Structure and Expression of Wild-Type and Suppressible Alleles of the Drosophila *purple* Gene

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Manuscript received October 4, 1995
Accepted for publication December 28, 1995

ABSTRACT

Viable mutant alleles of *purple* (*pr*), such as pr^{bw}, exhibit mutant eye colors. This reflects low 6-pyruvoyl tetrahydropterin (PTP) synthase activity required for pigment synthesis. PTP synthase is also required for synthesis of the enzyme cofactor biopterin; presumably this is why some *pr* alleles are lethal. The pr^{bw} eye color phenotype is suppressed by *suppressor of sable* [*su(s)*] mutations. The *pr* gene was cloned to explore the mechanism of this suppression. *pr* produces two PTP synthase mRNAs: one constitutively from a distal promoter and one in late pupae and young adult heads from a proximal promoter. The latter presumably supports eye pigment synthesis. The pr^{bw} allele has a *412* retrotransposon in an intron spliced from both mRNAs. However, the head-specific mRNA is reduced >10-fold in pr^{bw} and is restored by a *su(s)* mutation, while the constitutive transcript is barely affected. The Su(s) protein probably alters processing of RNA containing *412*. Because the intron containing *412* is the first in the head-specific mRNA and the second in the constitutive mRNA, binding of splicing machinery to nascent transcripts before the *412* insertion is transcribed may preclude the effects of Su(s) protein.

THE first *purple* mutation (pr^1) was isolated more than 75 years ago (BRIDGES 1919). pr^1 is a leaky spontaneous mutation that results in a purplish-ruby eye color phenotype as a consequence of low pteridine accessory pigment levels (HADORN and MITCHELL 1951; WILSON and JACOBSON 1977). The reduced pteridine pigment levels correlate with reduced levels of 6-pyruvoyl tetrahydropterin synthase (PTP synthase, originally called sepiapterin synthase A) in the heads of mutant flies (YIM *et al.* 1977; DORSETT *et al.* 1979). PTP synthase catalyzes conversion of dihydroneopterin triphosphate to PTP (SWITCHENKO and BROWN 1985), a key intermediate in the synthesis of pteridine pigments, and biopterin, an essential enzyme cofactor. PTP synthase has been purified from several organisms including Drosophila (PARK *et al.* 1990). The active form of the Drosophila enzyme has the same molecular weight as determined by gel filtration (83 kD) as the human and rat enzymes (TAKIKAWA *et al.* 1986; INOUE *et al.* 1991), although the K_m for dihydroneopterin triphosphate of the Drosophila enzyme (100 μM) is significantly higher than that of the human enzyme (10 μM).

In Drosophila, PTP synthase activity increases with *pr* gene dosage suggesting that *pr* encodes PTP synthase (YIM *et al.* 1977). Furthermore, two spontaneous *pr* alleles, pr^1 and pr^{bw}, are suppressed by mutations in *suppressor of sable* [*su(s)*] and *suppressor of purple* [*su(pr)*], and suppressed pr^{bw} mutants display a corresponding increase in the PTP synthase activity present in the heads of young adults (YIM *et al.* 1977; DORSETT *et al.* 1979). It has become evident that most mutations that are suppressed or enhanced by allele-specific modifiers such as *su(s)* and *su(pr)* are transposon insertions (RUTLEDGE *et al.* 1988). For example, *su(s)* mutations enhance several *gypsy* retrotransposon insertions in different genes (RUTLEDGE *et al.* 1988) and suppress a *412*-retrotransposon insertion in *vermilion* (v^1) (SEARLES and VOELKER 1986) and a *P*-element insertion in *yellow* (y^{76d28}) (GEYER *et al.* 1991).

The *su(s)* gene encodes a protein containing motifs found in RNA-binding proteins (VOELKER *et al.* 1991). In v^1 the *412* insertion is antiparallel and in the first exon upstream of the initiator codon, resulting in loss of the wild-type transcript (SEARLES *et al.* 1990). However, v^1 produces trace amounts of a wild type-size transcript that results from splicing out of most of the *412* element using cryptic splice sites near the ends of the *412* long terminal repeats (LTRs), and the level of this transcript is increased fivefold by *su(s)* mutations (FRIDELL *et al.* 1990; PRET and SEARLES 1991). In constructs containing a single *412* LTR, *su(s)* mutations increase the levels of both the spliced and unspliced RNAs, and substituting a consensus 5′ splice site for the cryptic donor mimics these effects (FRIDELL and SEARLES

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1994). Similarly, in y^{76d28}, an antiparallel *P* near the 5′ end of the transcript is spliced out at low efficiency using a variety of cryptic splice sites and the levels of both these and unspliced transcripts are increased by *su(s)* mutations (GEYER *et al.* 1991). It has been proposed that *su(s)* mutations stabilize the mutant precursor RNAs by increasing recognition of cryptic splice sites by the splicing apparatus without increasing splicing efficiency (FRIDELL and SEARLES 1994).

To explore further the mechanism of suppression by *su(s)* mutations, we undertook to isolate the *purple* gene. In this report we describe the structure and expression of the PTP synthase gene and provide compelling evidence that it is *pr*. The *pr* gene has two promoters that produce overlapping transcripts that encode the same 19-kD PTP synthase monomer similar in size and sequence to the rat and human PTP synthase monomers. The distal promoter produces a transcript present throughout development at low to moderate levels and the proximal promoter produces a transcript present at high levels in late pupae and the heads of young adults. We posit that the constitutive transcript supports enzyme cofactor synthesis and that the head-specific transcript is for eye pigment synthesis. The suppressible pr^1 and pr^{bw} alleles, which display only an eye color phenotype, have identical antiparallel *412* insertions and polymorphisms in an intron common to both the head-specific and constitutive transcripts, suggesting that they may be the same mutation. Accumulation of the head-specific transcript is reduced >10-fold by the *412* insertion and is partially restored by *su(s)* mutations, while the constitutive transcript is only slightly affected. We propose that the *412* insertion and wild-type *su(s)* destabilize the head-specific mRNA precursor, possibly by reducing binding of the splicing machinery to nascent transcripts. Because the intron with the *412* insertion is the second intron in the constitutive mRNA precursor, we also suggest that the splicing apparatus may have increased opportunity to recognize and stabilize the nascent transcript before the *412* sequences are transcribed.

MATERIALS AND METHODS

Drosophila culture: Flies were cultured at 25° on cornmeal-molasses-yeast media (WIRTZ and SEMEY 1982) or for isolation of RNA, on instant Drosophila food (Carolina Biological).

38BC chromosome walk: A chromosomal walk to locate *pr* was initiated from the tAP-19 *P* element insertion (provided by JAMES POSAKONY) at the boundary of 38BC. Genomic DNA was isolated from tAP-19 adults as described elsewhere (LEVIS *et al.* 1982), and the insertion site was cloned by inverse PCR (SAIKI *et al.* 1988). tAP-19 genomic DNA was restricted with *Xho*I and religated, and a 0.8-kb fragment containing the insertion site was amplified using oligonucleotide primers hybridizing to *P*-element sequences [5′-ATTAGGATCCGTGACTGTGCGTTAGGTCCTGTT-3′ (containing *P* nucleotides 5′-485–508-3′; 5-ATTAGAATTCTAGGTACGGCATCTGCGTTGAG-3′ (containing *P* nucleotides 5′-376–354-3′)]. The amplified fragment was subcloned into pGEM-1 (Promega Biotec) using the *Bam*HI and *Eco*RI sites in the primers. By *in situ* hybridization to salivary gland polytene chromosomes as described elsewhere (ENGELS *et al.* 1986), the tAP-19 insertion site was found to hybridize to 38B6 in the 38B3–C1 region to which *purple* has been localized (BRITTNACHER and GANETZKY 1983).

The tAP-19 insertion site was used to probe an Oregon R genomic library in λDASH (provided by JOSEPH JACK). The ends of the isolated phage inserts were used to probe the library and initiate a chromosomal walk (BENDER *et al.* 1983) in both directions from the tAP-19 insertion site that eventually encompassed a total of 120 kb (Figure 1). Radioactive probes were synthesized using [α-^{32}P]dATP and random priming.

Isolation and characterization of PTP synthase genomic clones: Selected phages covering the entire 38BC chromosomal walk were examined for PTP synthase coding sequences using PCR primers [5′-GTGGATCCCACAATGTTGTTGTCAG-3′ (antisense); 5′-GCGAATTCCTTGATCACAAGAACCT-3′ (sense)] directed against a C terminal region conserved in the human (THONY *et al.* 1992; ASHIDA *et al.* 1993), rat (INOUE *et al.* 1991) and salmon PTP synthase protein sequences (HAUER *et al.* 1992). One phage (λAP3.13) gave an amplification product (1.1 kb) that was subcloned into pGEM-1 using the *BamH*I and *EcoR*I sites in the primers, and sequenced using *Sequenase* v2 (U.S. Biochemicals) according the manufacturer's recommendations. Genomic DNA (2.7 kb) containing the amplified region was sequenced after subcloning appropriate restriction fragments of the λAP3.13 phage insert into pGEM-1. Both strands were sequenced.

Isolation and characterization of PTP synthase cDNA clones: The PTP synthase gene fragment isolated by PCR amplification as described above was used to probe a Drosophila head cDNA library in λgt11 (provided by PAUL SALVATERRA, Beckman City of Hope). Two identical cDNA clones were isolated and sequenced as described above for the genomic clones after subcloning into pGEM-1.

Preparation of RNA and characterization of PTP synthase transcripts: Isolation of total RNA from organisms of the indicated genotypes and developmental stages, and Northern blot hybridization analysis were conducted as described elsewhere (DORSETT *et al.* 1989). The 5′ ends of the PTP synthase mRNAs were determined by primer extension (MANIATIS *et al.* 1982) using total RNA from 0–2-day-old adults. The most distal primer used was ps74B (5′-ACTTTACGAGTGCGGCTA-3′). The ps74B products were amplified by a RACE protocol (FROHMAN *et al.* 1988) using the 5′AmpliFinder kit (Clontech) and sequenced. The other primers used were ps459B (5′-GTGGATCCCACGGACGGTTATCTC-3′, the first six nucleotides are not homologous to the cDNA) and psPRB (5′-GACGATGGCAGGCGC-3′).

To examine the intron-exon structure of PTP synthase mRNAs in the coding region, RT-PCR experiments were performed as described elsewhere (KIM *et al.* 1992) using the sense ps293E (5′-GCGAATTCATGTCGCAGCAACCTGT-3′) and antisense p876S (5′-AAGGCCTTAAGAGGTTGGCTGAGT-3′) primers to amplify the randomly primed reverse transcription products. The RT-PCR products were cloned into pGEM-1 for sequencing.

Characterization of the pr^1 and pr^{bw} alleles: Genomic DNA was isolated from pr^1 and pr^{bw} adults as described above and used to construct libraries in the EMBL3 λ vector (Promega) after partial digestion with *Sau*3AI. The libraries were screened using wild-type PTP synthase probes to isolate phage containing the PTP synthase gene (λpr^1G-2, λpr^{bw}G-2). The phage inserts were characterized by restriction digestion and Southern blot hybridization using the PTP synthase cDNA clone and *412* as probes. In both cases this revealed the pres-

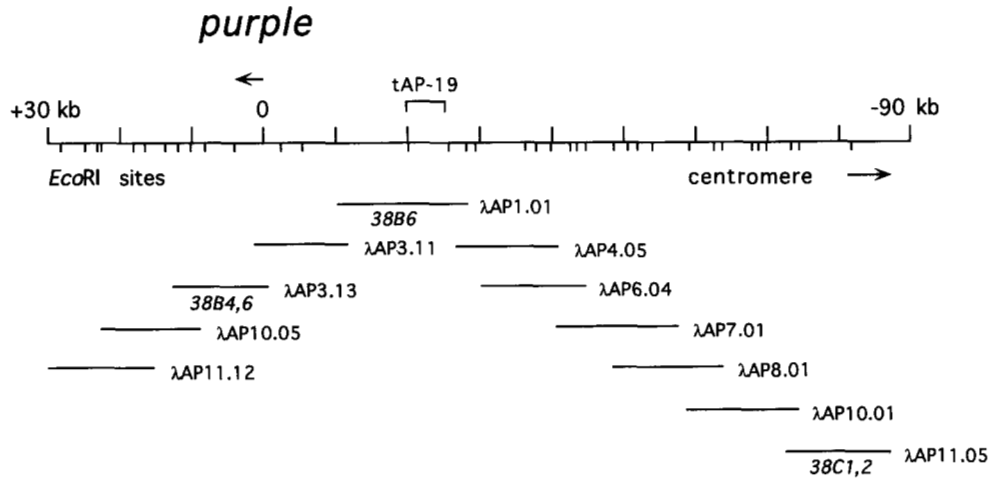

FIGURE 1.—Chromosome walk in the 38BC region. Shown is the *Eco*RI restriction map of the 120-kb region cloned in overlapping phage inserts. The walk is numbered in kb relative to the start of transcription of the *purple* gene. The walk was started with the tAP-19 *P* element at approximately −20 to −25. The individual phage insertions are shown with their names and, where tested, the cytogenetic positions to which they hybridize is indicated underneath.

ence of antiparallel *412* insertions within the same intron. To determine the exact insertion sites in both alleles, restriction fragments containing the *412*-PTP synthase gene junctions were subcloned into pGEM-1 and sequenced.

RESULTS

38BC chromosome walk: We cloned the *purple* gene to determine if it encodes PTP synthase and to explore the mechanism of suppression of the pr^1 and pr^{bw} alleles by *su(s)* mutations. Cytogenetically *purple* is in 38B3,6-38C1 (BRITTNACHER and GANETZKY 1983). On the basis of electron microscopy, 38B3–38B6 is estimated to contain ~40 kbp, and 38C1 is estimated to contain slightly >30 kbp (SORSA 1988). Therefore it was reasoned that *purple* must be within 70 kbp of the tAP-19 *P*-element insertion (obtained from JAMES POSAKONY, University of California, San Diego) determined to be at the 38BC boundary. The genomic sequences flanking the tAP-19 insertion were amplified by inverse PCR and were found to hybridize to 38B6. The cloned insertion site sequences were then used to clone the λAP1.01 phage (Figure 1) from an Oregon R genomic library. Both ends of the λAP1.01 insert were used as probes to initiate a chromosomal walk that eventually spanned 120 kbp (Figure 1).

Identification of the PTP synthase gene: The initial strategy was to locate *pr* in the 38BC chromosome walk using restriction fragment length polymorphisms associated with the $ln(2L)pr^2$ or $ln(2L)pr^{40}$ breakpoints. However, the lack of the parental strains for the inversion alleles and the presence of repetitive DNA stretches made this impractical. As an alternative approach, based on the hypothesis that *pr* encodes PTP synthase, the phage inserts were scanned for PTP synthase encoding sequences by PCR using primers designed to detect a region highly conserved in the human (THONY *et al.* 1992; ASHIDA *et al.* 1993), rat (INOUE *et al.* 1991), and salmon (HAUER *et al.* 1992) PTP synthase sequences. Only one phage, λAP3.13 (Figure 1) produced an amplification product. The amplification product was 1.1 kb, larger than the 0.15 kb expected if there were no introns between the 5′ and 3′ primers. The amplified region was cloned and sequenced and found to encode a peptide containing the expected portion plus the entire carboxy terminus of PTP synthase, indicating that the amplified fragment was larger than expected because the 3′ primer had actually hybridized downstream of the PTP synthase open reading frame. In retrospect this was not surprising because the region the 5′ primer was directed against (amino acids 88–93 in the human protein sequence; Figure 2) is completely conserved in the Drosophila protein, while the region the 3′ primer was directed against (amino acids 135–140 in the human sequence; Figure 2) is only half conserved in the Drosophila protein. Indeed, based on the DNA sequence, the 3′ end of the 3′ primer cannot hybridize to the Drosophila sequence in the appropriate position within the open reading frame.

The PCR-generated fragment was used to probe a head-specific cDNA library. Two identical clones were isolated containing a complete open reading frame and a poly(A) stretch at the 3′ end with a potential 5-AAUAAA-3′ polyadenylation signal 26 bp upstream. The encoded protein sequence closely matches the complete PTP protein sequences from rat (INOUE *et al.* 1991) and human (THONY *et al.* 1992; ASHIDA *et al.* 1993), and the partial salmon sequence (HAUER *et al.* 1992) (Figure 2). The predicted Drosophila sequence matches at least one of the available vertebrate sequences in 96 out of 168 residues (Figure 2). The conserved residues include a cysteine residue predicted to be part of the active site on the basis of biochemical (BURGISSER *et al.* 1994) and

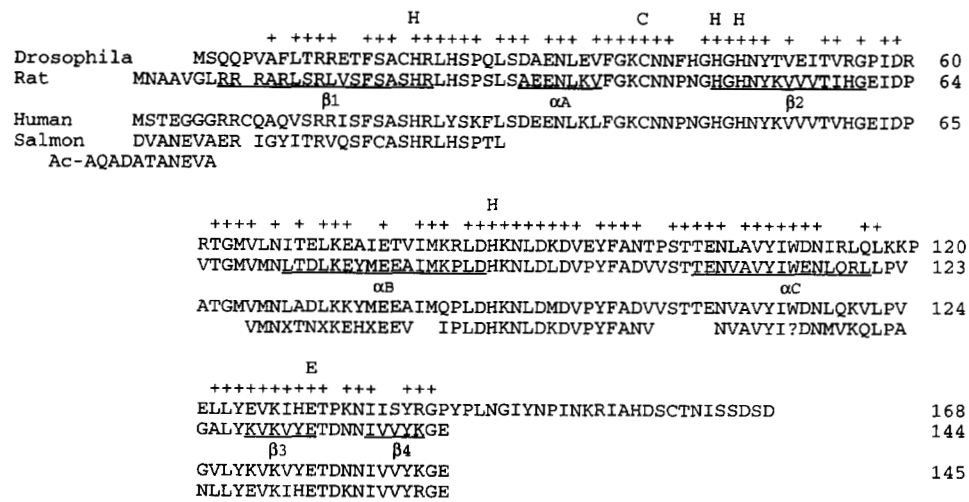

FIGURE 2.—Alignment of the predicted Drosophila PTP synthase protein sequence with the rat (INOUE *et al.* 1991), human (THONY *et al.* 1992; ASHIDA *et al.* 1993), and partial salmon (HAUER *et al.* 1992) sequences. Both of the *N* termini determined for the salmon protein are shown. Residues in the Drosophila sequence that match at least one of the vertebrate sequences are indicated with a +. The amino acid symbols above the sequence indicate putative active site residues (NAR *et al.* 1994). PTP synthase is active as a hexamer that forms an antiparallel β-barrel structure (NAR *et al.* 1994). The residues contained in the β-sheet and α-helical regions of the rat PTP synthase are underlined.

X-ray crystallography evidence (NAR *et al.* 1994) with mammalian enzymes, three histidine residues involved in coordinating a transition metal ion in the active site (NAR *et al.* 1994) and two other residues that the crystal structure suggests are involved in catalysis (NAR *et al.* 1994) (Figure 2).

The sizes of the Drosophila and mammalian PTP synthase subunits are very similar (~19 kD). Although the molecular weights of the active forms of the Drosophila, rat and human enzymes are all 83 kD as determined by gel filtration (TAKIKAWA *et al.* 1986; PARK *et al.* 1990; INOUE *et al.* 1991), the human (BURGISSER *et al.* 1994) and rat (NAR *et al.* 1994) enzymes form hexamers. The major difference between the predicted Drosophila sequence from the mammalian sequences is that the Drosophila N terminus is a few amino acids shorter, and the C terminus is nearly 30 amino acids longer. The longer C terminus in the Drosophila protein should not interfere with the interactions between individual subunits needed to form hexamers because X-ray crystallography of the rat enzyme (NAR *et al.* 1994) indicates that the C terminus protrudes into the solvent.

When the Drosophila cDNA clone is expressed in *Escherichia coli,* it produces a protein of the expected molecular weight (~19 kD) with PTP synthase activity (PARK *et al.* 1995). The measured pI (6.4) of the protein purified from *E. coli* agrees with that calculated from the predicted protein sequence (6.8), although it is higher than that determined for the native enzyme purified from Drosophila (4.6; PARK *et al.* 1990), and the pI's determined for rat liver (4.8; INOUE *et al.* 1991) and human liver (4.6; HASLER and CURTIUS 1989) PTP synthase. The K_m for Drosophila enzyme expressed in *E. coli* (590 μM) is also higher than that determined for the enzyme purified from Drosophila (100 μM; PARK *et al.* 1990), but both are significantly higher than the K_m values of ~10 μM determined for both the rat and human liver enzymes (TAKIKAWA *et al.* 1986; INOUE *et al.* 1991). Like the human enzyme (BURGISSER *et al.* 1994), the Drosophila PTP synthase expressed in *E. coli* is sensitive to reagents that modify cysteine residues (PARK *et al.* 1995).

The pI and K_m differences between native Drosophila PTP synthase and that expressed in *E. coli* suggests that the native enzyme is modified *in vivo.* When produced in *E. coli,* the Drosophila enzyme has a few nonnative amino acids at the amino terminus and is also not subject to the *in vivo* amino terminal processing detected for the rat enzyme (INOUE *et al.* 1991). The discrepancy between the molecular weight determined for the native subunit by SDS-PAGE (~37 kD; PARK *et al.* 1990) and the predicted molecular weight (19 kD) further suggests that native Drosophila PTP synthase is modified. For example, phosphorylation would both increase the apparent molecular weight determined by SDS-PAGE and reduce the pI. Despite the differences between the native and expressed forms, however, it is clear that the cloned Drosophila cDNA encodes PTP synthase. The similarities in the sizes of both the subunit and active forms of the Drosophila, rat, and human enzymes further suggests that Drosophila PTP synthase functions as a hexamer. Because the Drosophila cDNA clone encoding PTP synthase hybridizes to 38B4,6 in salivary gland polytene chromosomes (Figure 3), it is also likely that, as predicted, the PTP synthase gene is *purple.*

Structure and expression of the PTP synthase gene in wild-type flies: The PTP synthase cDNA clone was

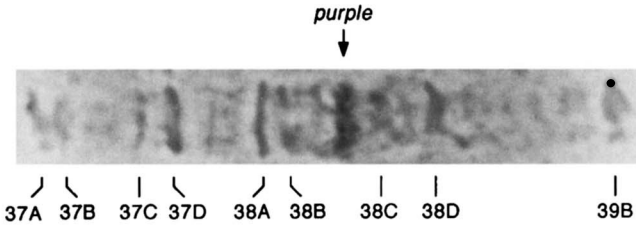

FIGURE 3.—*In situ* hybridization of the PTP synthase cDNA clone to salivary gland polytene chromosomes. Shown is a segment of chromosome 2L containing the only site of hybridization. Indicated below are the first bands in the lettered subdivisions. The arrow above points to the hybridization signal and the cytogenetic location of *pr*.

used to probe Northern blots, revealing the presence of at least two transcripts. A 1.3-kb transcript is present at low to moderate levels throughout development, and a 1.1-kb transcript is present at high levels in late pupae and young adults (Figure 4). In young wild-type adults, the 1.1-kb transcript was present only in the head, while the 1.3-kb transcript was found in the body (Figure 5, lanes 1 and 4). This suggests that the 1.1-kb transcript produces PTP synthase activity for synthesis of pteridine eye pigments, and that the 1.3-kb transcript supports synthesis of pteridine enzyme cofactors.

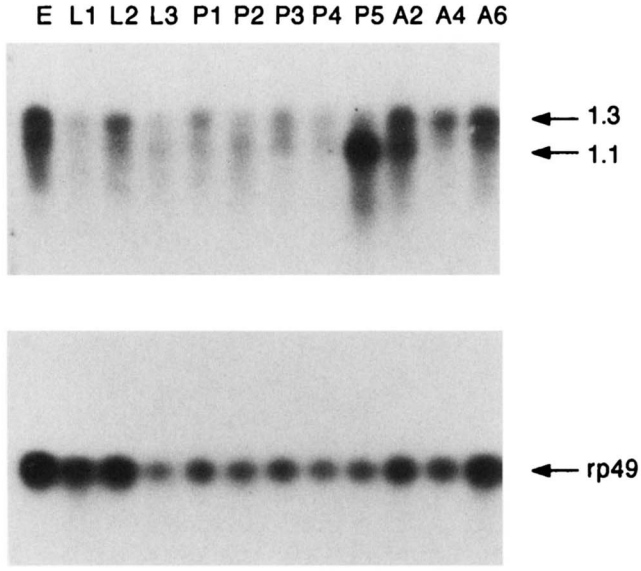

FIGURE 4.—Expression of PTP synthase gene transcripts in Oregon R wild-type flies at various developmental stages. (Top) An autoradiogram of a Northern blot hybridized to a radioactively labeled antisense RNA probe made using the PTP synthase cDNA clone as a template. (Bottom) The same blot hybridized to an rp49 gene (O'CONNELL and ROSBASH 1984) probe as a control for the amount of RNA. The sizes of the two transcripts detected by the PTP synthase probe as determined by comparison to standards (BRL) are indicated to the right of the top panel. The RNA samples were from the following: *E*, 0–24 hr embryos, L1, first instar larvae; L2, second instar larvae; L3, third instar larvae; P1, 0–1-day-old pupae; P2, 1–2 day pupae; P3, 2–3 day pupae, P4, 3–4 day pupae; P5, 4–5 day pupae; A2, 0–2 day adults; A4, 2–4 day adults; A6, 4–6 day adults.

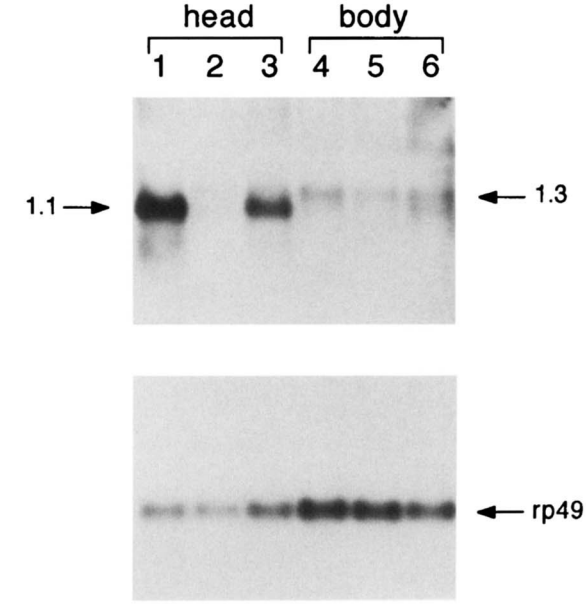

FIGURE 5.—Expression of PTP synthase RNA in the heads and bodies of 0–2-day-old wild-type and *pr* mutant adults. (Top) An autoradiogram of a Northern blot hybridized to a cDNA probe. (Bottom) The same blot hybridized to an rp49 control probe. Total RNA was isolated from heads and bodies of the following genotypes: lanes 1 and 4, Oregon R; lanes 2 and 5, pr^{bw}; lanes 3 and 6, $su(s)^2;pr^{bw}$. Although it appears that there may be transcripts smaller and larger than the 1.3-kb transcript in the $su(s)^2;pr^{bw}$ body (lane 6), these are artifacts apparently caused by the high background in this particular lane. Only the 1.3-kb transcript was detected in $su(s)^2;pr^{bw}$ bodies in separate experiments.

Several experiments were performed to determine the structures of the two PTP synthase transcripts. To help determine the exon-intron structure, 2.7 kb of the genomic region hybridizing to the cDNA clone was sequenced (Figure 6) and compared to the cDNA sequence. This revealed the presence of two introns in the coding sequence (Figures 6 and 7).

Primer extension experiments were performed using RNA isolated from 0–2-day-old adults to locate the transcription start sites. Three primers hybridizing to different positions in the cloned cDNA sequence were used (Figures 6 and 7). A primer directed against sequences near the 5′ end of the cDNA clone (ps74B; Figures 6 and 7) generates products ~170 nucleotides in length based on comparison to the migration of markers of known sizes in denaturing polyacrylamide gels. These products were amplified by a RACE protocol (FROHMAN *et al.* 1988) and sequenced, revealing a small intron upstream of the coding region (Figures 6 and 7).

Primer extension using a primer directed against cDNA sequences further downstream (ps459B; Figures 6 and 7) generates products ~240 nucleotides in size and additional products >500 nucleotides. A third primer between ps74B and ps459B (psPRB; Figures 6 and 7) gives products of ~130 and 450 nucleotides in

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TCTAGATTATTATTTAGGTATCTCTGCTCGTTTTTTAAATAACGCCGAAGAGTTTCGAATTATTGCGCGGCGTCGTTTGTTATTTTTTTTTCTAGTGAAG  100
GGCGGCGATCGATAGCAAAGCAGGGTTGATTACAGTGAGCGGCGTGAGGTCCGGCAACGCCACCACGGGCCAGGGATGAACAGCACTTACATACTCTGTT  200
ACGTAACTCTGTTAATCAGCTCATTTAAATTATGAGATAATTGACAAAGAACTAACATGAAATAAATAAATATATAGCGAAATTGTAATAGATGAAAGGA  300
TTTCTACCCATACAATAGTCTATTTGTGCAGTTGTAAATCACTGAGAACAGTTGTGGCGGGAAACTGCTCAGCTCTGGCCGATAACCGATACCGCTGCCT  400
                                                                                *P1

TGTTATCGGGAGCAGCTGTTGACGAGCAACGTAGACAAAAGTAAATATTATTGAAAATCCATAGCAATTAAAGgtgagttccttgctgaattgagtttgt  500
taccatggctatagcataccacaccctgtctttgcagCACGCATAGGCACTTGCAACAGAAAACGAGGGAACATCAATCGCTAATCAGCTGCAATTAGCC  600
GCACTCGAAAAGTTCAGAGAAACTAGTTCCGAGCGTGTTGATATCGCAGTGGCCGCATTTGTGGCTTATATTTCCTTGTTCCCTCGATCCAAGTACCATA  700
ps74B

TAAATAAGCGCGGCTCAAAAGCAGGTTTTGCATTCATTGTAAAACATTGCCAAAGCCGCGCAGCTGATCAGCCGCATATATAAATCCACATCCAGATACA  800
                                                                *P2
                                ps293E
GATCCAGATATATAGTATAAATCGGACTTGAA ATG TCG CAG CAA CCT GTT GCC TTT TTA ACC CGT CGC GAA ACT TTC AGC GCC  883
                                  M   S   Q   Q   P   V   A   F   L   T   R   R   E   T   F   S   A   17

TGC CAT CGT CTC CAT AG gtaattagattgaatcaaattgaatcattcaattgcctgattcaatcacggctggcaaaccaattcagttcgattta  977
 C   H   R   L   H   S                                                                                 23
psPRB

aattgattgctgcatctatttcctctccag T CCC CAA TTG AGC GAT GCC GAG AAT CTG GAA GTC TTC GGC AAG TGC AAC AAT 1059
      ^412                       P   Q   L   S   D   A   E   N   L   E   V   F   G   K   C   N   N   40

TTC CAC GGC CAC GGA CAC AAC TAT ACA G gtaaaaaaaaaatgccccttaaatacacttagtcgcatgattacctgcaaagtgccacactt 1149
 F   H   G   H   G   H   N   Y   T   V                                                                 50

attatcacataaccccggctcaatcaggcttaacccccactaaactggtaatttcagcccaaagtggtgcctaaccatagcaaaaccttggccgacaag 1249
ctcgcttatcaaacccatgttcaagttcaacgctcaaaggcaatcaatatgttgactgacgaccgagaattgataatgaataatggttctgaacgagcat 1349
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caaattataccaaaaaatgtgaatagactacgaaagatgttcttttataatcctttgtgatcttttcag TT GAG ATA ACC GTC CGT GGT CCC 1541
                                                                        E   I   T   V   R   G   P   57
                                                                            ps459B

ATC GAT CGG CGA ACC GGA ATG GTG CTA AAC ATC ACC GAG CTG AAA GAG GCT ATA GAA ACT GTG ATT ATG AAG CGC 1616
 I   D   R   R   T   G   M   V   L   N   I   T   E   L   K   E   A   I   E   T   V   I   M   K   R   82

CTG GAC CAC AAG AAT CTC GAT AAG GAT GTC GAA TAC TTT GCC AAT AC agtaaacaaccagcaagcacaacacaacttaccgtac 1700
 L   D   H   K   N   L   D   K   D   V   E   Y   F   A   N   T                                     98

ttatcctttatatctatcccccccca G CCA AGC ACC ACA GAA AAC TTG GCC GTT TAC ATC TGG GAC AAC ATC CGT CTG CAG 1780
                            P   S   T   T   E   N   L   A   V   Y   I   W   D   N   I   R   L   Q   116

CTG AAG AAG CCG GAG TTG CTG TAC GAG GTG AAG ATC CAT GAG ACC CCA AAG AAC ATT ATC AGC TAC CGC GGC CCG 1855
 L   K   K   P   E   L   L   Y   E   V   K   I   H   E   T   P   K   N   I   I   S   Y   R   G   P   141

TAT CCG CTC AAT GGC ATC TAC AAC CCC ATC AAC AAA CGC ATC GCT CAC GAT TCG TGC ACC AAC ATC TCG TCG GAT 1930
 Y   P   L   N   G   I   Y   N   P   I   N   K   R   I   A   H   D   S   C   T   N   I   S   S   D   166

TCG GAT TGAACATTCGAAGATTGTGACATCGCCTAACGAATTTTTTGATTGGACCAATGATCAGCGAAGACTCAGCCAACCTCTTAAGCACGCGCGTA 2028
 S   D                                                                                 ps876S        168

GGAAAGGACACTTACCATGTAAGGGAATGAAAAGTTATACGAATTACACAGTATGTAGGCGTAAAATATGTTAATGTAACATAATATTGAATAAAACAAA 2128
GGCAACTGAAATACTATTCTTTATAGCGATTAAACAAGAGAGCTTGCATTCCTAACACAGTACATTTGTCGACATTCGTTTAGCCTAAGTAAATCATACA 2228
TACTTCCTAAACTATCGCTCCATATGGCAGCTATACATAATTTGGTTTTCGCGCTGAGCAGGCGAGCTTTGTAGTTAATAAATAATTTAGTTGTTCCACC 2328
TTCTGCAACTCTAACTTATATCACGCTGTGTATGTCTCTGTTTGGAGAAGGCTCTCCACCCGATTTCTTGCCTTAGTTCATTCGGTGCTTGTAGTCATAG 2428
TCATAGTCTGCATGTACAAGCTGCAGTAGACGAGCCAATCTTTCGAATCTGTGGAATCAGCAAGGTAGCGGGTTGAATGATTTGGATTGCATAATACTTT 2528
GAGCAACGATCTCTACTTTGTTTAGTGTTTTAGTTATGGTTACTGCTACATGCTAGTAGATTTCCAATCCAGAATAAGTGTAGGTGTTAGTTGGTTAGTT 2628
TGCGTAAATTAGATAGCCATAAAACAATTAACAAATTCAGGTACTGACAACCTAATACGAA                                          2689
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FIGURE 6.—DNA sequence of the PTP synthase (*pr*) gene. Introns are in lowercase and encoded amino acids are indicated beneath the open reading frame. The two approximate cap sites (P1 and P2) are indicated by asterisks (*), and the site of the *412* insertions in pr^1 and pr^{bw} is indicated with an arrow. A polyadenylation signal starting 26 nucleotides upstream of the poly(A) tail in the cDNA clone is italicized and underlined. The sequences complementary to the primers (ps74B, psPRB, ps459B) used for primer extension experiments are also underlined. The sense (ps293E) and antisense (ps876S) primers used for RT-PCR experiments are overlined and underlined, respectively. The GenBank accession number for this sequence is U36232.

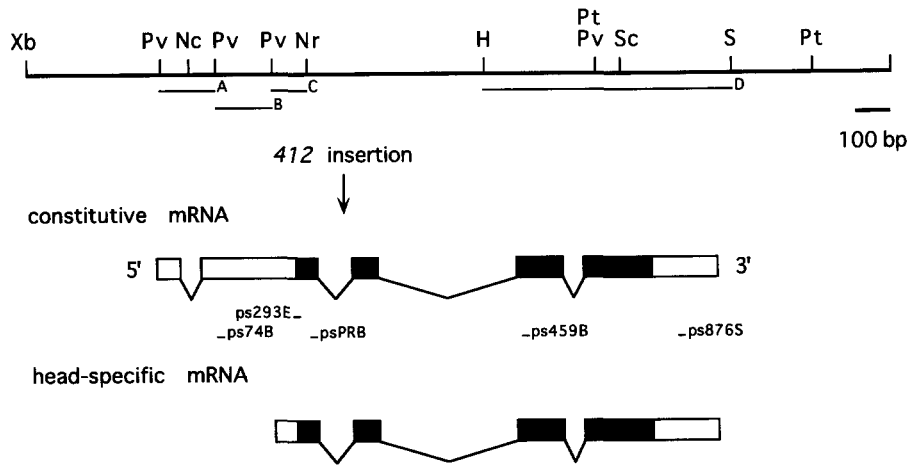

FIGURE 7.—Map of the PTP synthase transcripts. The two primary transcripts of the PTP synthase (*pr*) gene are shown beneath a restriction map of the genomic region that has been sequenced. Boxes indicate exons and filled regions indicate the open reading frame. The site of the *412* insertions in pr^{1} and pr^{hw} is indicated with an arrow. The genomic fragments (A–D) used as templates for synthesis of antisense RNA probes for Northern hybridization experiments are shown under the restriction map. The locations of the three antisense primers (ps74B, psPRB, ps459B) used in primer extension experiments, and the sense (ps293E) and antisense (ps876S) primers used for RT-PCR experiments are shown underneath the map of the constitutive transcript.

size. These experiments suggest that the PTP synthase gene contains two transcription start sites, at approximately nucleotides 379 and 764 in the genomic sequence (P1 and P2, Figure 6). The P1 start site represents the 170 nucleotide products obtained with primer ps744B, the 450 nucleotide products obtained with the psPRB primer, and the larger product (>500 nucleotides) obtained with the ps459B primer. The P2 start site represents the 130 nucleotide product obtained with the psPRB primer and the 240 nucleotide product obtained with the ps459B primer.

Northern blot experiments using four antisense RNA probes (A, B, C and D; Figure 7) confirmed that the primer extension products used to deduce the location of P2 reflect a true transcription start site instead of primer extension artifact. Probe A was transcribed from a *Pvu*II genomic DNA fragment containing nucleotides 416–588 (Figure 6); probe B was transcribed from a *Pvu*II genomic DNA fragment containing nucleotides 589–763; probe C was transcribed from a *Pvu*II to *Nru*I genomic DNA fragment containing nucleotides 764–867; probe D was transcribed from a *Hin*dIII-*Sal*I genomic DNA fragment containing nucleotides 1425–2196. Using RNA from 0–2-day-old adults, it was found that probes A and B only hybridize to the 1.3-kb transcript and that probes C and D hybridize to both the 1.3- and 1.1-kb transcripts. These are the results expected if the P1 and P2 transcription start sites determined by primer extension are correct.

The positions of two promoters in the PTP synthase gene are sufficient to explain the size difference between the two transcripts observed by Northern blots. Based on the Northern hybridization data, the 1.3-kb constitutive transcript presumably initiates at the upstream promoter (P1; Figure 7) and the 1.1-kb head-specific transcript at the downstream promoter (P2; Figure 7). The cDNA clone was produced from a transcript initiated at the upstream promoter because it contains sequences upstream of the P2 start site. Therefore it represents a constitutive transcript even though it was isolated from a head cDNA library. This library, however, has been amplified, and the abundance of different cDNA clones does not reflect the *in vivo* abundance of the corresponding transcripts. For example, one of a few aldolase gene cDNAs isolated from the same library represents a very rare abnormally spliced transcript that was only detected in adult RNA by RT-PCR (KIM *et al.* 1992).

The primer extension and Northern hybridization experiments demonstrate that the 1.3- and 1.1-kb transcripts differ in the transcription start site, and the Northern hybridization experiments further indicate that most of the sequences downstream of the the P2 start site are present in both transcripts. However neither experiment rules out the possibility of small differences between the two transcripts within the coding region. To examine this, RT-PCR experiments with total RNA isolated from 0–2-day-old adults were conducted using primers (ps293E and ps876S; Figure 6) flanking the coding sequence. The ps293E primer is in the middle of the region covered by the C probe that hybridizes to both transcripts and contains the putative translation initiation codon (Figure 6). The ps876S primer is in the region covered by the D probe that hybridizes to both transcripts and is between the translation termination codon and the polyadenylation site used in the cDNA clone (Figure 6). The ps293E primer must hybridize to both transcripts, although it is a formal but unlikely possibility that ps876S primer site could be ex-

cluded from the 1.1-kb transcript if it contains an unknown different 3′ exon than the 1.3-kb transcript. The RT-PCR reactions produced one major 0.6-kbp product. This is the size expected based on the cDNA sequence. Furthermore, when more than 20 cloned RT-PCR products were sequenced, all were found to have the same intron-exon structure as the original cDNA clone. Therefore, assuming that the 1.1-kb transcript does not have an alternative 3′ exon that lacks the ps876S primer site, these results indicate that both transcripts have the same structure downstream of the P2 transcription start site (Figure 7).

Structure and expression of the PTP synthase gene in suppressible *pr* mutants: Mutations in *pr* reduce PTP synthase enzyme activity and increased copies of wild-type *pr* increase PTP synthase activity, consistent with the hypothesis that *pr* encodes PTP synthase (YIM *et al.* 1977). The observation that the PTP synthase gene is contained within the region to which *pr* maps cytogenetically strongly supports this hypothesis. To further explore this question, we characterized the structure and expression of the PTP synthase gene in the pr^1 and pr^{bw} mutants. It has previously been suggested that pr^1 might be a *412*-retrotransposon insertion because the v^1 mutation, which is also suppressed by *su(s)* mutations, is a *412* insertion and the 38BC region in pr^1 salivary gland polytene chromosomes hybridizes to a *412* probe (SEARLES and VOELKER 1986).

Phages containing the PTP synthase gene were cloned from λ libraries made from pr^1 and pr^{bw} genomic DNA. Restriction mapping and Southern blot hybridization analysis using PTP synthase cDNA and *412* probes revealed the presence of an antiparallel *412* retrotransposon insertion in the same intron in both mutants. The *412*-PTP synthase gene junction fragments were subcloned and sequenced to determine the exact insertion sites. This revealed that the insertion sites are identical in the pr^1 and pr^{bw} alleles (Figure 6). No differences between the two alleles were detected and the expected insertion site duplications and *412* LTR sequences were found. The presence of *412* insertions in the PTP synthase gene in the suppressible *pr* alleles is additional strong evidence that the PTP synthase gene is *pr*.

If the two alleles are truly independent *412* insertions, it is somewhat surprising that both have exactly the same insertion site. Furthermore, although the exon sequences flanking the *412* insertion from both of the mutants are identical to those in the Oregon R, the intron sequences flanking the *412* insertions in both mutants contain the same single base polymorphisms relative to the Oregon R sequence. We think it is possible that pr^{bw} is a "reisolation" of pr^1. Both alleles were isolated by the same laboratory many years ago (BRIDGES 1919; LINDSLEY and ZIMM 1992). The pr^1 and pr^{bw} alleles used in these experiments were obtained from very different sources, minimizing the chances that they have been confused or mislabeled. The pr^{bw} chromosome carries the *cn* mutation while the pr^1 chromosome does not, avoiding confusion within our laboratory. The pr^1 stock (obtained from GUNTER REUTER, Martin Luther University) was maintained for several years in eastern Germany. The phenotype matches the original description of pr^1, and because pr^{bw} is not nearly as widely distributed as pr^1, it seems very unlikely that this stock is actually pr^{bw}. The pr^{bw} *cn* stock (provided by E. H. GRELL and K. BRUCE JACOBSON) is from the Oak Ridge National Laboratory collection and was the stock used to determine the suppressibility of pr^{bw}. Photographs taken in 1979 show that the phenotype has not changed. We cannot rule out the possibility that pr^1 was accidently substituted for pr^{bw} before the first suppression experiments. In this case it is possible that the "original" pr^{bw} is not suppressible. It may be possible to find an alternative source of pr^{bw} to examine this possibility. We will continue to refer to the Oak Ridge stocks, which were used in virtually all previous suppression studies, as pr^{bw} until there is evidence that the "original" pr^{bw} allele is different.

The pr^{bw} mutant has 10–20% wild-type PTP synthase activity in young adult heads, and when suppressed by *su(s)* mutations, PTP synthase activity is restored to near wild-type levels (YIM *et al.* 1977; DORSETT *et al.* 1979). To determine if these effects occur at the level of PTP synthase transcript accumulation, the levels of the constitutive and head-specific transcripts in pr^{bw} and $su(s)^2;pr^{bw}$ flies were examined by Northern blots. Indeed, the levels of the 1.1-kb head-specific transcript are dramatically reduced in pr^{bw} heads (Figure 5, lane 2). This was observed in all of several independent experiments, and quantitation by scanning densitometry indicated that the head-specific transcript is 5–10% of wild-type levels. In $su(s)^2;pr^{bw}$ flies the head-specific transcript is restored to 30–50% of wild-type levels (Figure 5, lane 3). In contrast, the 1.1-kb constitutive transcript appears to be only slightly reduced in pr^{bw} flies (Figure 5, lane 5). Although slight, the reduction was observed in all of several independent experiments. Because of the lower levels and similarity in size to the head-specific transcript, it proved more difficult to quantitate reliably the 1.3-kb constitutive transcript by Northern blots. Instead the levels were quantitated by primer extensions using the ps74B primer (Figures 6 and 7) that detects only transcripts initiated at the upstream P1 promoter. In the autoradiogram shown in Figure 8, scanning densitometry indicated that the constitutive transcript is 75% of wild-type levels in pr^{bw} adults (lane 2) and is restored to >90% wild-type levels in $su(s)^2;pr^{bw}$ adults (lane 3). Therefore, the head-specific transcript is affected much more dramatically by the *412* insertion and the *su(s)* mutation than is the constitutive transcript, even though the *412* element is an intron common to both.

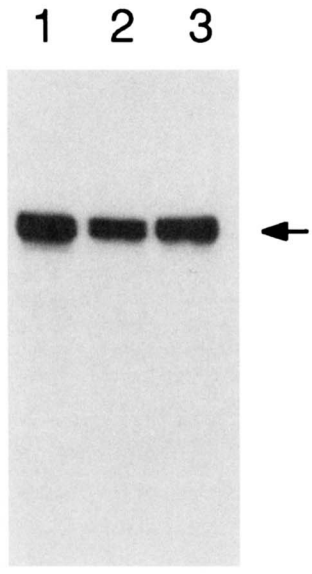

FIGURE 8.—Expression of the constitutive PTP synthase transcript in wild-type and *pr* mutant 0–2-day-old adults. The panel is an autoradiogram of a denaturing polyacrylamide gel used to separate primer extension products (arrow) made using primer ps74B (Figures 6 and 7) that only detects transcripts from the distal promoter P1. Total RNA was isolated from the following genotypes: 1, Oregon R; 2, pr^{bw}; 3, $su(s)^2;pr^{bw}$. Equal amounts of RNA (20 μg) were used for each lane, and scanning densitometry indicates that the level of product in lane 2 is 75% of the amount of product in lane 1, and that the amount of product in lane 3 is 90% of the amount in lane 1. Similar results were obtained in independent experiments and by Northern blots (Figure 5).

We considered the possibility that the transcripts present in the pr^{bw} and $su(s)^2;pr^{bw}$ flies might actually differ in structure from wild-type transcripts even though they are the same size. However, when the ps293E and ps876S primers (Figures 6 and 7) were used in RT-PCR experiments with RNA from young adults with these genotypes, the expected 0.6-kbp product was produced, and all of the several RT-PCR products sequenced from each genotype were identical to wild type. Therefore, the PTP synthase transcripts present in pr^{bw} and $su(s)^2;pr^{bw}$ flies have wild-type structure.

In pr^{bw} flies, the effects of the *412* insertion and *su(s)* mutation on the head-specific PTP synthase transcript correlate well with the effects on PTP synthase activity and the eye color phenotypes. If we presume that the constitutive transcript supports biopterin synthesis, then the small effect of the *412* insertion on the constitutive transcript explains why, in contrast to other *pr* alleles, pr^{bw} is viable. The effects of the *412* insertion and *su(s)* mutation on PTP synthase transcripts in pr^{bw} flies provide compelling evidence that the PTP synthase gene is *pr*.

DISCUSSION

Several lines of evidence lead to the conclusion that *pr* encodes PTP synthase. *pr* mutations reduce PTP synthase activity and increasing the number of copies of wild-type *pr* increases PTP synthase activity accordingly (YIM *et al.* 1977). As shown here, the PTP synthase gene is located in the region to which *pr* has been mapped cytogenetically (BRITTNACHER and GANETZKY 1983). Furthermore, the pr^{bw} allele is a *412* retrotransposon insertion in an intron of the PTP synthase gene. The *412* insertion dramatically reduces accumulation of a head-specific PTP synthase mRNA, and this mRNA is partially restored by a *su(s)* mutation that suppresses pr^{bw}.

The PTP synthase gene produces two transcripts, a constitutive transcript at low to moderate levels from a distal promoter and a transcript present at high levels in late pupae and the heads of young adults from a proximal promoter. These two transcripts explain both the homozygous lethal and viable alleles of *pr*. If we assume that the constitutive transcript supports synthesis of biopterin, then mutations that block expression of this transcript will be lethal. If we also assume that the head-specific transcript functions primarily to support synthesis of pteridine eye pigments, then mutations such as pr^{bw}, which reduces primarily the head-specific transcript, should be viable and display only an eye color phenotype.

pr^{bw} is suppressed by *su(s)* mutations and contains an antiparallel *412* retrotransposon insertion in an intron. *su(s)* mutations also suppress an antiparallel *412* insertion in the first exon of *vermillion* (v^1) (SEARLES and VOELKER 1986) and an antiparallel *P*-element insertion in the first exon of *yellow* (y^{76d28}) (GEYER *et al.* 1991). It has been proposed that *su(s)* alters RNA processing because it encodes a protein with motifs associated with RNA binding in other proteins (VOELKER *et al.* 1991).

The *412* insertion in v^1 virtually abolishes accumulation of normal transcripts and leaves only trace amounts of a transcript in which the antiparallel *412* insertion has been spliced out by cryptic splice acceptor and donor sites contained in the *412* LTRs (FRIDELL *et al.* 1990; PRET and SEARLES 1991). *su(s)* mutations increase accumulation of this abnormal but functional v^1 transcript approximately fivefold. In *vermillion* constructs containing a single *412* LTR, *su(s)* mutations increase the levels of both the spliced and unspliced RNAs (FRIDELL and SEARLES 1994), similar to the effects *su(s)* mutations have on the transcripts of y^{76d28} with a *P*-element insertion near the 5′ end (GEYER *et al.* 1991). On the basis of these observations it has been suggested that *su(s)* mutations stabilize the mutant precursor RNAs without increasing splicing efficiency (GEYER *et al.* 1991; FRIDELL and SEARLES 1994). However, the precursor stabilization appears likely to involve increased recognition of the cryptic splice sites because substitution of consensus 5′ donor sites for the cryptic 5′ donor in the *412* LTR in the *v* constructs increases accumulation of both the spliced and unspliced products and eliminates the response to *su(s)* mutations (FRIDELL and SEARLES 1994).

In contrast to v^1 and y^{76d28}, in pr^{bw} the transposon insertion is in an intron. In the primary *pr* transcript initiated at the distal promoter, this intron is the second intron and accumulation of the processed transcript is only slightly reduced. In the case of the transcript initiated at the proximal promoter, however, the intron with the *412* element is the first intron and the processed RNA level is reduced >10-fold in the presence of wild-type *su(s)*. Therefore, *su(s)* does not have the same effects on different transcripts containing the same *412* insertion.

Because the constitutive and head-specific *pr* mRNAs display different tissue distributions and developmental profiles, we cannot entirely rule out the possibility that the different effects of *su(s)* on the two mutant precursor RNAs reflect different levels of *su(s)* activity. However, *su(s)* is expressed constitutively throughout development (VOELKER *et al.* 1991) and has the same effects on splicing of *412* from *v* when the strong and widely expressed metallothionein promoter is substituted for the native *v* promoter (FRIDELL and SEARLES 1994). Furthermore, *su(s)* mutations affect mutations in several different genes expressed in a wide variety of tissues (LINDSLEY and ZIMM 1992).

A striking observation is that the precursors of the all RNAs downregulated by wild-type *su(s)* contain transposon insertions close to the 5′ end. Furthermore, the head-specific pr^{bw} mRNA precursor, the v^1 precursor RNA, and the y^{76d28} precursor all lack a complete intron upstream of the transposon insertion, in contrast to the pr^{bw} constitutive mRNA precursor that is not substantially affected by the *412* insertion and *su(s)*. It is an intriguing possibility, therefore, that the presence or absence of a complete intron upstream of the transposon insertion determines whether or not *su(s)* will influence transcript processing. Components of the splicing apparatus can bind to nascent transcripts (OSHEIM *et al.* 1985; BEYER and OSHEIM 1988) and therefore an intron upstream of the transposon insertion increases the opportunity for the splicing apparatus to bind and stabilize a nascent transcript before the transposon sequences are transcribed (Figure 9). Thus, the order in which factors recognize the precursor RNA may determine its fate. If factors recognizing the transposon sequences (possibly the Su(s) protein) bind first, then the splicing apparatus does not recognize the RNA and the RNA enters a degradation pathway. If, however, the splicing apparatus binds first, then the RNA enters the processing pathway and is eventually transported in mature form to the cytoplasm. This model is consistent with the hypothesis that *su(s)* mutations increase the extent to which precursor RNAs are recognized and stabilized by the splicing apparatus (FRIDELL and SEARLES 1994). The observation that transcripts containing *412* LTR sequences but lacking the cryptic splice sites are still unstable in *su(s)* mutants

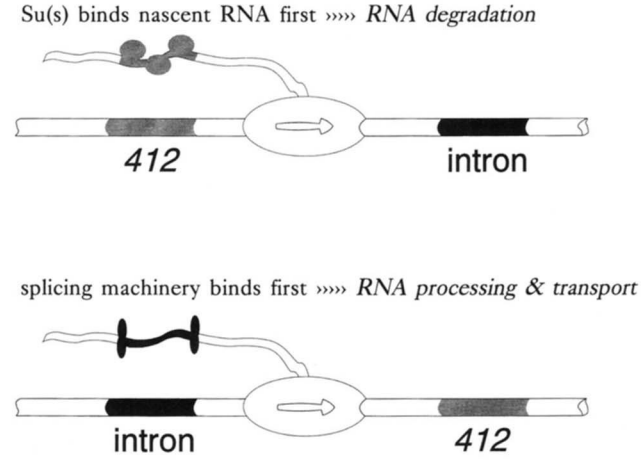

FIGURE 9.—Model for competition between the Su(s) protein and splicing machinery. We propose that if an antiparallel *412* insertion is upstream of all introns, such as in v^1 allele (SEARLES and VOELKER 1986), or is in the first intron, such as in the pr^{bw} head-specific transcript (Figure 7), then the Su(s) protein has the first opportunity to bind the nascent transcript and prevent the binding of splicing machinery. When the splicing machinery fails to bind, the transcripts enter a degradation pathway. If, however, the *412* insertion is downstream of a complete intron, such as in the pr^{bw} constitutive transcript (Figure 7), then the splicing machinery has the first opportunity to bind the nascent transcript and target it to a processing and transport pathway. This model for the action of Su(s) is consistent with those proposed by FRIDELL and SEARLES (1994) and GEYER *et al.* (1991).

(FRIDELL and SEARLES 1994) suggests that the primary role of Su(s) is to prevent recognition and stabilization of the nascent transcript by the splicing apparatus, rather than to actively destabilize the transcripts.

Although the hypothesis that Su(s) protein binds to antiparallel *412* and *P*-element sequences in nascent transcripts and prevents them from being recognized and stabilized by the splicing apparatus provides an explanation for suppression of v^1, pr^1 and y^{76d28} by *su(s)* mutations, it does not explain how *su(s)* mutations enhance the subclass of *gypsy* transposon insertion alleles that are also enhanced by *suppressor of white-apricot* [$su(w^a)$] mutations and suppressed by *suppressor of forked* [*su(f)*] mutations (RUTLEDGE *et al.* 1988). Although the effect of *su(s)* is opposite to that observed with *412* and *P*-element insertions, the observation that *su(s)* mutations only affect the same *gypsy* insertions modified by $su(w^a)$ and *su(f)* is further evidence that *su(s)* is involved in RNA processing. $su(w^a)$ encodes an RNA-binding protein (LI and BINGHAM 1991) and *su(f)* encodes a homologue of a human polyadenylation factor (MITCHELSON *et al.* 1993; TAKAGAKI and MANLEY 1994). Both $su(w^a)$ and *su(f)* modify processing of transcripts containing *gypsy* (DORSETT *et al.* 1989).

At least two of the *gypsy* insertion alleles enhanced by *su(s)* and $su(w^a)$ mutations and suppressed by *su(f)* mutations, f^1 and bx^{34e}, have *gypsy* insertions in an in-

tron. In f^1 the insertion is parallel and in the second intron (MCLACHLAN 1986; HOOVER *et al.* 1993), while in bx^{34e} the *gypsy* insertion is in the third intron and antiparallel (PEIFER and BENDER 1986). It is possible, therefore, that Su(s) protein recognizes both antiparallel and parallel *gypsy* sequences in nascent transcripts and by altering interactions with RNA processing factors reduces the inappropriate RNA processing events promoted by *gypsy* sequences (DORSETT *et al.* 1989). Indeed, it is possible to resolve the apparent paradoxical effects of *su(s)* on *412* and *gypsy* insertions by proposing that the normal function of Su(s) protein is to limit access of other RNA processing factors to nascent transcripts. In the case of *412* (and *P*-element) insertions in the first exon or first intron this would prevent the primary transcript from entering the normal processing pathway and therefore reduce stability of the precursor RNA and accumulation of mature transcript, while in the case of *gypsy* insertions it would reduce inappropriate processing events promoted by the transposon and thereby increase the relative amount of functional mature transcript.

The authors thank JIM POSAKONY and GUNTHER REUTER for providing fly stocks, JO JACK for providing a genomic library, P. SALVATERRA for providing a head cDNA library, and LILLIE SEARLES for helpful discussions. The authors also thank an anonymous reviewer for insightful comments on the model for Su(s) action. J.Y. and D.D. pay special thanks to BRUCE JACOBSON and ED GRELL who introduced them to the puzzle posed by the pr^1, pr^{bw}, and *su(s)* mutations before splicing was discovered. This work was supported by grants from Korean Science and Engineering Foundation and the Genetic Engineering Research Program of the Korean Ministry of Education to J.Y. and the American Cancer Society to D.D.

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Communicating editor: J. A. BIRCHLER