig. 5d, an example of an early gastrula) and early germ-band extension (data not shown).

The germ band of a *Drosophila* embryo is the ventral region between the cephalic furrow and the posterior pole that gives rise to the segments of the head, thorax, and abdomen. Between 3.5 and 5.5 hr of embryogenesis, the germ band extends around the posterior end of the embryo such that the former posterior pole is located near the head. Around the time of full germ-band extension, a dramatic change in the pattern of mAbI19 staining takes place: stripes of antibody staining appear

Figure 5. Indirect immunofluorescence staining of early embryos with mAbI19. Whole *Drosophila* embryos were stained with mAbI19 after removal of the chorion and vitelline membrane and subsequently stained with fluorescent second antibody. (a) Surface focal plane of a cellular blastoderm embryo. Anterior is to the left; (b, top) A higher magnification view of a cellular blastoderm embryo showing the reticular pattern of mAbI19 staining; (bottom) cellular blastoderm embryo stained only with fluorescent second antibody (outlined by hash marks). No significant antibody staining is observed; (c) A higher magnification view of a cellular blastoderm embryo at the focal plane of the middle of the embryo. Immunofluorescence is observed surrounding cells of the ectoderm; (d) Surface focal plane of the anterior end of an early gastrula (stage 7 or early stage 8); (e,f) Two different embryos with fully extended germ bands (stage 11). The dorsal side is up in both embryos. Anterior is to the right in e and to the left in f. (g) Higher magnification view of mAbI19 staining stripes.

over the germ band (Fig. 5e–g). In addition to staining of portions of the head region, 13 or 14 stripes are observed routinely (Fig. 5e,f). The uncertainty in the number of antibody staining stripes is due to variable observation of the most posterior stripe.

Three points about the nature of the *Dsrc28C* stripes should be emphasized. First, mAbI19 staining still is restricted to the periphery of ectodermal cells at this stage (Fig. 5g). Second, the striped pattern is extremely transient in nature. The first suggestion of stripes is observed in embryos with a completely extended germ band and the striped pattern fades within an hour, as determined by examination of synchronized embryo populations. By the time germ-band retraction begins (2 hr after full germ-band extension) and the stripes have faded, mAbI19 staining of the periphery of ectodermal cells, in general, has decreased greatly. Third, the stripes are faint, even at their peak expression and are separated by interstripe regions characterized by less intense but observable staining. Although these factors make it difficult to assess stripe width accurately, we believe that the most intense mAbI19 staining defines stripes of approximately two to three cells, separated by less intensely stained interstripes of three to four cells (Fig. 5g).

Dsrc28C is expressed in the nervous system

After mAbI19 staining of ectodermal cells fades, totally different pattern of antibody staining is observed: mAbI19 specifically stains cells of the nervous system. In addition, the antibody staining is no longer restricted to the periphery of the cell but, instead, seems to be redistributed throughout the cytoplasm. Two typical mAbI19-stained embryos are shown in Figure 6a. In the embryo on the left, mAbI19 staining is distributed evenly along the central nervous system (CNS). In the embryo on the right (~5 hr older), substantial central nerve cord condensation has occurred and all portions of the CNS still have stained uniformly with mAbI19. Uniform staining of the CNS is observed until the end of embryogenesis and in first instar larvae as well (data not shown).

Staining of embryos with antibody recognizing horseradish peroxidase (HRP) was carried out in parallel to identify the nervous system (data not shown). Anti-HRP fortuitously recognizes an antigen present in the *Drosophila* CNS and the peripheral nervous system (PNS) (Jan and Jan 1982). Closer examination revealed that mAbI19 not only stains the CNS but also stains neurons of the PNS. Ventral groups of segmental peripheral neurons staining with mAbI19 can be seen in Figure 6b, whereas other more lateral groups are shown in Figure 6c. No attempt was made to identify precisely the peripheral neurons staining with mAbI19. The diffuse cytoplasmic nature of mAbI19 staining of the nervous system also can be seen in the peripheral neurons. Thus, a large fraction of cells within the CNS and PNS express *Dsrc28C* proteins.

The Dsrc28C p66 protein represents the cell periphery form

The results presented so far allowed us to conclude that two major forms of the *Dsrc28C* protein are present during embryonic development and that *Dsrc28C* proteins are distributed in two different patterns. To determine which mAbI19 staining pattern was correlated with the p66 form of *Dsrc28C*, we made use of the transformed fly strain bearing the pCasper-hs28C expression vector shown in Figure 1C. Heat-shock-induced transcripts from this gene encode only the p66 *Dsrc28C* protein (Fig. 4A). Only low levels of p66 protein expression from genomic *Dsrc28C* are detectable by the immunoblot assay in third-instar larvae (data not shown). Therefore, mAbI19 staining of tissues from third-instar larvae of the pCasper-hs28C strain should represent a sensitive assay for the intracellular distribution of p66. The periphery of heat-shocked salivary gland cells from the pCasper-hs28C transformants (Fig. 7a) stain with mAbI19, reminiscent of the staining of early embryonic cells (Fig. 5). Staining of salivary glands from the P-element transformant alone, used as a control (Fig. 7b), yielded only background immunofluorescence equivalent to that obtained with second antibody alone. Most of what appears to be background fluorescence in the salivary gland in Figure 7a is due to out-of-focus peripheral mAbI19 staining.

The Dsrc28C p55 protein is the cytoplasmic nervous system form

A similar strategy was used to define the intracellular distribution of p55. Transformants bearing the pCasper-hs28C-S expression vector produce a protein equivalent to the p55 embryonic protein (Fig. 4). The cytoplasm of heat-shocked salivary gland cells from the pCasper-hs28C-S transformant stains with mAbI19 (Fig. 7c), in contrast to the peripheral staining of salivary gland cells expressing p66 (Fig. 7a).

An alternate strategy was employed to identify the form of *Dsrc28C* protein in the nervous system. This strategy relied on another monoclonal antibody, mAbB3, that recognizes the p66, but not p55, form of *Dsrc28C*. mAbB3 reacts with the bacterially produced forms of *Dsrc28C* shown in Figure 2: plac28C, pWV404, and pWV406, but not pWV405 (data not shown). mAbB3 detects the p66 protein from 10-hr embryos (Fig. 8A, lane 2) but not p55 in either 10- or 18-hr embryos (Fig. 8A, lanes 2 and 1, respectively). That mAbB3 recognizes only the p66 form of *Dsrc28C* was substantiated further by the reactivity of the antibody with bacterially produced forms of *Dsrc28C*. Two different bacterial expression plasmids [Fig. 1B(1),(2)] were used for these experiments. The major protein directed by the plac28C expression vector is longer than p66 *Dsrc28C* due to additional amino-terminal amino acids from the expression vector [Fig. 1B(1)]. Both mAbB3 and mAbI19 react with plac28C protein (Fig. 8A, lanes 3,6). mAbI19 also reacts with a protein not detected by mAbB3, which could result from initiation at the p55 initiator methio-

nine codon (Fig. 8A, lane 6), although the identity of this protein has not been investigated further. The expression vector diagramed in Figure 1B(2) directs the synthesis of two proteins, plac28C-S and p28C-S, which differ with respect to the initiator methionine codon used. A termination codon is located midway between the initiator codon in the plasmid and the initiator codon in the cDNA. Translational termination at this codon is suppressed partially in *Escherichia coli* (XL1-Blue) by the *SupE44* mutation, which encodes an amber suppressor tRNA. Suppression results in incorporation of tyrosine at the position of the amber codon and gives rise to the longer protein referred to as plac28C-S. The shorter form, referred to as p28C-S, is encoded solely by the truncated *Dsrc28C* cDNA and arises from efficient initiation at the *Dsrc28C* methionine codon downstream from the termination codon. Therefore, this protein is identical to p55 encoded by pCasper-hs28C-S. plac28C-S is longer than p28C-S due partially to amino-terminal amino acids from the expression vector and 15 amino acids encoded by the full-length *Dsrc28C* DNA directly upstream from the initiator methionine codon used by the p55 *Dsrc28C* protein. The difference between plac28C-S and p28C-S is diagramed in Figure 8B. The tyrosine residue encoded by the suppressible amber codon is indicated by an asterisk (*). In wild-type *E. coli*, translational suppression does not occur and only the shorter p28C-S protein is synthesized upon IPTG induction (data not shown). mAbB3 and mAbI19 both react with plac28C-S (Fig. 8A, lanes 4,5). However, the critical difference between the two antibodies is that mAbI19 recognizes p28C-S corresponding to p55 but mAbB3 does not (Fig. 8A, lanes 4,5). Therefore, the epitope recognized by mAbB3 is included in the amino acid sequence SPTLLNGNGNLLDAN.

Thus, cells in the embryo staining with mAbI19, but not mAbB3, would represent cells expressing the p55 *Dsrc28C* protein but not the p66 *Dsrc28C* protein. Cells of the nervous system have exactly this pattern of staining. A population of late embryos was divided after fixation and processing and then stained with either mAbI19 or mAbB3. mAbI19 stains the central nervous system (Fig. 9a), whereas no staining of the CNS is observed with mAbB3 (Fig. 9b).

Figure 6. Indirect immunofluorescence staining of the embryonic nervous system with mAbI19. Whole *Drosophila* embryos were stained with mAbI19 after removal of the chorion and vitelline membrane and subsequently stained with fluorescent second antibody. (a) Low magnification view of mAbI19 staining of the CNS. The embryo on the right with substantial ventral nerve cord condensation is ~5 hr older than the embryo on the left. (b) Ventral view of mAbI19 staining of the CNS and neurons of the PNS. (c) Lateral view of mAbI19 staining of the CNS and of the lateral and dorsal groups of peripheral neurons (PN). (d, left) An embryo stained with mAbI19 and fluorescent second antibody; (right) an embryo processed in parallel with the one on the left but stained with only fluorescent second antibody.

Discussion

The *Dsrc28C* gene encodes two proteins: p66, predicted from the cDNA sequence, and p55, revealed by the results presented in this discussion. Two striking features of p66 are that it is a highly basic protein and that it has

Figure 7. Intracellular localization of the p66 and p55 *Dsrc28C* proteins expressed in salivary glands. Third-instar larvae from either the strain bearing the pCasper-hs28C construct (*a*), a strain bearing only the Casper P element (*b*), or a strain bearing the pCasper-hs28C-S construct (*c*) were heat shocked. Salivary glands were then dissected out, fixed, and stained with mAb119 under the same conditions used for embryos. The salivary glands in *a* and *b* were present in the same microscopic field and were photographed simultaneously. The salivary gland shown in *c* was photographed under identical conditions.

a predominantly glycine amino-terminal domain. Analyses of p55 suggest that it lacks approximately the first 125 amino acids of p66. Therefore, p55 would retain the kinase domain and would still be a basic protein (pI 9.8) but would lack the glycine-rich domain.

As well as differing in their primary sequences, the two *Dsrc28C* proteins differ in their intracellular localization. p55 appears to be distributed throughout the cytoplasm of neuronal cells, whereas p66 localizes to the periphery within ectodermal cells. These *Dsrc28C* proteins, when expressed ectopically in salivary gland cells, appear to be located in regions of the cell analogous to their normal embryonic cell locations. These results suggest that some feature of the primary structure of either one or both proteins is responsible for their localization. A relatively straight forward speculation is that the amino acids uniquely present in the p66 protein might direct the protein to the periphery of the cell. Tight association with the inner layer of the cell membrane through an amino-terminal myristic acid added post-translationally is a property of many *src* family proteins (Buss and Sefton 1985; Buss et al. 1988; Kaplan et al. 1988). We have noted before (Gregory et al. 1987) that the p66 protein predicted from the cDNA sequence would lack glycine in the second position, which is required for post-translational myristylation. It is interesting to note that the proteins predicted from *Dsrc* and

Dabl cDNA sequences have glycine as the second amino acid (Simon et al. 1985; Henkemeyer et al. 1988). For p66 to undergo conventional post-translational myristylation, cleavage of the protein to expose an amino-terminal glycine residue would have to take place. However, we have not seen evidence for cleavage of the p66 expressed from the pCasper-hs28C vector in embryos, larvae, or adults (R.J. Gregory and S.C. Wadsworth, unpubl.). To achieve localization to the cell periphery, p66 could interact with integral membrane proteins or could alternatively associate with components of the cell cortex.

What are the routes by which the two forms of *Dsrc28C* protein could be produced? Alternate splicing of a uniform *Dsrc28C* primary transcript in different cell types could generate two forms of mRNA with different initiator codons. Two introns are present within the genomic sequence between the first and third methionine codons: one is 64 nucleotides in length and the other is at least 5-kb in length (Gregory et al. 1987). Splicing to remove the first *Dsrc28C* amino-acid-coding exon could generate an mRNA wherein the former third methionine codon is now the first in-frame methionine codon. Loss of the first exon would reduce the size of the mRNA by maximally 600–700 nucleotides, depending on the position of the upstream splice donor. We have noted before that multiple, closely spaced *Dsrc28C* tran-

Figure 8. Specificity of mAbB3. (A) Reaction with mAbB3. (Lane 1) 16-hr-old embryo protein; (lane 2) 10-hr-old embryo protein. The identity of the ~70-kD protein detected by mAbB3 is unknown. (Lane 3) Protein from IPTG-induced bacteria bearing the plac28C expression vector [Fig. 1B(1)]. The major mAbB3-reactive protein in lane 3 is plac28C, which is longer than the p66 *Dsrc28C* protein. Additional antibody-reactive proteins are presumably breakdown products of plac28C. (Lane 4) Protein from IPTG-induced bacteria bearing the plac28C-S expression vector [Fig. 1B(2)]. The only detectable mAbB3-reactive protein is plac28C-S, which is longer than the p55 *Dsrc28C* protein. Reaction with mAbI19. Lanes 5, 6, and 7 are identical in protein loading to lanes 4, 3, and 2. (Lane 5) The major mAbI19-reactive protein is plac28C-S. The other mAbI19-reactive protein is p28C-S, which corresponds to embryonic p55 *Dsrc28C* protein. (Lane 6) The major mAbI19-reactive protein is plac28C. Additional antibody-reactive proteins are presumed to be breakdown products of plac28C. (Lane 7) The mAbI19-reactive proteins are the p66 and p55 *Dsrc28C* embryo proteins. (B) The junction region between the plasmid vector and the truncated *Dsrc28C* cDNA clone. The first 19 amino acids shown are encoded by the plasmid vector. The arrow labeled plac28C-S indicates the initiator methionine in the protein. When translational termination is suppressed, a tyrosine residue (*) is incorporated into the protein at the position indicated. The arrow labeled p28C-S indicates the initiator methionine codon used for the protein p28C-S. In boldface type, between the above noted regions, are the *Dsrc28C* amino acids present in plac28C-S but not in p28C-S. Therefore, the epitope recognized by mAbB3 must be contained within these 15 amino acids.

scripts are observed in embryos (Gregory et al. 1987). There is ample precedent for alternate splicing in *Drosophila*, giving rise to variant protein products or as a mechanism for on/off regulation (Bingham et al. 1988). A germ-line-specific splice regulates whether P-element

transposase or a carboxy-terminally truncated protein of unknown function is produced (Laski et al. 1986; Rio et al. 1986). A neuronal cell-specific splice, in concert with cell-specific promoter elements, has been shown to regulate the expression of alternate forms of dopa decarboxylase (*Ddc*) in the hypoderm and in the CNS (Morgan et al. 1986). Like the alternative splicing pathways shown for P-element transposase and *Ddc*, the hypothetical *Dsrc28C* alternative splicing events would each give rise to a unique protein that functions in a specific tissue. It is interesting to note that a neuronal-specific form of vertebrate pp60src is generated by alternative splicing (Levy et al. 1987; Martinex et al. 1987).

Other mechanisms for expression of two *Dsrc28C* proteins also can be envisaged. Transcription of the *Dsrc28C* gene could be initiated from a neuronal-specific promoter residing in the large intron downstream from the first two methionine codons. Alternate splicing would not have to be invoked in this model because the first in-frame methionine codon would be the one used

Figure 9. Differential reaction of the nervous system with mAbI19 and mAbB3. Using the conditions described in Methods, a common batch of late embryos were stained with either mAbI19 (a) or with mAbB3 (b).

to initiate the p55 neuronal protein. Finally, it is also formally possible that translational initiation at the first and closely spaced second methionine codons in the mRNA could be suppressed by an unknown mechanism in neuronal cells. Isolation and characterization of *Dsrc28C* cDNA clones from late embryonic stages will be required to define the structure of the mRNA encoding p55.

Results of mAb19 staining suggest an involvement of *Dsrc28C* in segmentation. From the cellular blastoderm stage until full germ-band extension, p66 is expressed in all cells of the ectoderm. Shortly thereafter, 13 or 14 stripes of p66 staining within the germ band are seen, as well as additional staining in the head region that has not been well characterized. After peak stripe expression, mAb19 staining of the cell surface fades away rapidly by the initiation of germ-band retraction at the end of stage 11. As we have noted, highly synchronized embryo collections are required to observe a large fraction of embryos with the stripe pattern, indicating that this pattern of expression is extremely transient. As to the key questions of how the stripes arise and their relationships to known body segments, we can only speculate. The striped pattern of p66 expression could arise from selective turnover of the protein in the interstripe cells or from selective gene activation in the stripe cells. In situ hybridization experiments have not been helpful in resolving this question because *Dsrc28C* RNA is expressed at low levels in all cells during early embryogenesis. Similarly, the generally low level of *Dsrc28C* protein expression and the transient nature of the stripe pattern make it difficult to assess the number of cells participating in the stripes and to correlate the stripes with known segments. However, the number of stripes and the fact that they are distributed over the full length of the germ band suggest a correlation with embryonic parasegments (Martinez-Arias and Lawrence 1985; Scott and Carroll 1987). Simultaneous staining with antibodies specific for known segmentation gene proteins will be required to orient the *Dsrc28C* stripes with those defining body segment.

The pattern of expression of *Dsrc28C* is similar in another respect to the pattern of expression of many segmentation gene proteins. *Dsrc28C* and segmentation proteins are expressed in segmentally repeating patterns during early embryogenesis and appear later on in the nervous system. A clear difference, however, is that each of these segmentation genes is expressed in a small subset of segmental CNS neurons (Doe and Scott 1988), whereas *Dsrc28C* is expressed widely in cells within the CNS and PNS. The pattern of *Dsrc28C* expression is also superficially similar to that reported recently for fasciclin III, a set of closely related neuronal glycoproteins; ectodermal striped expression, followed by nervous system expression (Patel et al. 1988).

We began this study with the hypothesis that proteins encoded by the *Dsrc28C* gene might perform regulatory roles during embryonic development mediated through protein kinase activity. From the results presented in this discussion, it is clear that expression of the *Dsrc28C*

gene is regulated tightly with regard to the forms of the protein expressed, the cell type expressing the different forms of the protein, and the intracellular distribution of the protein. Little is known of the gene products whose expression is controlled by segmentation genes either in the ectoderm or in the nervous system. Proteins encoded by segmentation genes are thought to be DNA-binding proteins that almost certainly initiate regulatory cascades resulting in segment specialization. It is predictable that many such downstream functions might be controlled indirectly by segmentation proteins and that controls at the protein level also might represent part of the regulatory cascade. It would obviously be premature to suggest that the complicated pattern of *Dsrc28C* expression we have documented is insignificant with respect to the regulation of development of the segmented body pattern or the nervous system. However, by analogy to other members of the *src* family, it seems extremely likely that *Dsrc28C* proteins in ectodermal and neuronal cells serve in regulatory roles. The fact that two distinct forms of the protein exist—one within the periphery of the cell, perhaps associated with the cell membrane, and the other cytoplasmic—suggests that *Dsrc28C* could have two related yet distinct functions. Genetic and molecular genetic studies will be required to determine whether altered patterns of *Dsrc28C* expression or alterations in its primary sequence affect segmentation of nervous system development.

Methods

Fly culture and collection

D. melanogaster strain Oregon R, and the *Df(1)w, y w^{67c23(2)}* strain were maintained in population cages at 25°C. Embryos generally were collected for a 2-hr period, following a precollection to purge females of retained fertilized eggs, and allowed to develop at 25°C. For developmentally staged embryos, the time of development is designated from the midpoint of the collection period. Thus, a 4-hr-old embryo preparation represents embryos from 3–5 hr postegg deposition. Developmental stages are those described by Campos-Ortega and Hartenstein (1985).

Plasmid construction, RNA, and protein expression

DNA isolation, ligations, and transformations were performed using standard methods (Maniatis et al. 1982). The host for all protein expression vectors was XL1-Blue (Stratagene). Specific details on the construction of *Dsrc28C* expression vectors from pWV411 (restriction map shown in Fig. 1A), the original cDNA expression subclone (Gregory et al. 1987), are described below.

plac28C expression vector A 2.15-kb *MluI* fragment containing the entire *Dsrc28C* open reading frame was subcloned from pWV411 into pIBI25 to produce pRG411. Oligonucleotide-directed mutagenesis was performed on single-stranded pRG411 to introduce a *BalI* site 4 nucleotides upstream of the first initiator methionine in the open reading frame. Mutagenesis was performed essentially as described by Zoller and Smith (1983), except that T4 DNA polymerase was used instead of the Klenow enzyme, the reaction was treated with *dam* methylase to increase the efficiency of mutagenesis, and closed circular DNA was isolated by agarose gel electrophoresis. The

single-base change creating the *BalI* site and the bases complementary to the initiator methionine are underlined in the oligonucleotide sequence CTTCATGTGGCCAAT. Potential mutants were screened by restriction digest. A clone containing the mutation was digested with *BalI* and *MluI*, and the 2-kb fragment containing the open reading frame was isolated and inserted into the *HincII* and *MluI* sites of pIBI24 to create the plac28C expression vector. IPTG induction of bacterial cells containing this vector resulted in the production of a 68-kD protein found entirely in the insoluble fraction of cell lysates.

plac28C-S expression vector Oligonucleotide-directed mutagenesis of pRG411 also was used to introduce a *HindIII* site 50 nucleotides upstream of the third methionine of the open reading frame. The mutagenesis was performed exactly as described above using the oligonucleotide sequence GTTGGGGAAAGCTTGAAG, where the two nucleotides directing the mutation are underlined. A clone containing the mutation was digested with *HindIII* and *MluI* and the 1.65-kb fragment containing the majority of the open reading frame was cloned between the *HindIII* and *MluI* site of pIBI25. The resulting plasmid was then digested with *HindIII*, treated with Klenow enzyme and dNTPs to create blunt ends, and religated to generate plac28C-S.

β -Galactosidase-fusion vectors Fragments from pWV411 were cloned into the β -galactosidase fusion vector pWR590-1 (Guo et al. 1987) to generate a series of *lacZ*-*Dsrc28C* fusion proteins. pWV404 contains the 1.8-kb *EcoRI*-*HincII* fragment, pWV405 contains an 800-bp *BamHI*-*HincII* fragment, and pWV406 contains an *EcoRI*-*PstI* fragment and was generated by deleting the *PstI* fragment internally from pWV404. Upon IPTG induction, these plasmids produce insoluble proteins of ~130,000, 95,000, and 115,000 daltons, respectively.

pCasper-hs28C *Drosophila* *Dsrc28C* germ-line transformations were performed with a vector based on the Casper P element (kindly supplied by V. Pirrotta). A 340-bp *XbaI*-*PstI* fragment containing the hsp70 promoter was coupled to the 2.15-kb *MluI* fragment from *Dsrc28C*. A 240-bp *SalI*-*XhoI* fragment containing the polyadenylation signal from the hsp70 gene was then coupled to the 3' end of the *Dsrc28C* *MluI* fragment. Short segments of polylinker from pIBI24 and pIBI25, used to facilitate the cloning, separate the three gene fragments. A 2.75-kb *XbaI* fragment containing all of these elements was then inserted into the *XbaI* site of Casper. Germ-line transformations of Df(1)w, yw^{67c23(2)} embryos were performed essentially as described (Rubin and Spradling 1982; Spradling and Rubin 1982), using helper plasmid p25.7wc (Karess and Rubin 1984). The progeny of injected flies were scored for the presence of any eye pigmentation.

Production of soluble plac28C

Bacterial cultures containing the plac28C expression vector were grown to mid-log phase, induced with 1 mM IPTG and incubated 5–6 hr. The cells were then pelleted by centrifugation, resuspended in 200 mM Tris-HCl (pH 7.6), 250 mM NaCl, 10 mM MgCl₂, 10 mM β -mercaptoethanol, 5% glycerol, and 0.2 mg/ml lysozyme. After incubation at room temperature for 30 min, the cell were subjected to two freeze-thaw cycles, sonicated to reduce viscosity, and then centrifuged to separate the soluble and insoluble cellular fractions. The insoluble pellet was then washed with 5 M urea in 20 mM Tris-HCl (pH 8.0), 5 mM EDTA, and 5% glycerol (TEG). After centrifugation, the

pellet was resuspended in TEG containing 6 M guanidine-HCl and 100 mM β -mercaptoethanol and incubated 0.5–1 hr at 37°C. This solution was centrifuged to remove particulates and then diluted gradually 1 : 100 into 50 mM Tris-HCl (pH 8.5), 50 mM NaCl, 1 mM EDTA, 0.1% Tween 80, 1 mM reduced glutathione, and 0.1 mM oxidized glutathione. After overnight incubation at 4°C, the solution was centrifuged to remove insoluble protein and the soluble fraction was concentrated by dialysis versus PEG-8000 or by precipitation with 50% ammonium sulfate.

Antibody generation

Dsrc28C protein for antigen was isolated from cell cultures following induction with IPTG. Insoluble plac28C protein was obtained by lysis in 7 M guanidine HCl (pH 8.0) and by precipitation by dilution with 10 mM Tris-HCl (pH 7.5) (Guo et al. 1984). The insoluble pellet was rinsed with buffer and dissolved in SDS sample buffer at 95°C. Following analysis on SDS-PAGE (Laemmli 1970), a strip at either edge of the gel was stained with Coomassie blue and destained. The region of the remaining gel corresponding to the plac28C protein was excised and protein was extracted by soaking the slice in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1 mM EDTA, 0.1% SDS, and 5 mM DTT overnight at 4°C. The eluted material was pelleted, mixed with Freund's adjuvant and water (1 : 1), and sonicated to form an emulsion. Approximately 50 μ g of protein was used to inoculate BALB/c mice subcutaneously. Booster injections of either the same material or total bacterial insoluble protein in Freund's incomplete adjuvant followed at 2-week intervals until the serum titer of antibody to plac28C protein reached 1 : 1000 by Western blot analysis. The animals then were sacrificed for monoclonal antibody production. This antigen yielded the antibody designated mAbI19 following cloning. Alternatively, mice were injected in a similar regimen using the soluble plac28C protein fraction described above. Cloning of spleen cells from one of these mice yielded the antibody mAbB3. Hybridomas with NS-1 myeloma cells were obtained by PEG fusion and cloned according to standard procedures (Goding 1986).

Protein blotting

Proteins analyzed by SDS-PAGE were transferred to nitrocellulose in 25 mM ethanolamine/25 mM glycine/20% methanol to facilitate elution and binding of basic proteins (Szewczyk and Kozloff 1985). The membranes were incubated in 1% gelatin in Tris-buffered saline/Tween [TTBS: 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.05% Tween 20] for at least 30 min to block nonspecific binding and then rinsed in TTBS prior to reaction with antibody. Primary antibody was either cell culture supernatant diluted to 1 : 10 in TTBS or affinity-purified IgG at 10 μ g/ml in TTBS. Second antibody was HRP-conjugated goat anti-mouse IgG (Jackson Immuno Research), 1 : 2000 in TTBS. Color development used 0.3% 4-chloro-1-naphthol in 0.03% H₂O₂.

Protease digestions

V8 protease and chymotrypsin digestions in gel slices were performed essentially as described (Cleveland et al. 1977). The proteins indicated in the text were isolated on 8% SDS-polyacrylamide gels. For each sample, the segment of the gel containing proteins in the 66-kD or 55-kD range was identified by comparison with prestained electrophoresis standards in parallel lanes, excised from the gel, and soaked in stacking gel buffer for 30

min. The gel slices were then placed in the wells of a second SDS–polyacrylamide gel (12.5% polyacrylamide separating gel), and each gel slice was overlaid with sample buffer containing either 250 ng of V8 protease (Miles Laboratories) or 5 µg of chymotrypsin (Worthington). Electrophoresis was performed as usual, except that current was interrupted for 30 min when the dye front neared the bottom of the stacking gel. Subsequently, the digestion products were transferred to nitrocellulose and detected using mAb119 as described above.

Immunofluorescence

Embryos for whole-mount immunofluorescence histology were collected for one hr, aged at 25°C for the appropriate time period, and rinsed with distilled H₂O prior to dechorionation, fixation, and devitellinization, according to the method of Thomas et al. (1988). Primary antibody incubation was overnight at 4°C in phosphate-buffered saline (PBS) containing 0.1% Triton X-100 and 5% normal goat serum (PBSTN), at the same antibody concentration as for Western blotting, followed by several rinses in PBS/Triton X (PBST) for at least 2 hr. The embryos were then incubated in secondary antibody, fluorescein-conjugated goat anti-mouse IgG (Cappel) at 1 : 200 dilution in PBSTN, at room temperature for 2 hr. The embryos were rinsed in several changes of PBST and mounted in 2.5% 1,4-diazobicyclo-[2,2,2]-octane (DABCO)/90% glycerol/PBS (Johnson et al. 1982) for microscopy.

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References

- Banerjee, U., P.J. Renfranz, J.A. Pollock, and S. Benzer. 1987. Molecular characterization and expression of *sevenless*, a gene involved in neuronal pattern formation in the *Drosophila* eye. *Cell* **49**: 281–291.
- Basler, K. and E. Hafen. 1988. Control of photoreceptor cell fate by the *sevenless* protein requires a functional tyrosine kinase domain. *Cell* **54**: 299–311.
- Beach, D., B. Durkacz, and P. Nurse. 1982. Functionally homologous cell cycle control genes in fission yeast and budding yeast. *Nature* **300**: 706–709.
- Bingham, P.M., T.-B. Chou, I. Mims, and Z. Zacher. 1988. On/off regulation of gene expression at the level of splicing. *Trends Genet.* **4**: 134–138.
- Buss, J.E. and B.M. Sefton. 1985. The rare fatty acid, myristic acid, is the lipid attached to the transforming protein of Rous sarcoma virus and its cellular homolog. *J. Virol.* **53**: 7–12.
- Buss, J.E., C.J. Der and P.A. Solski. 1988. The six amino-terminal amino acids of p60src are sufficient to cause myristylation of p21^{ras}. *Mol. Cell. Biol.* **8**: 3960–3963.
- Campos-Ortega, J.A. and V. Hartenstein. 1985. The embryonic development of *Drosophila melanogaster*. Springer-Verlag, New York.
- Cavener, D.R. 1987. Comparison of the consensus sequence flanking translational start sites in *Drosophila* and vertebrates. *Nucleic Acids Res.* **15**: 1353–1361.
- Cleveland, D.W., S.G. Fisher, M.W. Kirchner, and U.K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252**: 1102–1106.
- Doe, C.Q. and M.P. Scott. 1988. Segmentation and homeotic gene function in the developing nervous system of *Drosophila*. *Trends Neurosci.* **11**: 101–106.
- Goding, J.W. 1986. *Monoclonal antibodies: Principles and practice*. Academic Press, New York.
- Gregory, R.J., K.L. Kammermeyer, W.S. Vincent III, and S.C. Wadsworth. 1987. Primary sequence and developmental expression of a novel *Drosophila melanogaster* src gene. *Mol. Cell. Biol.* **7**: 2119–2127.
- Guo, L.-H., P.P. Stepien, J.T. Tso, R. Brousseau, S. Narang, D.Y. Thomas, and R. Wu. 1984. Synthesis of human insulin gene. VIII. Construction of expression vectors for fused proinsulin production in *Escherichia coli*. *Gene* **29**: 251–254.
- Hafen, E., K. Basler, J.-E. Edstrom, and G. Rubin. 1987. *sevenless*, a cell-specific homeotic gene of *Drosophila*, encodes a putative transmembrane receptor with a tyrosine kinase domain. *Science* **236**: 55–63.
- Hanks, S.K., A.M. Quinn, and T. Hunter. 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* **241**: 42–52.
- Henkemeyer, M.J., R.L. Bennett, R.B. Gertler, and F.M. Hoffmann. 1988. DNA sequence, structure, and tyrosine kinase activity of the *Drosophila melanogaster* Abelson proto-oncogene homolog. *Mol. Cell. Biol.* **8**: 843–853.
- Jan, L.Y. and Y.N. Jan. 1982. Antibodies to horseradish peroxidase as specific neuronal markers in *Drosophila* and in grasshopper embryos. *Proc. Natl. Acad. Sci.* **72**: 2700–1704.
- Johnson, G.D., R.S. Davidson, K.C. McNamee, G. Russel, D. Goodwin, and E.J. Holborow. 1982. Fading of DABCO for FITC quench inhibition of immunofluorescence during microscopy: A study of the phenomenon and its remedy. *J. Immunol. Methods* **55**: 231–242.
- Kaplan, J.M., G. Mardon, J.M. Bishop, and H.E. Varmus. 1988. The first seven amino acids encoded by the *v-src* oncogene act as myristylation signal: lysine 7 is a critical determinant. *Mol. Cell. Biol.* **8**: 2435–2441.
- Karess, R.E. and G.M. Rubin. 1984. Analysis of P transposable element functions in *Drosophila*. *Cell* **38**: 135–146.
- Kozak, M. 1987. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* **15**: 8125–8148.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685.
- Laski, F.A., D.C. Rio, and G.M. Rubin. 1986. Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. *Cell* **44**: 7–19.
- Levy, B., T. Dorai, L.-H. Wang, and J.S. Brugge. 1987. The structurally distinct form of pp60c-src detected in neuronal cells is encoded by a unique *c-src* mRNA. *Mol. Cell. Biol.* **7**: 4142–4145.
- Lorincz, A.T. and S.I. Reed. 1984. Primary structure homology between the product of yeast cell division control gene CDC28 and vertebrate oncogenes. *Nature* **307**: 183–185.
- Maniatis, T.E., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

- Martinez, R., B. Mathey-Prevot, A. Bernards, and D. Baltimore. 1987. Neuronal pp60c-src contains a six-amino acid insertion relative to its non-neuronal counterpart. *Science* **237**: 411–415.
- Martinez-Arias, A. and P.A. Lawrence. 1985. Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**: 639–642.
- Morgan, B.A., W.A. Johnson, and J. Hirsh. 1986. Regulated splicing produces different forms of dopa decarboxylase in the central nervous system and hypoderm of *Drosophila melanogaster*. *EMBO J.* **5**: 3335–3342.
- Patel, N.H., P.M. Snow, and C.S. Goodman. 1988. Characterization and cloning of Fasciclin III: A glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. *Cell* **48**: 975–988.
- Reed, S.I., J.A. Hadwiger, and A.T. Lorincz. 1985. Protein kinase activity associated with the product of the yeast cell division cycle gene *CDC28*. *Proc. Natl. Acad. Sci.* **82**: 4055–4059.
- Rio, D.C., F.A. Laski, and G.M. Rubin. 1986. Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. *Cell* **44**: 21–32.
- Rubin, G.M. and A. Spradling. 1982. Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**: 348–353.
- Scott, M.P. and S.B. Carroll. 1987. The segmentation and homeotic gene network in early *Drosophila* development. *Cell* **51**: 689–698.
- Simon, M.A., T.B. Kornberg, and J.M. Bishop. 1985. The nucleotide sequence and tissue-specific expression of *Drosophila c-src*. *Cell* **42**: 831–840.
- Spradling, A. and G.M. Rubin. 1982. Transposition of cloned P element into *Drosophila* germ line chromosomes. *Science* **218**: 343–347.
- Szewczyk, B. and L.M. Kozloff. 1985. A method for the efficient blotting of strongly basic proteins from sodium dodecyl sulfate-polyacrylamide gels. *Anal. Biochem.* **150**: 403–407.
- Thomas, B., S.T. Crews, and C.S. Goodman. 1988. Molecular genetics of the *single-minded* locus: A gene involved in the development of the *Drosophila* nervous system. *Cell* **52**: 133–141.
- Tomlinson, A. and D.F. Ready. 1986. *Sevenless*: a cell-specific homeotic mutation of the *Drosophila* eye. *Science* **231**: 400–402.
- Van Beveren, D. and I.M. Verma. 1986. Homology among oncogenes. *Curr. Top. Microbiol. Immunology* **123**: 91–98.
- Wadsworth, S.C., K. Madhavan, and D. Bilodeau-Wentworth. 1985. Maternal inheritance of transcripts from three *Drosophila src*-related genes. *Nucleic Acids Res.* **13**: 2153–2170.
- Zoller, M.J. and M. Smith. 1983. Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. *Methods Enzymol.* **100**: 468–500.

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