Isolation and Characterization of the prune Locus of Drosophila melanogaster

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ABSTRACT

The complementary lethal interaction between the prune (pn) and Killer of prune loci of Drosophila melanogaster is an unusual and highly specific phenomenon. A lesion in pn results in a brownish-purple color of the compound eyes, while the conditional dominant Killer of prune mutation exhibits no phenotype by itself. However, a hemizygous or homozygous pn mutant carrying a copy of the Killer of prune gene dies during the late second to third instar stage of larval development. As a step toward understanding the molecular nature of this lethality and the role of pn in pigment biosynthesis, we have cloned the pn locus by using a transposon tag in the P element-induced allele, pn-neutral. In addition, seven independent revertant lines were generated by the remobilization of transposons in pn-neutral. The pn gene is located in a region that is transcriptionally active, and the isolated cDNAs that correspond to this area fall into three transcription units: I, II and III. Southern analysis shows that the restriction fragment length polymorphisms in five pn alleles are localized within a 1.2-kilobase genomic fragment. Of only transcription unit II is a part. The cDNA of this unit recognizes 1.65- and 1.8-kilobase messages in wild-type Drosophila adult head and body tissues that are absent or extremely reduced in pn mutants. Taken together, the results suggest that transcription unit II defines a part of the pn locus and its cDNA encodes a putative structural gene of pn.

The study of genetic interactions has been important in understanding the processes involved in physiology and development. Mutations in genes that play a role in the same or interdependent pathways often result in phenotypes which are similar. Additionally, genes may interact to cause an enhancement, suppression or alteration of a given phenotype. The characterization of such genes and their protein products provide valuable insights into the molecular mechanisms of the pathway.

Several eye-color mutations in the fruit fly, Drosophila melanogaster, have genetic interactions with other loci that result in lethality. Such lethal systems that have been previously described include the following: prune and Killer of prune; purpureoid and Purpureoider; deep orange and rosset; Henna-recessive-3 and rosset (Lucchesi 1968). It is intriguing that apart from Killer of prune (K-pn; 3-102.9), the other six complementation groups are involved in the production or maintenance of pteridine pigments. A mutation in the pn (1-0.8) locus causes the compound eyes of the fly to change from a characteristic wild-type red color to brownish-purple. Biochemical analysis has shown that this eye-color phenotype is a result of a reduction in the concentration of drosopinogens, the red pteridine pigments (Naryanan and Weir 1964; Evans and Howells 1978). Specifically, pn alters the level of activity of GTP cyclohydrolase (Evans and Howells 1978; Mackay and O'Donnell 1983), the enzyme that catalyzes the initial reaction in pteridine biosynthesis (Brown 1985). In this step, GTP cyclohydrolase converts GTP to dihydronopterin triphosphate with the release of formic acid. Gene dosage studies suggest that the pn+ product may participate in the developmental regulation of GTP cyclohydrolase, while the Punch (Pu; 2-97) locus encodes the structural gene of the enzyme (Mackay and O'Donnell 1983; McLean, Boswell and O'Donnell 1990). The recessive pn gene exhibits a highly specific, complementary lethal interaction with K-pn, a conditional dominant mutation that has no phenotype of its own (Sturtevant 1956; Lifschytz and Falk 1969b). When homozygous pn females are crossed to homozygous K-pn males, all of the pn K-pn/+ progeny die during the late second to third instar period of larval development. No other eye-color mutation tested in combination with K-pn has exhibited this phenomenon.

Killer of prune is an allele of the cell autonomous lethal mutation abnormal wing discs (hence, K-pn is known as awd-pn), which is located on the right tip of the third chromosome at 100C-D (Biggs et al. 1988; Dearolf et al. 1988b). Various abnormalities have
been detected in awd homozygotes during the extended third larval instar stage of their development, which terminates in prepupal death (Dearolf, Hesperger and Shearn 1988a). These mutants have aberrations in the larval brain, proventriculus as well as ovaries, while improper differentiation and cell death occurs to varying degrees in the wing, leg and eye imaginal discs. The awd* locus codes for a 17-kD polypeptide that appears to be a nucleoside diphosphate kinase (Biggs et al. 1990; Wallet et al. 1990), an enzyme that facilitates the interconversion of NDPs and NTPs. Interestingly, the deduced amino acid sequence of the Awd product is 78% identical to Nm23, a protein that suppresses metastasis in mammalian tumor cells (Rosengard et al. 1989; Biggs et al. 1990).

A comparison of nucleotide sequences has shown that Killer of prune is a missense mutation of awd that changes the proline residue at position 97 to serine (A. Shearn, personal communication). This single amino acid substitution results in the neomorphic lethal activity of awdK-pn in combination with pn. The nature of this lethality, however, is still unclear. Further insight into the interaction between pn and awdK-pn, as well as the role of pn in the biosynthesis or metabolism of pteridines, may be achieved by elucidating the function of the pn+ product. As a step toward this end, we have initiated a molecular analysis of pn. In this paper, we report the isolation of the genomic fragments corresponding to the pn locus, along with the analysis of transcripts and cDNAs within this region.

**MATERIALS AND METHODS**

**Materials:** Bluescript KSII phagemid was purchased from Stratagene. DH5αF+ Escherichia coli hosts were obtained from Bethesda Research Laboratories. nylon-66 membrane, was purchased from Schleicher and Schuell. Oligo-dT cellulose type 7 resin was obtained from Schuell. Oligo-dT cellulose type 7 resin was obtained from Schuell. Oligo-dT cellulose type 7 resin was obtained from Schuell.

**Fly genomic libraries:** The preparation of genomic DNA from flies was performed according to the procedure described by Levis, Bingham and Rubin (1982). The genomic DNA of pn38 bw st was digested with BamHI and the resulting fragments were cloned into the BamHI site of the λ phage replacement vector, EMBL3 (Frischauf et al. 1983). The wild-type genomic library was made from a partial MboI digest (Tsujimoto et al. 1989). The libraries were subsequently screened by the plaque hybridization method (Maniatis, Frisch and Sambrook 1982).

**Southern analysis:** To prepare genomic DNA, 100 mg of flies were homogenized in 1 ml of homogenization buffer (60 mM NaCl, 5% sucrose, 0.15 mM spermine, 0.15 mM spermidine, 10 mM Tris, 10 mM EDTA, pH 7.5). An equal volume of freshly made Proteinase K buffer (30 mM EDTA, 2% SDS, 200 μg/ml Proteinase K, 0.2 M Tris, pH 9.0) was mixed gently with the homogenate and incubated at 37°C for 1 hr. The suspension was extracted twice with 2 ml of phenol followed by an extraction with 2 ml of chloroform. One twentieth the volume of 4 M NaCl and two volumes of 100% ethanol were added to the aqueous phase retrieved, mixed and placed at -20°C for more than 3 hr. The DNA was pelleted at 12,000 × g, 4°C and washed with 70% ethanol. The DNA was then dried in vacuo, resuspended in 150 μl

<table>
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<tr>
<th>Strain</th>
<th>Phenotype</th>
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<tr>
<td>Canton-S</td>
<td>+</td>
<td>M</td>
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<tr>
<td>Oregon-R</td>
<td>+</td>
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<tr>
<td>4A/(2)A(C1)DX, yP</td>
<td>+</td>
<td>M</td>
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<tr>
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<td>pn86 bw st</td>
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<td>pn6(1)C1)DX, yP</td>
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<td>pn8+/(1)DX, yP</td>
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<td>pn8(1)C1)DX, yP</td>
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<td>pn7</td>
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<td>M</td>
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<td>pn58</td>
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In the C1)DX, yP lines, only males were used for the purification of DNA or RNA. Since the remaining lines were maintained as homozygous stocks, both males and females were used. The symbol + denotes wild-type eye color; - indicates the absence of a mutation at the pn locus.

**General procedures:** Standard molecular cloning techniques were performed according the procedures described by Maniatis, Frisch and Sambrook (1982). Genomic and cDNA fragments were subcloned into Bluescript KSII vector. DNA probes were made by labeling gel-purified fragments with [α-32P]dCTP or [α-32P]UTP to a specific activity of 5 × 10^7–10^8 cpm/μg by a nick translation reaction (Rigby et al. 1977). Single-stranded RNA probes, labeled with [α-32P]UTP, were made by the in vitro transcription of linearized plasmids with T3 or T7 RNA polymerase according to Melton et al. (1984).

**Chromosomal in situ hybridization:** Larval salivary gland chromosome preparations and hybridization of [α-32P]dCTP labeled DNA to the chromosomes were performed according to the method described by Banerjee et al. (1987) with the following modification: after hybridization, the slides were extensively washed in Formamide Wash (50% formamide, 0.6 M NaCl, 1 mM EDTA, 10 mM Tris, pH 7.5) at 37°C for 16–18 hr.

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RESULTS

P transposon tagging of the pn locus: A pn mutation, pn38 bw st, isolated from a dysgenic cross (ROBERTSON et al. 1988; refer to MATERIALS AND METHODS) was investigated to determine if the lesion was caused by a P element insertion. In situ hybridization of pr25.1 (O’HARE and RUBIN 1983) probe, which contains a complete 2.9-kilobase (kb) P element, to pn38 bw st larval salivary gland chromosomes showed the presence of three transposons in the X chromosome (data not shown). One of the sites of insertion corresponded to the 2E1–2 cytological location of pn (PERRIMON, ENGSTROM and MAHOWALD 1984), suggesting that a P element was present within or nearby the pn locus. In order to determine whether the pn mutation of pn38 bw st was caused by the insertion of a transposon, a reversion analysis was performed. The defective P elements in females of M' cytotype pn38 bw st were remobilized in a dysgenic cross with P cytotype 4A/ (2)A male flies (Figure 1). In the remobilization screen, nine red-eyed flies were obtained out of the 11,600 F2 males scored, thus corresponding to a recovery of revertants at an average frequency of 7.8 × 10^{-4} (Table 2). This frequency of reversion suggested that the lesion in pn38 bw st was an insertional mutation.

Cloning of pn: A genomic library of pn38 bw st was screened with pr25.1 probe. Two of the positive clones obtained, 38PN.13 and 38PN.15, hybridized with the latter had a 0.7-kb insert with identical restriction patterns. The insert of 38PN.15 was used to obtain homologous clones from a wild-type genomic library and four of the overlapping clones isolated were subjected to restriction analysis (Figure 3). A comparison of the restriction maps of the wild-type clones with 38PN.15 showed that the latter had a 0.7-kb P element inserted into a 1.7-kb EcoRI-XhoI fragment in this region. Since the size of a complete P element is 2.9 kb (O’HARE and RUBIN 1983), the transposon in 38PN.15 is incomplete. This is consistent with the fact that the M' cytotype Birm2 Mutator parent used to generate pn38 bw st contains defective P elements (ROBERTSON et al. 1988).

<table>
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<tr>
<td>pn38 bw st</td>
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Seven revertant lines were established since two of the nine red-eyed F2 males obtained were sterile.

Northern analyses: Genomic DNA isolated from flies of wild-type, pn and revertant strains was probed with 7.1-kb BamHI-Sall fragment A (Figure 3) and the results are shown in Figure 4A. As expected, Canton-S (lane 2) showed a 7.1-kb hybridization band while that of pn38 bw st was 7.8 kb (lane 4), reflecting the presence of a 0.7-kb transposon insertion. A comparison of four revertant lines (lanes 5–8) with their parent, pn38 bw st, showed that their phenotypic reversion of pn corresponded with the loss of the complete P element from this region. Although only four of the revertant lines are shown in this analysis, the

FIGURE 1.—Remobilization of the P elements of pn38 bw st. Hybrid dysgenesis was induced in a cross between P cytotype 4A/ (2)A males and M' cytotype pn38 bw st females. In the F1 stage, 3–4 males were mated to 4–5 G(1)DX,yfP virgin females in vials. Each of the red-eyed F2 males obtained were mated to virgin G(1)DX,yfP females to establish a revertant line. Since 4A/(2)A/C(I)DX,$' flies have 30–50 transposons per genome and M' pn38 bw st has only three, the revertant lines generated have many transposons per genome, similar to their P cytotype progenitors.

The gel electrophoresis and RNA transfer onto nylon Southern hybridization was done according to the procedure described by MANDEL, RIVIN and WALBOT (1986). Fly heads were isolated from bodies according to the method described by OLIVER and PHILLIPS (1970). Total RNA was purified from fly tissues according to the procedure described by LEMEUR et al. (1981) and resuspended in binding buffer (0.4 M NaCI, 0.5% SDS, 5 mM EDTA, 10 mM Tris, pH 7.5). A final concentration of 2–3 mg/ml Poly(A)+ RNA was bound to oligo-dT cellulose resin (preequilibrated in binding buffer), washed with binding buffer without SDS, and eluted with 10 mM Tris, 5 mM EDTA, pH 7.5. Subsequently, one-tenth the volume of 3 M NaOAc, pH 7.0, and 2.5 volumes of 100% ethanol were added, mixed and kept at -20° overnight. The poly(A)+ RNA was pelleted at 11,000 g for 10 min at 4°, washed with 100% ethanol, dried in vacuo and resuspended in 50% formamide. The concentration of the RNA was determined by measuring the absorbance at 260 nm and it was used in northern analyses.

The gel electrophoresis and RNA transfer onto nylon were done according to the procedure described by MANIATIS, FRISCH and SAMBROOK (1982) with the following modifications: (i) the 10 × gel-running buffer was 100 mM MOPS, 50 mM NaOAc, 10 mM EDTA, pH 8.0; and (ii) after the electrophoresis was completed, the 1.4% formaldehyde-agarose gel was soaked in 20 × SSC for 40 min at room temperature, and the RNA was subsequently transferred onto nitrocellulose. The northern conditions were similar to those described for Southern with the following changes: DNA-RNA hybridizations were done at 50°, while the hybridization temperature was 60° when single-stranded RNA probes were used.

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remaining three lines have also been examined and showed a similar genotypic reversion to the hybridization pattern of wild-type DNA (data not shown). These results indicated that the insertion of the transposon in fragment \( \Delta \) was responsible for the \( pn \) lesion. Additional evidence substantiating this inference was the observation that the other \( P \) element-induced allele, \( pn^{w2} \) (lane 3), had a 0.5-kb insertion in \( \Delta \); its wild-type progenitor, 4A/(2)A \( P \) males (lane 1), showed a 7.1-kb band. Out of the five \( M \) cytotype alleles, \( pn^{1} \), \( pn^{2} \), and \( pn^{3c} \) exhibited large hybridization fragments between 14 and 20 kb. The remaining two alleles, \( pn^{7} \) and \( pn^{69} \), showed no detectable polymorphisms.

All of the restriction fragment length polymorphisms observed in fragment \( \Delta \) of \( pn \) flies were further localized to a 1.7-kb \( EcoRI \)-\( XhoI \) fragment \( B \) (Figures 3 and 4B). The presence of these molecular lesions within \( B \) indicated that a critical domain of the \( pn \) locus was located in this fragment. As a control, \( pr25.1 \) was utilized as a probe to confirm the cytotypes of the strains used in the analysis (Figure 4C). As expected, 4A/(2)A \( P \) males, \( pn^{w2} \), and the revertant lines showed multiple \( P \) elements with varying hybridization intensities, while \( pn^{58} \) \( bw \) \( st \) had three weak bands corresponding to its incomplete transposons.

**Detection of transcripts:** To identify the transcripts produced from the region within and around \( B \), poly(A)\(^+\) RNA purified from tissues of Oregon-R and \( pn \) adult flies was probed with the 4.0-kb \( XhoI \) fragment \( C \) (Figures 3 and 5A). The northern blot showed that this fragment recognized multiple messages, ranging from 1.3 to 9.5 kb in size. In comparison with Oregon-R, the three \( pn \) alleles had varying amounts of several novel mRNAs indicating that the lesions in \( pn^{58} \) \( bw \) \( st \), \( pn^{w2} \) and \( pn^{3} \) affected the expression of one or more transcription units within \( C \).

**Characterization of cDNAs:** The transcription units located in fragment \( C \) were defined by analyzing cDNAs produced from this region. This fragment was used to screen a Drosophila adult head cDNA expression library (Itoh et al. 1986) from which four clones were isolated. The homology of the cDNAs to each other, and to genomic DNA fragments, indicated that the cDNAs belonged to the following three classes of transcription units: (I) 1.75-kb TcD20, (II) 1.6-kb TcD37, and (III) 2.0-kb TcD12 and 1.8-kb TcD13 (Figure 6). When TcD12 and TcD13 were digested with \( SalI \) and \( XhoI \), the two clones had similar restriction patterns with the exception of the rightmost region near the \( SalI \) site, where TcD12 was longer by 200 base pairs (bp). The comparison of the nucleotide sequences of TcD12 and TcD37 revealed that the poly(A)\(^+\) tail ends of these two cDNAs overlapped by 93 bp and indicated that opposite strands of genomic DNA were used to transcribe their messages (our unpublished results). This suggested that the direction of transcription of TcD12 and TcD13 proceeded from right to left, while TcD37 was transcribed from left to right, as shown in Figure 6. Southern analysis indicated that TcD20 had no homology to the other three cDNAs, however, its nucleotide sequence will have to be determined to confirm this observation.
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Finer localization of pn mutations: The presence of two overlapping transcription units, II and III, in fragment B made it important to determine whether the restriction fragment length polymorphisms of pn38, pn2, pn' and pn54c fell specifically into one of these units, or were both affected. A comparison of the restriction maps of TcD37 and its P element-containing genomic homologue from pn38 bw st, THF-10XE, revealed that its transposon had inserted into transcription unit I1 (Figure 6). More specifically, the nucleotide sequences of TcD37 and THF-10XE showed that the P element was located in the open reading frame of the cDNA (our unpublished results).

To further localize the polymorphisms of the remaining pn mutants, TcD37 was used to probe fly genomic DNA that was double digested with EcoRI and HindIII (Figure 4D). As seen in lane 2, genomic DNA of wild-type Canton-S flies had the two expected 1.2- and 5.3-kb hybridization bands, however, the 50-bp HindIII fragment was not observed probably due to its relatively small size. While the 5.3-kb band was present in every strain tested, the aberrations of the five pn alleles of interest altered the 1.2-kb band (Figure 6). These results clearly showed that the mutations of pn2, pn', pn' and pn54c affected fragment D, in which most of transcription unit II is located.

Northern analyses using cDNA probes: To determine which transcripts corresponded to the isolated cDNAs, clones from the three transcription units were used to probe poly(A)+ RNA isolated from several strains of flies. A single-stranded RNA probe of TcD20 hybridized to several messages in the wild-type Oregon-R tissues (Figure 5B), indicating that its

FIGURE 4.—Localization of the pn locus by Southern analyses. Wild-type and pn genomic DNA were double digested with (A and C) BamHI and SalI, (B) EcoRI and XhoI, or (D) EcoRI and HindIII. The resulting fragments were separated by electrophoresis, transferred onto nitro, and subsequently probed with (A) fragment A, (B) fragment B, (C) pn25.1, or (D) TcD37. The genomic DNA in the lanes shown are from flies of the following strains: 1, 4A/(2)A P males; 2, Canton-S; 3, pn22; 4, pn38 bw st; 5, pn25.1 males; 6, pn25.1 males; 7, pn25.1 males; 8, pn25.1 males; 9, pn'; 10, pn2; 11, sc pn' g5 B2; 12, y pn' spl; and 13, pn'. In the case of (C), the genomic Drosophila sequences of the 17C region spanning the P element of pn25.1 recognized a 1.6-kb fragment.

FIGURE 5.—Detection of transcripts by northern analyses. A 5-µg aliquot of poly(A)+ RNA isolated from head or body tissues of flies was probed with (A) fragment C, (B) TcD20, (C) TcD12, or (D) TcD37. In the case of TcD20 and TcD37, single-stranded RNA probes were used. Lanes: 1, Oregon-R head; 2, Oregon-R body; 3, pn38 bw st body; 4, pn22 body; and 5, sc pn' g5 B2 body. In (D), the 1.65- and 1.8-kb wild-type messages recognized by TcD37 are arrowed, while the 2.35- and 2.2-kb aberrant messages of pn38 bw st and pn'2, respectively, are indicated with arrowheads. The insert next to the lower left corner of (D) shows a shorter exposure of the two wild-type messages in the Oregon-R head and body lanes of this Northern blot.
Identification of a putative \( pn \)\(^{+} \) transcript: A single-stranded probe of TcD37 (Figure 6), the cDNA from transcription unit II, hybridized to 1.65- and 1.8-kb mRNAs in head and body tissues of Oregon-R flies (Figure 5D). These wild-type messages were absent or severely reduced in the \( pn \) mutants. Additional faint hybridizations that were observed in the \( pn \) tissues reflected the expression of aberrant messages. The two \( P \) alleles of \( pn \) showed altered mRNAs of particular interest as \( pn^{38} \) \( bw \) \( st \) had a 2.35-kb transcript, while \( pn^{pw2} \) had a 2.2-kb transcript. The sizes of these messages correlated with the transcription of the 1.65-kb TcD37 mRNA along with the insertion in each mutant; \( pn^{38} \) \( bw \) \( st \) had a 0.7-kb \( P \) transposon, while \( pn^{pw2} \) had a 0.5-kb insertion.

In summary, Northern analyses showed that the fragment \( C \) probe recognized multiple transcripts. The isolated cDNAs that corresponded to this region fell into three classes of transcription units. Of the three cDNAs used, TcD37 was the only one that detected messages in wild-type Oregon-R tissues that were absent or substantially reduced in \( pn \) tissues suggesting that transcription unit II encodes a \( pn^{+} \) product.

DISCUSSION

We have presented evidence for the molecular isolation of the \( pn \) locus. The \( P \) element-induced allele \( pn^{38} \) \( bw \) \( st \) shows the presence of a \( P \) transposon in the 2E1-2 cytological location of \( pn \). Reversion analysis of \( pn^{38} \) \( bw \) \( st \) suggests that its lesion in the \( pn \) locus is caused by a transposon insertion. In a Southern analysis, \( pn^{38} \), \( pn^{pw2} \), \( pn^{1} \), \( pn^{3} \) and \( pn^{+} \) show restriction fragment length polymorphisms in genomic fragment \( D \) (Figure 3) when compared to wild-type Canton-S.

The seven independent revertant lines of \( pn^{38} \) \( bw \) \( st \) show that their phenotypic reversion of \( pn \) corresponds with a genotypic reversion of this fragment to the DNA hybridization pattern of wild-type flies.

The polymorphisms exhibited by the five \( pn \) alleles have been further localized to the 1.2-kb genomic fragment \( D \) (Figure 6). This indicates that this fragment contains a critical domain of \( pn \). However, the extent of the \( pn \) locus in the 22 kb of genomic DNA isolated has not been defined. Three transcription units have been identified within this genomic region. Among the three, transcription unit II is the only one that is a part of fragment \( D \). A comparison of nucleotide sequences shows that the \( P \) element of \( pn^{38} \) \( bw \) \( st \) is inserted into the structural gene of this second transcription unit (our unpublished results), to which TcD37 belongs. Of the three cDNAs that have been used to probe northern blots, TcD37 is the only one that detects messages in wild-type Oregon-R tissues that are absent in \( pn^{38} \), \( pn^{pw2} \) and \( pn^{1} \) mutants. These results suggest that transcription unit II defines a part
of the pn locus and TcD37 encodes a pn+ product.

The Northern analyses of transcription units I and III reveal that although the wild-type mRNAs recognized by their cDNAs appear to be present in the pn38, pn-pw2 and pn3, altered messages are also observed. These results suggest that the lesions in these pn alleles have some effect on the expression of these two transcription units. Considering that the observed polymorphisms in the three pn mutants reside within genomic fragment D, it is interesting that the production of some of the messages from the flanking transcription units I and III might have changed. One possible explanation is that the isolated cDNAs that have been analyzed may be incomplete. Another possibility is that there may be other cDNAs from this region, including those corresponding to alternatively spliced messages, that have not been identified yet. This initial molecular characterization may therefore only reveal a part of the overall complexity of this region.

We interpret the data obtained so far in at least two ways. One possibility is that pn is a simple locus comprised of transcription unit II, therefore, TcD37 defines its structural gene. Alternatively, the pn locus may be complex and a critical domain of it is in fragment D. A rigorous molecular dissection of this region, along with gynandromorph transformation rescue experiments, will be necessary to define the extent of the pn locus and determine which cDNA(s) codes for the pn+ product(s).

Nature of the pn locus: The specificity and effectiveness of the pn-awdK-pn interaction have provided geneticists with a highly sensitive and selective system in which one can screen for rare mutational and recombinational events. In two separate studies in which over 105 embryos were examined, intragenic recombination was not detected between various pn mutations (Lifschytz and Falk 1969a; Orevi and Falk 1975), including several temperature-sensitive alleles that were generated with ethyl methylkülfonate. Moreover, to date, every allele of pn with an altered eye-color that has been tested, including pn38 bw st and pn-pw2 strains used in our analyses, is lethal in combination with awdK-pn. The only exceptions to this are the nine temperature-sensitive pn-awdK alleles isolated by Orevi and Falk (1975). At the restrictive temperature, the color of the compound eyes of these conditional mutants is light brown and they are insensitive to awdK-pn. However, the authors surmised that these pn-awdK mutants were hypomorphs and that their nonlethal combination with awdK-pn was due to a partial loss of the pn+ function rather than a localized lesion in an eye color-specific genetic domain. This implies that only pn alleles having a severe loss of activity of the pn+ product are affected by awdK-pn. Thus, the results of extensive genetic studies suggest that the pn locus is simple, and all characterized mutations of pn apparently affect the same single cistron (Lifschytz and Falk 1969a).

The interpretation that TcD37 defines the structural gene of pn is consistent with the finding that its locus appears to be genetically simple. If this is correct, then the P element insertion of pn38 bw st into the open reading frame of TcD37 should result in the production of a nonfunctional Pn protein. This inference is substantiated by the apparent lack of the wild-type messages corresponding to TcD37 in pn38 bw st mutants, therefore suggesting that pn38 is a null mutation of pn. Two other alleles, pn-pw2 and pn3, also show the absence or substantial reduction of the normal messages recognized by TcD37, suggesting that these are severe hypomorphic or null mutations as well.

Tissue distribution of the pn products: Imaginal disc transplantation experiments have shown that pn is required autonomously in the eye disc for the normal production of pteridine pigments (Beadle and Ephrussi 1936). Reciprocal temperature shift studies using pn+ alleles have revealed that the pn+ function was needed during the last tenth of pupal life for pteridine biosynthesis (Orevi and Falk 1975), while the lethal interaction between pn and awdK-pn has been shown to extend from the first larval instar period of development to eclosion (Orevi and Falk 1974). As the requirement of pn for pigment formation in the eyes occurs much later than the initial period of its lethal interaction with awdK-pn, it is plausible that the death of pn awdK-pn mutants is not necessarily the consequence of an eye disc or eye tissue-specific interaction. Indeed, gynandromorph mosaic experiments have suggested that the focus of pn awdK-pn lethality is in a region from which the mesodermal structures and nervous system develop (Orevi and Falk 1974). These tissues are present in both head and body regions of larvae and subsequently formed imagos. Thus, it is not unexpected to find the putative pn transcripts in the head and body tissues of adult flies.

The information available on pn and awdK-pn has generated intriguing questions. How does pn regulate the activity of GTP cyclohydrolase, and thereby control pteridine production? What is the neomorphic function of awdK-pn in combination with pn that causes the death of these double mutants? When the complementary lethal interaction between pn and awdK-pn was first discovered, Sturtevant proposed a simple hypothesis: pn mutants produce or accumulate some substance that is absent, or present in only small amounts, in pn+ flies, and awdK-pn converts this substance into a toxic product. Since a molecular handle is now available on the products of the pn and awdK-pn loci, it will be possible to test this and other possible hypotheses,
LITERATURE CITED


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