**deadpan**, an essential pan–neural gene in *Drosophila*, encodes a helix–loop–helix protein similar to the *hairy* gene product

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Neural precursor cells in *Drosophila* acquire their identity early during their formation. In an attempt to determine whether all neural precursors share a set of genetic machinery, perhaps to control properties of differentiation common to all neurons, we used the enhancer–trap method to identify several genes (pan–neural genes) that are expressed in all neurons and/or their precursors. One of the pan–neural genes is *deadpan*, which encodes a helix–loop–helix protein closely related to the product of the segmentation gene *hairy*. The function of *deadpan* is essential for viability and is likely to be involved in the functional rather than the morphological differentiation of neurons.

**Key Words:** *deadpan*, pan–neural gene, *Drosophila*, HLH protein, *hairy* gene product, neural precursor cells
et al. 1987; Kopezynski et al. 1988; Rao et al. 1990), whereas others encode putative transcription factors (Knüst et al. 1987; Hartley et al. 1988; Klambt et al. 1989, Smoller et al. 1990; Boulianne et al. 1991). The existence of neurogenic genes encoding membrane proteins is consistent with a role in mediating the cell-cell interactions involved in lateral inhibition. An important consequence of these cell-cell interactions is the activation of genes from the Enhancer of split [E(spl)] complex in cells assuming epidermal fates, including several genes encoding closely related bHLH proteins (Klambt et al. 1989).

Although much is already known about how the location and the spacing of neural precursors are accomplished (i.e., through the formation of proneural clusters and subsequent lateral inhibitory interactions), little is known about how this information converges to create a neural precursor cell. Given that different neural precursors acquire their identity by the action of different genes such as cut, pax-neural, and single-minded (Bodmer et al. 1987, Bloblinger et al. 1988, Nambu et al. 1991; Dambly-Chaudiere et al. 1992) and that the formation of different neural precursors depends on the activity of different upstream regulators (Dambly-Chaudiere and Ghysen 1987), it is not obvious a priori that all primary neural precursors need to share any common properties. In addition, the distinct identities of different neural precursors are manifested early on by their patterns of division (Doe and Goodman 1985, Bodmer et al. 1989). For example, central nervous system (CNS) neuroblasts divide according to a stem cell type of lineage in which all final progeny are neurons, whereas distinct lineages for the PNS external sensory organs and chordotonal (stretch receptor) organs yield one or a few neurons as well as support cells. To analyze early events directing nervous system formation, we asked whether genes exist that are expressed in all primary neural precursors and, if so, whether these genes control aspects of neural differentiation common to all neural precursors or their progeny. To simplify discussion of this group of genes we introduce the term pan-neural. We define a pan-neural gene as one that is expressed during some stage of neurogenesis (i.e., from the formation of primary neural precursors to the formation and differentiation of neurons) in all neural cells but not in epidermal cells.

We used the enhancer trap P element, P-lacW (Bier et al. 1989), to screen ~4000 independent P-lacW insertion lines and identified several pan-neural genes in this collection. Some correspond to known genes such as the elav gene, which is expressed postmitotically in neurons (Bier et al. 1988, 1989; Robinow and White 1988a,b); the prospero gene (Doe et al. 1991; Vaessen et al. 1991), which is expressed in secondary neural precursor cells; and the cyclin A (Lehner and O’Farrell 1989), and scabrous genes (Mlodzik et al., 1990), which are expressed early during neurogenesis in primary neural precursor cells. Other pan-neural genes, such as dph (described in this paper), are newly identified genes. We report here that dph encodes a HLH protein that shows sequence similarity with the Drosophila hairy gene product. dph is expressed in primary neural precursors as soon as they form, and loss of dph function results in weak motor activities, lethargic behavior, and death. Ectopic expression of dph causes elimination of sensory bristles in flies with reduced AS-C activities, suggesting a negative interaction between dph and genes of the AS-C complex. Similar negative interactions have been described in sex determination; whereas scute functions as a numerator, dph acts as a denominator in the determination of the X-chromosome to autosome ratio (Younger-Shepherd et al. 1992).

### Results

**dph, an essential pan-neural gene**

The dph gene is disrupted by a P-lacW insertion at 44C on the polytene chromosome, and the resulting recessive lethality is not complemented by a small deficiency, Df(2L)193A. The lethality is the result of the P-lacW insertion, as we have obtained viable revertants in which the P-lacW element has been excised by remobilization. We refer to this essential gene as dph as it has a pan-neural expression pattern and a lethal mutant phenotype. The mutation due to the P-lacW insertion, dph1, is likely to be a null mutation as it eliminates both detectable transcript and protein product (see below). Mutants homozygous for dph1 die at various stages of development. Under favorable conditions a few percent of the homozygotes survive to young adulthood. The dph1 mutant larvae are weak; many pupate in the food rather than crawl up the wall to pupate as do wild-type larvae. The rare homozygous dph1 mutant flies have drooped wings, are extremely sluggish, and do not show any spontaneous motor activities. Most of these mutant flies die within a few days after eclosion. In spite of the severe behavioral defects, homozygous dph1, homozygous Df(2L)193A, and trans-heterozygous dph1/Df(2L)193A embryos do not exhibit any obvious consistent morphological abnormalities when examined with a variety of tissue-specific markers, including the following antibodies: mAb44C11 [a neuronal nuclear antigen], anti-Hunchback [expressed in neuroblasts], anti-horseradish peroxidase (HRP) [a neuronal membrane antigen], anti-Cut [a nuclear protein expressed in external sensory organ precursors], mAb22C10 [a cytoplasmic PNS antigen], anti-Sna [expressed in all neural precursor cells and neurons], anti-Eve [expressed in a pair-rule pattern and then in a subset of neurons], anti-Engrailed [expressed in segmental stripes and then in a subset of neurons], and mAb6D5 [a muscle antigen]. Thus, it is likely that complete lack of zygotic dph function affects the function but not the gross morphology of the nervous system.

**The dph transcription unit**

Genomic DNA flanking the dph1 P-lacW insertion site was isolated by plasmid rescue (Fig. 1A; see also Materials and methods) and used as a probe for Northern blot
Figure 1. *dpn* genomic map and transcription unit. (A) The *dpn* genomic map was determined from a combination of restriction digests of clones derived from plasmid rescue of *Drosophila* sequences flanking the P-lacW insertion at 44C, λ-Dash clones of *Drosophila* wild-type genomic DNA, and Southern blots of genomic DNA extracted from wild-type and *dpn* flies. Restriction fragments hybridizing to the 2.1-kb *dpn* cDNA clone are indicated by the stippled boxes below the map. Restriction enzymes are abbreviated as follows: (X) XbaI; (G) BglII; (E) EcoRI; (H) HindIII. (B) Northern blot of staged embryo RNA probed with the genomic EcoRI-rescued plasmid (see diagram in A). A single transcript of ~2.3 kb is present at all stages but is significantly more abundant in 4- to 10 hr embryos during which time neurogenesis is occurring. Only traces of the transcript are present in embryos >10 hr old. A band at ~1 kb is seen in this blot as well as parallel Northern strips hybridized with probes for other pan-neural genes and is likely to be the result of nonspecific plasmid hybridization.

analysis of poly(A)⁺ RNA from staged embryos [Fig. 1B]. Only one transcript of 2.3 kb was detected in embryos of 0–4 hr and 4–10 hr [embryogenesis takes 22 hr at room temperature]. Ten cDNA clones corresponding to this transcript were isolated, and the two longest cDNA clones were further characterized. A digoxigenin-labeled probe synthesized from one of these clones hybridized to mRNA in all neural precursors in the embryonic PNS and CNS, revealing a pattern similar to the *lacZ* expression pattern due to the *dpn* P-lacW insertion. The *dpn* mutant embryos showed no expression of this mRNA, as revealed by in situ hybridization to whole-mount embryos [not shown]. Consistent with these in situ results, antibodies raised against a Dpn fusion protein revealed staining of all neural precursors in wild-type but not in *dpn* mutant embryos [not shown]. Taken together, these results strongly indicate that the 2.3-kb transcript corresponds to the *dpn* gene.

Embryonic expression patterns of the *dpn* transcript and protein product

Zygotic expression of *dpn* RNA is first detected early during nuclear cycle 12 at low levels throughout the embryo, with the exception of the pole cells at the posterior end. This broad expression peaks near the end of nuclear cycle 12 and then fades after the completion of nuclear division cycle 13, first in anterior regions leaving a transient gap gene-like pattern [Younger-Shepherd et al. 1992]. *dpn* transcripts then reappear during the middle of cycle 13 in a pattern of eight stripes of cells [Fig. 2B] (at ~3 hr of embryogenesis). *dpn* transcripts are concentrated in the apical region of expressing cells [Fig. 2B], as are the transcripts of several pair-rule genes [Davis and Ish-Horowicz 1992]. *dpn* stripes overlap with corresponding *hairy* stripes, which extend more posteriorly [Ingham et al. 1985b; Carroll et al. 1988], as well as localization of both *hairy* transcripts and Dpn protein in the same embryos [not shown]. The most anterior *dpn* stripe corresponds to the most anterior full *hairy* stripe and the most posterior *dpn* stripe overlaps a weak *hairy* stripe in the hindgut primordia [see diagram in Fig. 3D]. The order of appearance of *dpn* and *hairy* stripes are different, however [see Fig. 2 legend]. The pair-rule expression of *dpn* RNA and protein begins to fade rapidly as gastrulation commences and is almost entirely gone as the germ band begins to elongate.
Figure 2. Expression of dpn transcripts and protein. Embryos are all oriented with anterior to the left and dorsal up unless otherwise indicated. (A) Dpn protein at approximately preblastoderm cycle 12 is expressed in all nuclei. (B) A sagittal view of dpn transcripts expressed in a pattern of eight stripes during middle to late cycle 13 (3½–4 hr of development). Note that transcripts are concentrated near the apical surface (arrow), as has been observed for several pair-rule gene transcripts (Davis and Ish-Horowicz, 1992). The order of appearance of dpn stripes is 5, 7 + 2, 3 + 4, 1, 8, and 6. This differs from the reported order of hairy stripe appearance, which is 1, 2 + 3, 4, 7, 5, and 6. (For determination of the register of dpn stripes with respect to engrailed-expressing cells, see Fig. 3.) (C) A ventral view of dpn transcripts in neuroectodermal patches from which the innermost and outermost rows of CNS neuroblasts will form before neuroblast segregation at ~5½ hr of development. Expression in neuroectodermal patches is not observed, however, in all regions giving rise to neuroblasts (e.g., neuroblasts in row 2). In very good staining preparations Dpn protein can also be observed at low levels in similar neuroectodermal patches. (D) A ventral view of dpn transcripts in the first wave of neuroblasts at ~6 hr of development. At the end of the first wave of neuroblast segregation, continuous rows of neuroblasts (four per hemisegment) are visible in row 1 (the innermost row) and row 3 (the outermost row) and in one neuroblast per segment in row 2. (E) A ventral view of a 6½-hr-old embryo stained for Dpn protein showing that Dpn expression is limited to the neuroblast cell layer. Dpn is nuclear during the first wave of neuroblast segregation, but later it can also be found throughout the cell including the cytoplasm in some cells (arrowhead) as well as being strictly localized in the nucleus in other cells (arrow). dpn transcripts are also limited to neuroblasts as soon as they begin to segregate from the neuroectoderm. (F) A sagittal view of dpn RNA expression when all neuroblasts have segregated (6½–7 hr of development). dpn transcripts and Dpn protein are not observed in ganglion mother cells of the CNS after neuroblast divisions, suggesting that both dpn RNA and protein are very labile. (G) A lateral view of Dpn protein expression in sensory organ precursor cells in the PNS. dpn transcripts are transiently expressed in neuroectodermal patches before the segregation of the first PNS precursors. Subsequently, dpn transcripts and protein are found only in primary neural precursor cells. dpn expression is not observed in secondary PNS precursor cells after division of primary sensory organ precursor cells. (H) Lateral view of Dpn protein expression in neurons of the PNS (~10 hr of development). Dpn is also expressed in CNS neurons at this time. Dpn is likely to be expressed briefly in most neurons as lacZ expression derived from lacZ–dpn promoter fusions labels all PNS neurons (J. Emery and E. Bier, unpubl.). Although Dpn expression is transient in most neurons, it is maintained in a small subset of the CNS neurons for several hours longer (not shown). Abbreviations in this and the following figures: (A) Abdominal segments, (cf) cephalic furrow, (ect) ectoderm, (G) gnathal segments, (ms) mesoderm, (nb) neuroblast, (PNS prec.) primary sensory organ precursor cell, (r1, r2, r3) rows 1, 2, and 3, respectively, of first wave neuroblasts; (T) thoracic segments; (vml) ventral midline.

Expression of the most posterior dpn stripe is maintained for the longest period but is gone before full germ band extension. We should note here that dpn1 mutant embryos did not show any detectable abnormality in the segmentation pattern.

dpn transcripts are expressed briefly in patches of neuroectodermal cells just preceding the first wave of neuroblast segregation in the CNS [Fig. 2C]. This expression rapidly becomes confined to the neuroblasts before their delamination [Fig. 2D]. The Dpn protein follows a similar course of neural expression but is expressed at very low levels in the neuroectodermal patches before neuroblast formation. By 6½–7 hr of development all neuroblasts have segregated and express Dpn [Fig. 2E,F]. The same pattern of brief dpn transcription in patches of ectodermal cells followed by strong expression of mRNA and protein in sensory organ precursor cells is observed in the PNS [Fig. 2G]. The expression of dpn RNA and protein in primary neural precursors disappears soon after they divide. dpn is expressed transiently again at a later stage of nervous system development in the PNS and CNS as neurons begin to differentiate [Fig. 2H], suggesting that dpn may act during two distinct phases of neuronal development.

It is clear from antibody staining that Dpn is a nuclear protein, although some cells also exhibit cytoplasmic
staining (Fig. 2E). The very first wave of neuroblast expression, however, seems to be restricted to the nucleus. Perhaps subsequent cellular events lead to a phase of less strict localization. The dynamic expression pattern suggests that dpn transcripts and Dpn protein turn over rapidly. With the exception of the nuclear staining before cellularization (Fig. 2A), the antibody staining is eliminated in embryos homozygous for dpn1 or Df(2L)193A. Whether the nuclear staining at the nuclear cleavage stages represents maternal contributions or background staining remains to be determined.

The pattern of lacZ expression derived from the dpn1 P-lacW insertion corresponds precisely to that of the dpn gene itself at all stages mentioned above (data not shown). The only difference in these patterns is that lacZ expression is maintained for longer periods of time, presumably because lacZ RNA and protein are more stable than dpn transcripts and protein.

In summary, the expression patterns of dpn during embryogenesis include three components. The very early expression is consistent with the functional role of dpn in sex determination (Younger-Shepherd et al. 1992), and the subsequent transient, pair-rule pattern resembles that of hairy. The Dpn protein is then expressed in primary neural precursors and in neurons as they begin to differentiate. This latter pattern differs drastically from the hairy expression pattern and is likely to be responsible for the phenotype of lethargy and lethality in homozygous dpn mutants.

dpn expression in imaginal tissue

We examined dpn expression in the larval CNS and the imaginal discs to determine whether the later expression patterns of dpn were also pan-neural or resembled the hairy expression pattern. Comparison of the dpn and hairy patterns in discs is of particular interest because of the conspicuous absence of hairy expression in neural precursors and neurons. dpn is expressed in neuroblasts of the larval CNS and in precursors of sensory neurons in the imaginal disc (Fig. 4). dpn expression is restricted to the primary neural precursor cell. This can be seen most easily in Figure 4, B and D, in which CNS neuroblasts are labeled with the anti-Dpn antiserum, whereas the gan-
dpn expression in larval CNS and imaginal discs. Expression of Dpn protein (B,D) is compared with lacZ expression owing to the dpn¹ P-lacW insertion (A,C) in larval CNS (A,B,C,D). At lower magnification (A,B), expression in the larval CNS is evident in neural precursors at the ventral side of the thoracic ganglia (Truman and Bate 1988), as well as in the larval brain (Br). Neurons in the eye disc (E) also express dpn. (A) At the lateral view, the thoracic ganglia are to the right of the brain and the abdominal ganglia (below the thoracic ganglia) contain no neural precursors or cells that express dpn. The ventral side is to the right. (B) A dorsal view of the thoracic and ventral ganglia and the two brain hemispheres (Br) on either side. The disc is connected to the brain by the eye stalk. The neuroblasts on the ventral side of the thoracic ganglia can be seen through the entire thickness of the ganglia, owing to the staining for Dpn. Anterior is up for both A and B. At high magnification (C,D), Dpn protein (C) is clearly localized to the primary neuronal precursor, the neuroblast (NB), whereas the long-lived lacZ product (D) persists in the secondary neuronal precursor, the ganglion mother cell (GMC), and neurons derived from the neuroblast. Brackets indicate groups of cells, each derived from a single neuroblast. In the leg disc, shown at two different focal planes (E,F), dpn is expressed in chordotonal organ (Cho) precursors and other sensory organ precursors (arrows), as well as stripes of epidermal cells.

glion mother cells and their neuronal progeny do not stain. In contrast, the bacterial β-galactosidase protein encoded by the lacZ gene in P-lacW has a much longer lifetime; therefore, lacZ expression in heterozygous dpn¹ larvae led to lacZ staining of all CNS neuroblasts as well as their progeny, the ganglion mother cells, and the neurons (Fig. 4C).

In leg imaginal discs dpn is also expressed in additional non-neuronal cells. Thus, dpn is expressed in stripes at the distal edge of each leg segment primordium (Fig. 4E,F). Expression in these cells is very similar to the pattern of hairy expression in imaginal discs (Carroll and Whyte 1989). The major difference between dpn and hairy expression in the leg imaginal discs is that hairy is excluded from neural precursor cells.

dpn expression in embryos lacking proneural and neurogenic gene functions

The expression of Dpn in neuroblasts serves as a criterion for neuroblast differentiation, in addition to the morphological criterion of neuroblast enlargement and delamination. This has provided a tool to investigate the function of proneural genes [e.g., da and AS-C]. In embryos lacking zygotic da function, the PNS is missing completely and the CNS is greatly reduced (Caudy et al. 1988a). As expected from the absence of the PNS, neural precursors for the PNS do not form in da mutants (Caudy et al. 1988a). In contrast, many of the CNS neuroblasts delaminate in a roughly normal pattern (Jimenez and Campos-Ortega 1990), even though far fewer than the normal number of final neurons form (Caudy et al. 1988a; Jimenez and Campos-Ortega 1990; M. Brand, pers. comm.). This suggests that either only a fraction of the neuroblasts follow their normal patterns of division or all neuroblasts behave abnormally and produce fewer neurons than normal. We found that the remaining CNS neuroblasts in da mutants did not express any detectable levels of dpn RNA or protein, although the ventral midline cells in the same da mutant embryos expressed rhomboid transcripts (Fig. 5B). Thus, all neural precursors in da mutant embryos are abnormal; they do not express dpn and they produce very few neurons. Although early dpn expression in neuroblasts appears likely to be directly activated by da, later expression of dpn was found in some CNS neurons in da/da mutant embryos, indicating that more than one mechanism controls dpn expression at different developmental stages.

The CNS phenotype in embryos deficient for AS-C is similar to that in da mutant embryos; fewer neuroblasts are formed than in wild-type embryos and far fewer than normal number of CNS neurons are found later during neurogenesis (Jimenez and Campos-Ortega 1990). This similarity in the CNS phenotype no longer holds when the dpn expression patterns are examined. In contrast to
homzygous da mutants, embryos homzygous for the sc857 deletion [sc857 eliminates all known AS-C transcription units] express normal levels of Dpn in a reduced number of neuroblasts. The Dpn-expressing neuroblasts in sc857 mutants are most numerous near the midline during the first wave of neuroblast formation and, in general, are observed in groups of cells that are mirror symmetric with respect to the midline [Fig. 5C]. Thus, although da is required for expression of detectable levels of Dpn in all neuroblasts, AS-C function appears to be dispensable for the formation of a subset of CNS neuroblasts that express dpn.

The very early expression of dpn in primary neural precursors also offers an opportunity to examine the effects of neurogenic mutations. Embryos homzygous for the neurogenic mutations E(spl), Notch, Delta, and neuralized form an excess of Dpn-expressing cells [data not shown]. This hyperplasia of the nervous system is evident when the first wave of neuroblasts delaminate from the neuroectoderm, consistent with the early actions expected of neurogenic genes in the determination of neural precursors.

The dpn gene encodes a protein with a bHLH domain

The two longest dpn cDNA clones were sequenced. The 2.1-kb clone is nearly full length and includes a complete open reading frame, whereas the 2.2-kb clone is likely to represent a partially spliced version of the gene. The predicted protein encoded by the 2.1-kb cDNA (Dpn) contains 435 amino acids [Fig. 6]. Consistent with its nuclear localization, the Dpn protein contains a HLH motif and a preceding basic residue-rich sequence [bHLH] near the amino terminus. bHLH domains in proteins such as E12/E47, Da, and MyoD have been shown to bind to specific DNA sequences [Murre et. al. 1989a,b]. Among the known bHLH proteins, the Dpn bHLH domain shows the highest degree of sequence similarity with the product of the Drosophila segmentation gene hairy [Hairy] [there is 84% amino acid identity and 92% similarity permitting only the most conservative changes] over 61 amino acids in the bHLH region [Fig. 7A]. It appears likely that dpn and hairy arose from a common ancestral gene because the additional sequence that is present in the 2.2 kb dpn cDNA, but not in the 2.1-kb dpn cDNA, contains the appropriate consensus sequences for mRNA splicing, suggesting that it is an intron of the dpn gene; and the location of this putative splice site corresponds precisely to an intron–exon boundary in the hairy gene. The Dpn and Hairy bHLH domains are also similar to the bHLH domains of the highly related proteins of the E(spl) complex than to other known bHLH proteins. For example, the bHLH domains of Dpn, Hairy, and the gene products of the E(spl) complex all have a characteristic proline residue in the basic domain.

Dpn, Hairy, and the gene products of the E(spl) complex also share structural features outside the bHLH domain [Fig. 7]. In the 53-amino-acid residues immediately carboxyl to the bHLH domain, Dpn and Hairy share 42% amino acid identity. After this region, both proteins are rich in proline, serine, and threonine residues, although the sequences do not align. The bHLH proteins encoded in the E(spl) complex also show sequence similarity to Dpn and Hairy in these carboxyl domains, although the degree of similarity is less marked. Finally, proteins in this group all possess the same four carboxy-terminal amino acids WRPW. This last feature is not found in any other known bHLH proteins.

dpn mutations interact genetically with known genes required for neurogenesis

Genes acting in common developmental pathways often exhibit dosage sensitive interactions. In such cases, mu-
Figure 6. Sequence analysis of dpn cDNA clones. The sequence of the 2.1-kb [2023 bp long, not including the poly(A) tail] and 2.2-kb (2223 bp) dpn cDNAs were determined by single-stranded dideoxy sequencing of both strands of each clone. The 2.1-kb cDNA contains a single open reading frame encoding a predicted protein (Dpn) of 435 amino acids (represented by the standard single-letter code). The predicted Dpn protein contains a bHLH domain near the amino terminus, followed by a region rich in proline, serine, and threonine, and terminating in the tetrapeptide WRPW. The sequence of the 2.2-kb cDNA is identical to the 2.1-kb cDNA after nucleotide 492.

DPN PROTEIN

C TERMINUS

BASIC DOMAIN

HELIX 1

LOOP

HELIX 2

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Figure 7. Alignment of Dpn and other bHLH protein sequences. (A) Alignment of the Dpn bHLH region with other bHLH domains. Vertical lines indicate amino acid identity between Dpn and Hairy bHLH sequences, and broken lines indicate highly conservative substitutions. Boxed and bold sequences are identical or highly similar (italicized) to Dpn. Asterisks (*) denote conserved positions in similarities outside the conserved bHLH domains. In particular, there is a region of higher sequence similarity directly after the Basic Domain is most similar to that of Hairy, it is also more similar to E(spl) proteins in this region than to other bHLH domains.

(B) Alignment of complete Dpn, Hairy, and E(spl) protein sequences. The Dpn, Hairy, and E(spl) proteins share additional sequence similarities outside the conserved bHLH domains. In particular, there is a region of higher sequence similarity directly after the HLH domain. This region is followed by segments of differing length that are rich in the amino acids proline, serine, and threonine. These proteins also all end in the tetrapeptide WRPW.
have inserted the protein-coding region of the 2.1-kb \textit{dpn} cDNA into the pWH1 heat shock P-element vector (Schnewly et al. 1987) and introduced this heat shock--\textit{dpn} (HS--\textit{dpn}) construct into flies by P-element-mediated transformation [Spradling 1986]. When heterozygous \textit{sc^{657}} female flies that carry one copy of this construct are heat shocked during early pupation, many of the innervated small bristles (microchaete) are missing (in some of these flies there are only four rows of microchaete instead of the normal eight rows between the large dorsocentral macrochaetes). This interaction between HS--Dpn and AS-C could reflect a normal interaction of Dpn and AS-C products or might be nonphysiological. We favor a specific interaction between \textit{dpn} and AS-C as similar heat shock experiments performed with a heat shock--\textit{Hairy} (HS--\textit{Hairy}) construct in \textit{sc^{657}} heterozygotes results mainly in the loss of dorsocentral macrochaete, whereas HS--Dpn affects mainly microchaete. These observations indicate that like \textit{hairy}, \textit{dpn} acts in a manner antagonistic to genes of the AS-C.

**Discussion**

**Neural precursors: a tissue type with individual properties**

The existence of early pan–neural genes suggests that the diverse genetic paths by which different ectodermal cells become specified as neural precursors all converge to activate a common genetic program. Thus, although different neural precursors rely on different genes, such as \textit{cut} [Bodmer et al. 1987; Bloehlinger et al. 1988], and \textit{pox–neural} [Dambly-Chaudiere et al. 1992], to acquire their own identity, they all express pan–neural genes. Pan–neural genes may therefore integrate the varied upstream regulatory pathways specifying different neural precursors and allow the collection of many individual neurons to differentiate as a coherent neural tissue type. The consolidation of complex early positional pattern information into a relatively small number of pan–neural genes may simplify the problem of activating functions common to all neural precursors and their neuronal progeny. In this view, pan–neural genes would function analogously to the pair–rule class of segmentation genes that transform global positional information provided by the gap genes and the maternal morphogen into regularly spaced stripes.

The functions of some pan–neural genes have been implicated from the sequence of their products. Pan–neural transcription factors are likely to regulate genes required for executing neural functions or to repress genes functioning in other tissues such as mesoderm or epidermis. For example, the \textit{prospero} gene function is required for proper axonal outgrowth and path finding of both central and peripheral neurons [Doe et al. 1991; Vaessin et al. 1991]. Whether these pan–neural regulatory factors individually control subsets of neuronal functions, such as axonogenesis and electrical excitability, or collaborate in some combination to regulate neuronal differentiation remains to be determined. Besides nuclear proteins potentially involved in gene regulation, as in the case of \textit{dpn} and \textit{prospero} [Vaessin et al. 1991], other pan–neural gene products may be involved in cell–cell communication [Mlodzik et al. 1990], control of the cell cycle [Lehner and O’Farrell 1989], and RNA processing [Bier et al. 1988, 1989; Robinow and White 1988b].

**Possible function of the \textit{dpn} gene**

\textit{dpn} is an essential gene, as loss of \textit{dpn} function results in lethality. \textit{dpn} does not appear to be necessary for normal morphogenesis of the nervous system, as \textit{dpn} mutant embryos or escaper flies do not exhibit any consistent morphological defects. One possibility is that \textit{dpn} regulates a subset of events in neural precursors unrelated to morphogenesis. We are currently screening through more markers specific to various subsets of the CNS, PNS, and epithelium to identify potential target genes that may be positively or negatively regulated by Dpn. Another possibility is that other genes (perhaps encoding bHLH proteins) act in parallel with \textit{dpn} in neural precursors. This would not be surprising given the known examples of the overlapping function of subgroups of bHLH proteins (e.g., AS-C and \textit{E(spl)} complex) functioning in neurogenesis [Cabrera et al. 1987; Dambly-Chaudiere and Ghysen 1987; Romani et al. 1987, 1989; Jimenez and Campos-Ortega 1990; Campos-Ortega and Jan 1991]. This possibility may be explored by further analyzing the interaction of \textit{dpn} with AS-C and possibly with genes encoding other bHLH proteins that can be isolated based on their sequence similarity to \textit{dpn} [E. Bier and J. Feder, unpubl.].

The bHLH motif in Dpn suggests that this nuclear protein is involved in transcription regulation. The presence of a proline residue in the basic domain of Dpn raises questions concerning the ability of Dpn to bind DNA, as a proline for alanine mutation in MyoD at a position corresponding to the neighboring residue of the proline found in Dpn, Hairy, and proteins encoded by the \textit{E(spl)} complex rendered the mutant MyoD protein unable to bind to the cognate DNA sequence [Lassar et al. 1989; Davis et al. 1990]. Moreover, ectopic expression of the \textit{hairy} product, which also contains a proline in the basic domain and shows strong overall sequence similarity with the \textit{dpn} product, has been found to interfere with sex determination, presumably owing to the formation of nonfunctional heterodimers of Hairy and the AS-C T4 gene product [Parkhurst et al. 1990]. The male-specific lethality caused by the negative interaction between AS-C and \textit{dpn} in sex determination indicates that \textit{dpn} and \textit{hairy} interact with AS-C genes in a similar manner [Younger-Shepherd et al. 1992]. According to this view, \textit{E(spl)} proteins might also act to inactivate proneural bHLH heterodimers in cells assuming epithelial fates. On the other hand, the protein encoded by the m8 gene of the \textit{E(spl)} complex binds a specific DNA target sequence, even though it contains a proline in the basic domain [Tietze et al. 1992]. Preliminary data suggest that the Dpn protein also binds DNA [H. Vaessin, unpubl.].
How Dpn might interact with other bHLH proteins is an important question to be addressed in future studies. One possibility is that Dpn functions in neural precursor cells to turn off AS-C expression (AS-C expression in neural precursors is very transient) by interfering with the autoregulatory loop of AS-C activation, which has been demonstrated in the adult PNS (Martinez and Modolell 1991). In this model Dpn would carry out the same function in neural precursors that Hairy executes in epidermal precursors, whereas Hairy is expressed in cells surrounding the neural precursor, thereby preventing these cells from adopting the neural precursor fate by suppressing AS-C expression. Dpn is expressed in neural precursors but with sufficient delay to allow the initial steps of neurogenesis to proceed before the suppression of AS-C expression.

Potential implications for the evolution of neural patterning

Recent observations suggest that certain developmental pathways have been conserved throughout large branches of phylogeny. For example, the strong homology in sequence and expression pattern between Drosophila homeotic genes and their vertebrate counterparts suggests that positional values are likely to be specified by similar mechanisms in these organisms (Akam 1989; Malicki et al. 1990; McGinnis et al. 1990). There are also striking examples of homologous regulatory genes expressed in the same tissues throughout phylogeny. This is well documented for genes regulating myogenesis such as MyoD, twist, and snail (Boulay et al. 1987; Thissie et al. 1988; Hopwood et al. 1989; Michelson et al. 1990; Sargent and Bennett 1990) and is likely to be the case for genes determining neural identities, as vertebrate homologs of the proneural AS-C genes are expressed in neural precursor cells (Johnson et al. 1990; Lo et al. 1991; W. Harris, unpubl.). Finally, the Drosophila emc gene, which acts to suppress neurogenesis, may be a homolog of the vertebrate Id gene (Benezra et al. 1990; Ellis et al. 1990; Garrell and Modolell 1990). It is therefore of substantial interest to determine whether Drosophila pan–neural genes have vertebrate homologs that are also expressed in pan-neural patterns.

Within a given organism, developmental strategies often share significant features. For example, pattern formation is achieved by mechanisms of progressive refinement both during neurogenesis and body axis formation in Drosophila (Ingham 1988; Ghysen and Dambly-Chaudiere 1989; Jan and Jan 1990). Furthermore, these genetic programs employ common genes. Thus, early in development, hairy, fushi tarazu, and even-skipped function to specify anterior–posterior positions, and rhomboid acts to establish dorsal–ventral cues (Jürgens et al. 1984; Nüsslein-Volhard et al. 1984; Ingham et al. 1985a; Mayer and Nüsslein-Volhard 1988). These same genes then function again later to assign various cell fates in the nervous system (Doe et al. 1988a, b; Kushlow et al. 1989; Bier et al. 1990). The observation that dpn is expressed in a pair–rule pattern at the cellular blastoderm stage and then again in a pan–neural pattern may represent yet another example of a gene functioning at multiple distinct stages of development. Alternatively, the dpn blastoderm pattern may reflect the common ancestry shared by dpn and hairy.

Materials and methods

Fly stocks

All genetic markers and chromosome balancers used are described in Lindsley and Grell (1968). The Df(2L)193A stock was kindly provided by J. Hooper.

Antibody staining of embryos

The following primary antibodies were used according to published conditions: rabbit anti-5-gal (Cappel); mAb44C11 (Bier et al. 1988); anti-Hunchback (provided by P. McDonald; see Small et al. 1991); anti-HRP (Jan and Jan 1982); anti-Cut (Blochlinger et al. 1998); mAb22C10 (Venkatesh et al. 1985); anti-Sna (Kosman et al. 1991); anti-Eve (provided by M. Frasch, see Small et al. 1991); anti-Engrailed (provided by T. Kornberg; see Patel et al. 1989); and mAb6D5 (Caudy et al. 1988a). To visualize primary antibodies we used biotinylated secondary antibodies followed by avidin–HRP from Vectastain ABC elite kits (Vector Laboratories, PK-4001). After reacting HRP with diaminobenzidine, the samples were dehydrated in ethanol, cleared in xylene, mounted on slides with Permoun (Fisher), and examined under a microscope with Nomarski optics.

Production of antibodies against Dpn

To raise antibodies against Dpn, an internal BamHI fragment (base pairs 594–1363) encoding amino acids 109–365 of the predicted Dpn protein, was cloned in the pet3 vector (Rosenberg et al. 1987) and expressed in bacteria. This part of Dpn was chosen, because it has only limited similarity to hairy. After lysis of the bacteria, the initially insoluble fusion protein was separated from soluble bacteria proteins by centrifugation at 10,000 rpm for 15 min and subsequently resolubilized in 8 M urea, 1 × PBS. Renaturation of the protein was done by stepwise dialysis against decreasing concentrations of urea (2 M urea increments, each step for 3 hr at room temperature). After two additional dialysis steps against 1 × PBS, the resulting soluble protein, which consisted at this stage of >50% Dpn fusion protein, was used for immunization of two rabbits. Serum from these animals was subsequently purified in two steps. First, the serum was preabsorbed against total bacterial protein, coupled to Affigel 10 + 15 (Bio-Rad) to remove the antibodies directed against bacterial proteins. Subsequently, anti-Dpn antibodies were purified by affinity purification against Dpn fusion protein coupled to Affigel 10 (Bio-Rad). Antibodies bound to the Dpn fusion protein were recovered by elution with 1.5% glycine/HCl (pH 2.3).

In situ hybridization to RNA in sections or whole-mount embryos

In situ hybridization to whole-mount embryos was done following the method of Tautz and Pfeifle (1989), using digoxigenin labeled DNA or RNA probes (Boehringer Mannheim; 1093 657).
Plasmid rescue of genomic sequences flanking the dpn^1 P-lacW insertion

We used the unique EcoRI and BamHI restriction sites in P-lacW to plasmid rescue genomic DNA flanking the 3' and 5' sides of the P-lacW insertion at 44C, respectively (see genomic map in Fig. 1). DNA fragments containing fly sequences from the two rescue plasmids were labeled with ^32P and hybridized to Southern blots of wild-type and dpn+ DNA and Northern blots of staged embryonic RNA. Hybridization of these fragments to Southern blots of wild-type genomic DNA was consistent with the restriction map deduced from the rescued genomic DNA (data not shown).

Northern blot analysis of RNA extracted from staged embryo collections using the labeled rescued genomic fragments as probe is shown in Figure 1B. Strong hybridization to the 2.3-kb transcript is observed in lanes corresponding to 0- to 4- and 4- to 10-hr embryos using the genomic fragment derived from the EcoRI-rescued plasmid. This same transcript is only weakly detected in 10- to 20-hr embryos. No hybridization is observed to a Northern blot processed in parallel using the genomic fragment contained in the BamHI-rescued plasmid as probe, although this same probe hybridized subsequently to a genomic Southern blot (data not shown).

Construction of the dpn heat shock vector

The coding region of dpn between nucleotides 266 and 1578 was amplified by the polymerase chain reaction and cloned into the Smal site of the Bluescript plasmid modified to have the Spel site converted to a Kpnl site (the EcoRV site was also converted to a BgIII site for other purposes). The dpn insert was removed from this plasmid with Kpnl and subcloned into the P-element transformation vector pWHL (Schnewly et al. 1987), cut with Kpnl in the 5' to 3' direction relative to the HSP70 promoter. This construct was injected into fly embryos, and transformed flies were isolated according to standard methods (Spradling 1986).

Other molecular techniques

Plasmid rescue from minipreparations of fly DNA was performed according to Pirrotta [1986]. Other cloning techniques followed standard procedures, as in Maniatis et al. [1982].

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