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Molecular and Genetic Analyses of Drosophila *Prat*, Which Encodes the First Enzyme of *de Novo* Purine Biosynthesis

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ABSTRACT

The Drosophila *Prat* gene encodes phosphoribosylamidotransferase (PRAT), the enzyme that performs the first committed step of the *de novo* purine nucleotide biosynthesis pathway. Using information from amino acid sequence alignments of PRAT from other organisms, a polymerase chain reaction-based approach was employed to clone *Prat*. Amino acid sequence alignment of Drosophila PRAT with PRAT from bacteria, yeast, and vertebrates indicates that it is most identical (at least 60%) to the vertebrate PRATs. It shares putative amino-terminal propeptide and iron-binding domains seen only in *Bacillus subtilis* and vertebrate PRATs. *Prat* was localized to the right arm of chromosome *3* at polytene band 84E1-2. Owing to the fact that this region had been well characterized previously, *Prat* was localized to a 30-kilobase region between two deficiency breakpoints. By making the prediction that *Prat* would have a similar "purine syndrome" phenotype as mutations in the genes *ade2* and *ade3*, which encode enzymes downstream in the pathway, five alleles of *Prat* were isolated. Three of the alleles were identified as missense mutations. A comparison of PRAT enzyme activity with phenotype in three of the mutants indicates that a reduction to 40% of the wild-type allele's activity is sufficient to cause the purine syndrome, suggesting that PRAT activity is limiting in Drosophila.

BIOSYNTHESIS of purine nucleotides *de novo* begins with 10 enzymatic steps that lead to the synthesis of IMP, the common precursor of AMP and GMP. An important regulatory point of the pathway is the first step, which is thought to be rate-limiting for the synthesis of IMP and is catalyzed by phosphoribosylamidotransferase (PRAT) (WYNGAARDEN and KELLEY 1983). PRAT performs the following reaction: 5-phosphoribosylpyrophosphate (PRPP) + glutamine $\rightarrow$ 5-phosphoribosylamine (PRA) + glutamate + PP_i, where NH_3 can be substituted for glutamine. The enzyme contains two major domains: approximately the first 200 amino acids from the amino terminus are required for glutamine amide transfer, and the remaining are for the synthesis of PRA with NH_3 (ZALKIN and DIXON 1992). The amino acid sequences have been determined from the *purF* locus of *Escherichia coli* (TSO *et al.* 1982), *purF* of *Bacillus subtilis* (MAKAROFF *et al.* 1982), ADE4 of *Saccharomyces cerevisiae* (MÄNTSÄLÄ and ZALKIN 1984), from chicken (ZHOU *et al.* 1990), and recently from rat (IWAHANA *et al.* 1993b) and humans (IWAHANA *et al.* 1993a).

PRAT activity is directly inhibited by purine nucleotides and stimulated by PRPP. This has been shown in mammalian systems (BECKER and KIM 1987), *E. coli* (MESSENGER and ZALKIN 1979), and *B. subtilis* (MEYER and SWITZER 1979). There are two features of PRAT that are not shared by all species studied. One feature is a propeptide that must be proteolytically processed for use of glutamine as a substrate and is found in *B. subtilis* and chickens but not in *E. coli* and *S. cerevisiae* (ZALKIN and DIXON 1992). The second feature is an iron-binding region found in *B. subtilis* and chickens that may play a role in protein stability (GRANDONI *et al.* 1989) and in propeptide processing (ZHOU *et al.* 1992).

Regulation of PRAT and other *de novo* purine pathway enzymes has been found at the level of gene expression in *E. coli*, *B. subtilis* and *S. cerevisiae*. In *E. coli*, all but one of the nine loci encoding the *de novo* purine pathway enzymes are regulated coordinately at the transcriptional level by the *purR* repressor (ZALKIN and DIXON 1992). *purR* responds to high levels of the pathway end-products adenine and hypoxanthine. Two *de novo* pyrimidine pathway genes, a pyrimidine salvage pathway gene and *glyA* (which encodes serine hydroxymethyltransferase) are also repressed by *purR*. In *B. subtilis*, all the enzymes in the *de novo* pathway are encoded in a single operon. Initiation of transcription of the operon is repressed by adenine and elongation is regulated by guanosine (ZALKIN and DIXON 1992). In *S. cerevisiae*, at least four genes of the purine pathway are positively regulated by the transcriptional activators BAS1 and BAS2 in response to adenine starvation (DAIGNAN-FORNIER

The sequence data presented in this article have been submitted to EMBL/GenBank Data Libraries under accession number L23759.

and FINK 1992). BAS1 and BAS2 are also required for basal expression of at least two genes in the histidine and one gene in the tryptophan biosynthesis pathways. The transcription factor GCN4, which is responsive to amino acid starvation and activates amino acid biosynthetic and aminoacyl tRNA synthetase genes, also activates transcription of the ADE4 gene which encodes PRAT (HANS-ULRICK *et al.* 1991). ROLFES and HINNEBUSCH (1993) have found that GCN4 also responds to purine starvation and its induction leads to increased expression of not only ADE4 but ADE5,7, ADE8, and ADE1, which all encode *de novo* purine biosynthesis enzymes. In summary, at least part of the regulation of expression of *de novo* purine pathway genes occurs at the transcriptional level. In the two bacteria, regulation is negative in response to the presence of pathway end products while in *S. cerevisiae* it is positive in response to a reduction in a pathway end product. In addition, not only is there coordinated expression of *de novo* purine pathway genes, but cross-pathway regulation has been demonstrated in both *E. coli* and *S. cerevisiae.*

Little is known about how the *de novo* purine pathway genes are regulated in multicellular eukaryotes. One exception is the human gene encoding inosine monophosphate dehydrogenase, one of the two enzymes required for the synthesis of GMP from IMP. It is regulated at the post-transcriptional level in the nucleus by guanine nucleotides (GLESNE *et al.* 1991).

In *Drosophila melanogaster,* two *de novo* purine pathway genes have been well characterized. The $ade2^1$ and $ade3^1$ (also known as *Gart*; *ade* for *ade*nine-requiring) mutations were isolated in screens for purine auxotrophs (JOHNSTONE *et al.* 1985). *ade2* encodes FGARAT (HENIKOFF *et al.* 1986b) and *ade3* encodes a trifunctional polypeptide with the three enzyme activities GARS, AIRS and GART (HENIKOFF *et al.* 1986a) (Figure 1). Whether the *ade2* and *ade3* genes are regulated with respect to pathway end products has not been addressed.

To begin to understand the regulation of *de novo* purine pathway genes in Drosophila, a study of the gene encoding PRAT (Figure 1) was initiated. Through sequence analysis, the structure of the gene, which has no introns, was inferred. A multiple alignment of the derived amino acid sequence with other PRAT sequences indicates that Drosophila PRAT possesses a propeptide and an iron-binding region and is most similar to chicken, human, and rat PRAT.

Previous work on the phenotype of *ade2* and *ade3* mutations provided the basis of a screen for *Prat* mutations. The first alleles of *ade2* and *ade3* were isolated as purine auxotrophic mutations which had no obvious visible phenotype except for a reduction in red eye pigments in $ade2^1$ mutants (JOHNSTONE *et al.* 1985). Subsequently, lethal and sublethal alleles were isolated in screens for noncomplementing alleles

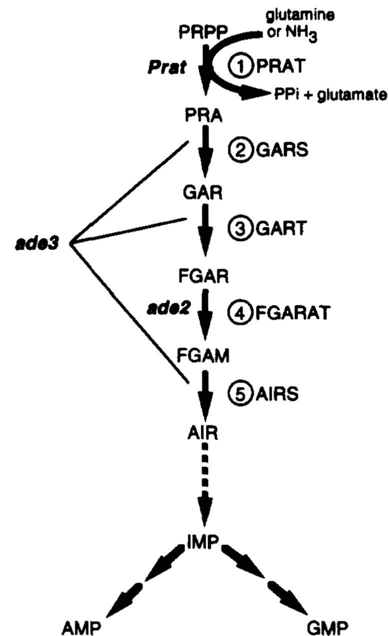

FIGURE 1.—The first five steps of *de novo* purine nucleotide biosynthesis. The Drosophila genes encoding these enzymes are indicated on the left of the pathway. The pathway intermediates are: PRPP = 5′-phosphoribosyl 1-pyrophosphate; PRA = 5′-phosphoribosylamine; GAR = 5′-phosphoribosylglycinamide; FGAR = 5′-phosphoribosyl *N*-formylglycinamide; FGAM = 5′-phosphoribosyl *N*-formylglycinamidine; AIR = 5′-phosphoribosylaminoimidazole. The enzymes, indicated on the right of the pathway are: PRAT = phosphoribosylamidotransferase (EC 2.4.2.14); GARS = GAR synthetase (EC 6.3.4.13); GART = GAR transformylase (EC 2.1.2.2); FGARAT = FGAR amidotransferase (EC 6.3.5.3); AIRS = AIR synthetase (EC 6.3.3.1).

of $ade2^1$ (TIONG *et al.* 1989) and of a deficiency of the *ade3* region (TIONG and NASH 1990). These mutations have a pleiotropic phenotype or "purine syndrome" characterized by some or all of the following, depending on the severity of the mutation: reduction in red eye pigments, finer bristles, smaller wings with venation defects, deformed legs, and pupal lethality (TIONG and NASH 1990; TIONG *et al.* 1989). The pupal lethal phenotype of null alleles of *ade3* is incompletely penetrant: a small percentage of individuals will eclose, and these always have the purine syndrome (TIONG and NASH 1990). Isolation and characterization of ethyl methanesulfonate (EMS)-induced mutations of *Prat* show that they have the "purine syndrome" phenotype that is found with mutations in the downstream genes *ade2* and *ade3*. It was found that a much smaller decrease in enzyme activity is required to produce a purine syndrome phenotype with *Prat* than with *ade2* or *ade3,* suggesting that PRAT activity is limiting in Drosophila. This finding is consistent with PRAT being an important regulatory point of the *de novo* purine pathway (WYNGAARDEN and KELLEY 1983).

MATERIALS AND METHODS

Amplification of a segment of *Prat*: Oligonucleotide primer sequences were based on conserved amino acids in

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GAATTCGAATATCGAATCAAGTACTTAAGCTAAACGTTTTCATTAAATTCCTACTGCTCATAATGACACCAACAAATTGAACCAATCGCATTTCAGTTTG -769
ATACTTATCAAATGTATAAAAGAAACCGTTTTTGTAAAAATGTTCCTACTGTGAATTTTTTCTAATATATTTATCTCTTAGTGGCGTTTTTATTGAGGGA -669
TTGCAGTGAACTTATTTTCAATTGTTATACATATGTAGTTAATTATTATTTATTTATTTCACGAAACATTTTTTATATTTTTGGTTTTAATTGCCTACGT -569
GTTAAAAAAAAAACTGTTAAATATTATATATACGAACATACACATACAATTTTTAATTAATTTTAAATGCGCAAAATAACTTTAATTCAATTTAAAATAA -469
GCTTTAGTTTGATTTAGTTTGATTGCGTATATTTTATATAAACGAATAAAATCAAATTATAATGTGTATATTGTTTTGTGTACTAAATTACAAATTAGGT -369
AAAATTCCAATTTGAAAATACAGACTATAGAATGCTATCGATCCCATCAGCACACCGCACTATCGATATCAGCGACCGATCGATATTGGGCTTGATCTCG -269
ACCGATTCGGGAAAAACTGAGCTGCGCGTGCGAGCGTGCGAGGATTTTTCTTGTATAAAACCGTTGCGTAAATCAGCAGGTAAATCAGATCGGCGTCAAC -169
TGCGCCACTGCCTGCAATCAGTTGCCTTCAACCAGCATATTGTACCGATTTTTCAGCAGCAACATGTCAGCGCCACAGCAACAACAACAGTCGCAGCAGA -69
AGCAACAACAACATGTGCGCGTGGTCGAGCAACAACAGGTGGAACCAGCTGAGGCGGTGACCTCCAGC ATG GAA TCG GAA TCC ATC TCG GCC 24
                                                                     M   E   S   E   S   I   S   A   8

AGC AAG GAG CTA ACC GGT TTG ACG CAC GAG TGC GGC GTT TTC GGG GCA ATC GCT TGC GGA GAT TGG CCC ACC CAA 99
 S   K   E   L   T   G   L   T   H   E   C   G   V   F   G   A   I   A   C   G   D   W   P   T   Q  33

ATG GAC ATT GCA CAC GTG ATC TGT TTG GGG CTG GTG GCA CTG CAG CAT CGT GGG CAG GAG TCT GCG GGC ATA GCG 174
 M   D   I   A   H   V   I   C   L   G   L   V   A   L   Q   H   R   G   Q   E   S   A   G   I   A  58

ACC AGC GAG GGA AAG TGC TCC AAG AAC TTC AAC GTG CAC AAG GGC ATG GGT ATG ATC AGC ACC CTG TTC AAC GAC 249
 T   S   E   G   K   C   S   K   N   F   N   V   H   K   G   M   G   M   I   S   T   L   F   N   D  83

GAC TCC ATG AAG AAG CTT CGT GGC AAC CTG GGC ATC GGT CAT ACA CGC TAC TCG ACC GCT GGC GGA TCC GGA GTG 324
 D   S   M   K   K   L   R   G   N   L   G   I   G   H   T   R   Y   S   T   A   G   G   S   G   V  108

GTT AAC TGT CAG CCC TTT GTG GTA CAT ACG ACC CAT GGA GCA TTG GCC CTG GCC CAC AAT GGT GAG CTT GTT AAC 399
 V   N   C   Q   P   F   V   V   H   T   T   H   G   A   L   A   L   A   H   N   G   E   L   V   N  133

AAC GAA TCT CTA AGA AGG GAG GTT TTG GCC AGA GGC GTG GGC TTA TCC AGC CAC AGC GAC AGT GAG TTG ATC GCC 474
 N   E   S   L   R   R   E   V   L   A   R   G   V   G   L   S   S   H   S   D   S   E   L   I   A  158

CAG TCG TTG TGC TGC GCC CCG GAG GAT GTT TCC GAA CTG GAT GGA CCC AAC TGG CCA GCT AGG ATC AGG CAT TTC 549
 Q   S   L   C   C   A   P   E   D   V   S   E   L   D   G   P   N   W   P   A   R   I   R   H   F  183

ATG ATG CTG GCG CCA CTC TCC TAC TCG CTG GTC ATC ATG CTA AAG GAC AAA ATC TAC GCC GTG AGG GAC ACC TAT 624
 M   M   L   A   P   L   S   Y   S   L   V   I   M   L   K   D   K   I   Y   A   V   R   D   T   Y  208

GGA AAT AGA CCG CTA TGC ATT GGC AAG ATA GTG CCC ATC AAT GCT GGG CAC GGA AAT AAC TTA GAC ACA CCT GCC 699
 G   N   R   P   L   C   I   G   K   I   V   P   I   N   A   G   H   G   N   N   L   D   T   P   A  233

GAT GGT TGG GTG GTG TCC AGT GAG AGC TGT GGC TTC CTG TCG ATC GGT GCC AGA TAC GTC CGC GAA GTG GAG CCT 774
 D   G   W   V   V   S   S   E   S   C   G   F   L   S   I   G   A   R   Y   V   R   E   V   E   P  258

GGA GAG ATA GTG GAG CTG TCG CGA AGT GGC TAC CGC ACG GTG GAC ATT GTG GAA AGA CCC GAC TTT AAG CGC ATG 849
 G   E   I   V   E   L   S   R   S   G   Y   R   T   V   D   I   V   E   R   P   D   F   K   R   M  283

GCC TTT TGT ATA TTT GAG TAC GTT TAC TTC GCC CGC GGA GAC AGC ATC TTC GAG GGT CAG ATG GTG TAC ACA GTG 924
 A   F   C   I   F   E   Y   V   Y   F   A   R   G   D   S   I   F   E   G   Q   M   V   Y   T   V  308

AGG CTG CAG TGC GGC CGG CAG CTG TGG CGA GAG GCT CCA GTG GAG GCG GAT ATT GTG AGT TCC GTT CCC GAG TCT 999
 R   L   Q   C   G   R   Q   L   W   R   E   A   P   V   E   A   D   I   V   S   S   V   P   E   S  333

GGC ACA GCG GCG GCG CAT GGC TAT GCC CGT GAG TCT GGC ATT GAA TTT GCC GAG GTT CTC TGC AGG AAT CGC TAC 1074
 G   T   A   A   A   H   G   Y   A   R   E   S   G   I   E   F   A   E   V   L   C   R   N   R   Y  358

GTG GGA CGG ACG TTT ATT CAA CCC TCT ACA CGG CTG CGG CAG TTG GGA GTA GCC AAG AAG TTC GGA GCC CTA TCC 1149
 V   G   R   T   F   I   Q   P   S   T   R   L   R   Q   L   G   V   A   K   K   F   G   A   L   S  383

GAG AAC GTG GCT GGC AAG AGA TTG GTC CTG ATA GAC GAT TCC ATT GTG CGC GGC AAC ACC ATT GGA CCA ATA ATC 1224
 E   N   V   A   G   K   R   L   V   L   I   D   D   S   I   V   R   G   N   T   I   G   P   I   I  408

AAG CTG CTA CGG GAT GCA GGC GCC CGG GAG GTG CAC ATC CGC ATT GCC AGC CCA CCG CTG CAG TAC CCA TGC TAT 1299
 K   L   L   R   D   A   G   A   R   E   V   H   I   R   I   A   S   P   P   L   Q   Y   P   C   Y  433

ATG GGT ATA AAT ATT CCA ACT CGA GAG GAA TTG ATT GCC AAC AAG CTG AAC CCC GAC CAA CTA GCT AGG CAT GTG 1374
 M   G   I   N   I   P   T   R   E   E   L   I   A   N   K   L   N   P   D   Q   L   A   R   H   V  458

GGC GCC GAC AGT CTG GCT TAT CTT AGT GTG GAG GGA CTA GTG GAG GCT GTC CAA CTA AAG CAC CGC GAT GCA GGC 1449
 G   A   D   S   L   A   Y   L   S   V   E   G   L   V   E   A   V   Q   L   K   H   R   D   A   G  483

GAT AGT AAA TCC AAG GGA ACG GGC CAC TGC ACC GCC TGT CTC ACT GGT GAA TAT CCC GGT GGG CTG CCC GAT GAG 1524
 D   S   K   S   K   G   T   G   H   C   T   A   C   L   T   G   E   Y   P   G   G   L   P   D   E  508

TTG AGC TGG TGA TACCGATTGAAGCTCATAAGTGCCAAAAGACGAAAAAGGCTTTTGGAAACCAGTTTTGCACGCAATTGCCCACTACGAAATAA 1619
 L   S   W   *                                                                                        511

CAAAAATTGCAGTATAAATAGCCATAGAGGGCTCGGAATTACTGTCACAAATTTTGCATTTAGTTTATCTAGCATTACAGCCCACCCATTCCTATTTACA 1719
CTCTACCTTATTAGATCTTAAGTAAGCAGCCCACGTGCTTCCCCAAGCTAAAAGACACCGATTTATGTCTGGTTTTGCCTCTGGAACTTTACTCATAGTA 1819
CAAAATACATAACTCTTTGTTGCCCATCTTGAATATCACTAAAATTTGTTGTTTGCAAATGCCAATAATAAAGACACTTAGACTATTGATTAAACAGACT 1919
CTATTTTCTCTCTCTAGCTATCGAAGATATGAAATTACGTGCATGATATCGAGCAGTATGCTCCAAGGTCCGAAGCCAGTTGCTCTGTGCGGCAATGCAC 2019
CATTTCCTAAGGAGGTCATGGGTTCCACAAGCACTGGAGGCATTCGGATATGATTCTTTTGAATTTTAGATGATTAATTGGTGATAAGTCCACTGCAATA 2119
AATACTTAAAAACAGATTCAATTGTCGTGCTCTGTTGCTTTGCAAATAATCATTCTTATCAATACTGAGGAAACACTTTAATTTTACACAAATAATGAGT 2219
TGACCAATGATATGGCAAAATTTCTTGTCTAAGGTTTACTTTAACAACAGAAACCGGCGTTGAAGTCGTAAATAGTTTGAAGTAACATCCGAATATGTAT 2319
GTACATAGTTTTTATTATAACTTATGCTAGTCCACCATTTGTATTATATCTGTATCTTAAGAGTCCAGCATATCTCGAAGTCCTTCAGCTCCAATTCCCT 2419
TTCAATAACCTGTGTGTAGAGATTATCAAGCGTTTCCTGCATTCGTTGTTTGAGTGTGGCATTCTGAATGGAAGCAATTTCCGTTGACTTTTGCACCTGC 2519
TGCGCTTTCAGTTCACGAATCTGGCGCGTCAGTTGATTGATCTTCTGCTGGACCTGTTGCTGCGTGTACTGCGGCTGCGGTTCGTCTATGAATTC      2614
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FIGURE 2.—Nucleotide sequence of the 3.5-kb *Eco*RI fragment containing *Prat*. Coordinates of the sequence are relative to the A in the initiator methionine codon, which is at +1. The translation of the single open reading frame is shown below the nucleotide sequence. Putative transcription start consensus sequences are boxed; polyadenylation sequences are underlined. The overlining arrows indicate position and orientation of primers used to sequence *Prat* mutations.

PRAT. The sequences of the 20-nucleotide degenerate primers DmPRPP-left and DmPRPP-right were 5′-YT(C/G)CARCAYCGNGG(T/C/A)CARGA-3′ and 5′-(C/G)A-RRTT(T/A/G)CCRTTRTGNGC(G/C)A-3′, respectively. The degeneracy of the primers was somewhat reduced by omitting rarely used codons (WADA *et al.* 1990). Using

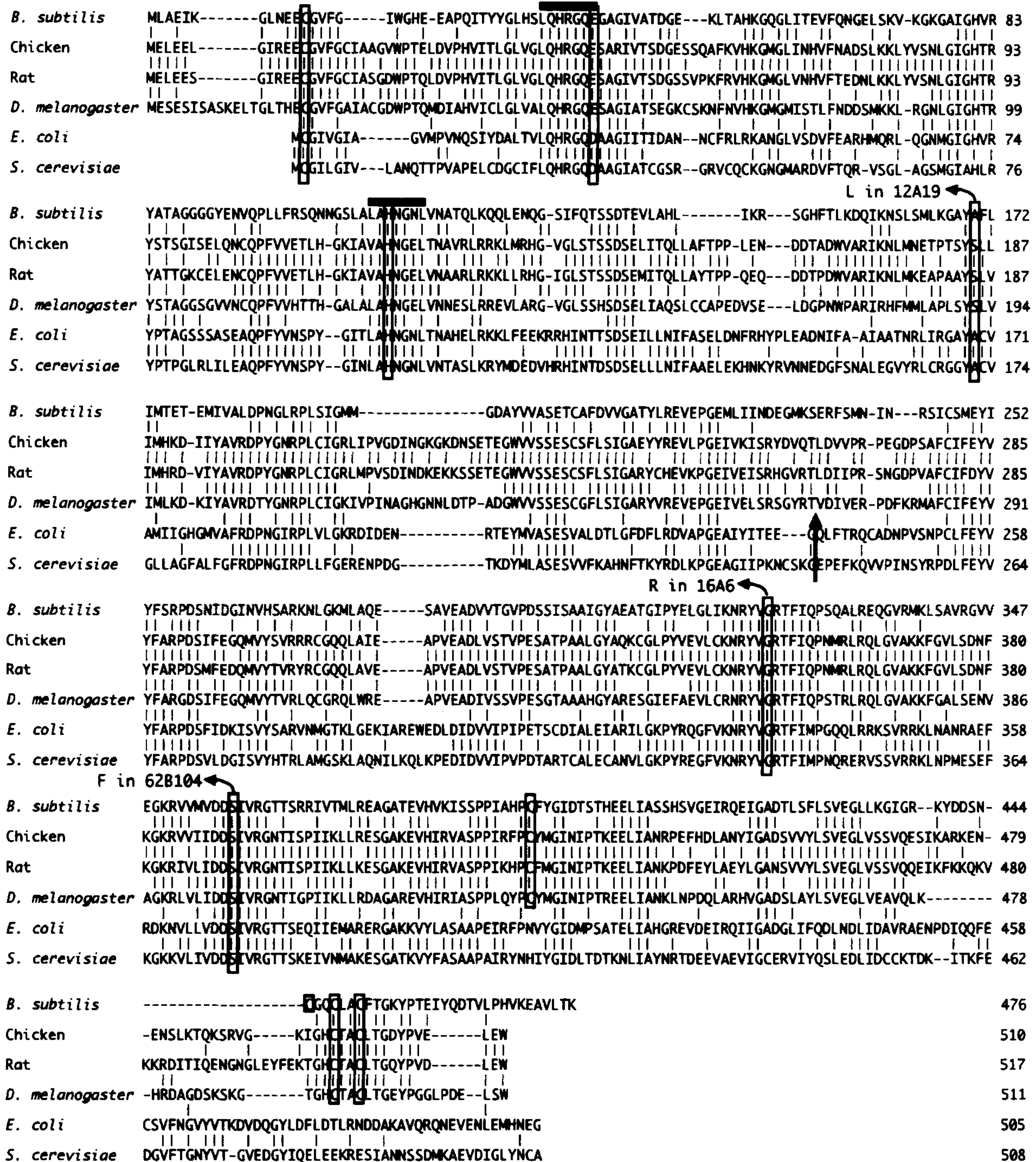

FIGURE 3.—Multiple alignment of PRAT sequences from *B. subtilis* (MAKAROFF *et al.* 1982), chicken (ZHOU *et al.* 1990), rat (IWAHANA *et al.* 1993b), *E. coli* (TSO *et al.* 1982), and *S. cerevisiae* (MÄNTSÄLÄ and ZALKIN 1984). The human sequence is not shown as it has 92% amino acid identity with the rat sequence (IWAHANA *et al.* 1993a). Vertical bars indicate sequence identity. The thick horizontal bars indicate the conserved residues that were the basis for degenerate oligonucleotide primer design. The sites of three Drosophila *Prat* missense mutations (12A19, 16A6 and 62B104) are boxed, and the amino acid substitution is indicated above each site. The three residues of the "catalytic triad" (MEI and ZALKIN 1989) of the glutamine amidotransferase domain toward the amino terminus of the proteins are boxed, as are the cysteines involved in iron binding toward the carboxyl terminus. The alignment of the *B. subtilis* iron-binding region with other sequences was performed manually subsequent to performing the multiple alignment by computer (see MATERIALS AND METHODS). A vertical arrow indicates the site of the amino-terminal deletion in *E. coli* that still retains catalytic activity (see DISCUSSION) (MEI and ZALKIN 1990).

Drosophila genomic DNA as the template, primers with phosphorylated 5′ ends, and Taq DNA polymerase (Stratagene), a polymerase chain reaction (PCR) was performed with 40 cycles of the following conditions: 30 sec at 94°, 30 sec at 48°, and 30 sec at 72°. The PCR product of expected size was gel-purified in low melt agarose and blunt end ligated into *Sma*I-digested pVZ1, a derivative of pBluescribe (Stratagene) (HENIKOFF and EGHTEDARZADEH 1987).

Library screening, subcloning and sequencing: λ Phage library screening, λ DNA isolation, restriction digests, Southern blots, probe hybridizations, and plasmid subclone production followed standard protocols (SAMBROOK *et al.* 1989).

To sequence both strands of the plasmid subclone pCs1-3.5E, exonuclease III-nested deletions were generated (HENIKOFF 1987).

The five *Prat* mutations and the wild-type *Prat* allele from the parent e^{11} chromosome were cloned by screening six genomic libraries for two clones of each allele. These libraries were constructed by isolating the 3–6-kilobase (kb) *Eco*RI fragments from the genomic DNA of each mutant hemi- or homozygote. These fragments were ligated to *Eco*RI-digested, phosphatase-treated λZAPII DNA (Stratagene). The phage were then packaged (Gigapack IIXL; Stratagene) and the primary phage clones were screened using the 3.5-kb *Eco*RI insert from pCs1-3.5E as a probe. One strand of the open reading frame of each of the five alleles was sequenced using a set of eight oligonucleotide primers. The primer sequences are indicated in Figure 2. Sequence differences were detected by comparison to the wild-type allele. Once a sequence difference was found in one clone, it was confirmed by sequencing the same region in a second clone.

The sequences of the PCR product clones, nested deletion clones, and mutant clones were determined using a Sequenase Version 2.0 kit (U. S. Biochemical Corp.). Sequencing data were entered directly into a computer file and verified using a sonic digitizer and editor software from Riverside Scientific Enterprises (Seattle, Washington). Sequence display, restriction site searches, and some alignments were performed using the GENEPRO software from Riverside. Multiple alignment was done using MULTALIN with default parameters (CORPET 1988). Sequence figures were prepared with the aid of the Eyeball Sequence Editor (CABOT and BECKENBACH 1989).

Drosophila strains and mutant screen: The strains carrying $Df(3R)dsx^{M+R2}$ and $Df(3R)dsx^{M+R43}$ (BAKER *et al.* 1991) were obtained from BRUCE BAKER. The strains carrying $Df(3R)Antp^{Ns+R17}$ and e^{11} were from the Bloomington, Indiana stock collection and *v* was from Bowling Green. The mutant screen and all experiments were conducted at 25° on Carolina Biological instant Drosophila medium. A dose of 0.025 M EMS (Sigma) was used to mutagenize males (see Figure 5) (ASHBURNER 1989). Ten mutagenized males were mated to 10 females per bottle. These parents were transferred to fresh bottles 4 days later, then again in 4 more days. The non-Sb, non-ebony F_1 individuals were screened for a reduction in red eye pigments, wing defects, and small bristles. Candidate mutant F_1's were backcrossed to the deficiency-carrying strain to establish heritability and to balance over TM3 (genotype $v\,;\,Prat^{-}\,e^{11}/TM3,Sb$).

Enzyme assays: Crude protein extracts were prepared from equal numbers of adult males and females (HENIKOFF *et al.* 1986a). PRAT assays were performed using the GARS-coupled reaction essentially as described by OATES and PATTERSON (1977). The 100-μl reaction mixture contained 100 mM Tris-HCl, pH 8.0, 4 mM $MgCl_2$, 10 μCi/ml [^{14}C]-glycine (Du Pont NEN), 1 mM glycine, 0.5 mM ATP, either 5 mM glutamine or 50 mM NH_4Cl, 40 μl protein extract and 3 mM PRPP. Background activity was determined from reactions without PRPP. The reactions were incubated at room temperature for 4 hr. The amount of ^{14}C incorporated into GAR was measured by spotting two 50-μl volumes of each reaction onto Whatman DE-81 filters, followed by 4 washes of 5 min each in 2 liters of water, followed by scintillation counting (MARTIN 1972). GAR, and not glycine, is selectively retained on the DE-81 filter. The PRAT activity of each extract (average counts for the two readings minus background) was normalized to the glucose-6-phosphate dehydrogenase (G6PDH) activity of each extract, which was assayed following the method of KUBY and NOLTMANN (1966). This was done to control for variability in protein quantity and quality from extract to extract.

Measurement of viability: The crosses employed to measure the survival to adulthood of *Prat* mutants were 1) $v\,;\,Prat^{-}\,e^{11}/TM6B,Tb$ males × $v\,;\,Df(3R)dsx^{M+R43}/TM6B,Tb$ virgin females, where the non-*Tb* vs. *Tb* individuals were scored and 2) $v\,;\,Prat^{-}\,e^{11}$ males × $v\,;\,Df(3R)dsx^{M+R43}/TM3,Sb$ virgin females, where the non-*Sb* vs. *Sb* individuals were scored (see Table 1). Viability was measured as the ratio of *Prat* mutants to wild-type siblings, which was then normalized to the ratio obtained for the parental e^{11} chromosome in a parallel cross.

RESULTS

Amplification of a segment of *Prat*: A multiple alignment of PRAT amino acid sequences from *E. coli*, *B. subtilis* and *S. cerevisiae* showed two regions in the glutamine amide transfer domain (MEI and ZALKIN 1990) where there is complete amino acid identity among the three species. These conserved regions, indicated in Figure 3, were used to design two degenerate oligonucleotide primers. The predicted size of the PCR product, excluding the possibility of introns, was approximately 250 base pairs. Using *D. melanogaster* genomic DNA as a template and the degenerate primers, a band of approximately this size was produced in a PCR (not shown). Five clones of this PCR product were sequenced, and one showed significant identity (62%) with the chicken PRAT sequence (ZHOU *et al.* 1990). The sequences of the other four clones did not show similarity to PRAT (data not shown). These are probably nonspecific PCR products that arose from the use of degenerate primers.

Isolation of the *Prat* gene: The PCR product clone encoding a segment of *Prat* was used as a probe to screen a Canton S genomic DNA λ library (MANIATIS *et al.* 1978) and three overlapping λ clones were obtained. The PCR product clone mapped to a 3.5-kb *Eco*RI fragment which was subcloned (pCs1-3.5E). This fragment was sequenced and a single, uninterrupted 511 amino acid open reading frame encoding PRAT was found entirely within this fragment (Figure 2).

The position of the initiator methionine is supported in two ways. First, multiple amino acid sequence alignment (Figure 3) indicates that there is a conserved cysteine near the amino terminus. This cysteine is found at the amino-terminus of the mature protein (ZALKIN and DIXON 1992) and is a necessary component of the catalytic triad that performs the removal of the amide group from glutamine (MEI and ZALKIN 1989). Since there is no methionine immediately upstream of the cysteine, as in *E. coli* and *S. cerevisiae* PRAT, Drosophila PRAT must have a pro-

peptide sequence. In addition, since the chicken and *B. subtilis* PRAT sequences have short propeptides of 11 amino acids, the first in-frame methionine upstream from the conserved cysteine was chosen as the initiator methionine for Drosophila PRAT. Second, the sequence just upstream of this initiator methionine fits well with translation start sequences found in Drosophila (CAVENER and RAY 1991), where the consensus nucleotides are C and A at positions −4 and −3, respectively (Figure 2).

Drosophila PRAT is most similar to chicken, human, and rat PRAT, with 62%, 61%, and 60% identity respectively. As discussed above, Drosophila PRAT appears to have a propeptide, although it is 18 rather than 11 amino acids long. Drosophila PRAT also has a domain at the carboxyl end of the sequence that includes three cysteine residues at positions 432, 493 and 500 that are also found in chicken, rat, human and *B. subtilis* PRAT (Figure 3). In the sequence containing cysteines 493 and 500, 11 out of 12 residues are conserved among Drosophila and the vertebrates. This cysteine-containing domain is responsible for the formation of an iron-sulfur cluster in *B. subtilis* (ZALKIN and DIXON 1992) and is important for propeptide processing in chickens (ZHOU *et al.* 1992).

Some features of the nucleotide sequence are as follows. Potential transcription initiation sequences found in Drosophila (HULTMARK *et al.* 1986) are at positions −185 and −152 (Figure 2). Two potential polyadenylation signal sequences (WICKENS 1990) are at sites 350 and 372 nucleotides downstream from the translation stop (Figure 2). Although the second sequence of AUUAAA is less commonly found than AAUAAA, it is almost as efficient as a polyadenylation signal in *in vitro* studies (WICKENS 1990), and in this case it is followed by a U-rich sequence which is important for cleavage (HUMPHREY and PROUDFOOT 1988). Taken together, the above data predict a transcript of approximately 2.1 kb. Northern blot analysis of polyadenylated RNA isolated from different stages of development shows a single message of this size (D. CLARK, unpublished data).

Localization of *Prat*: *In situ* hybridization of the PCR product clone to polytene salivary gland chromosome squashes showed a single site of hybridization on the right arm of chromosome three at polytene band 84E1-2 (not shown). This region had been extensively characterized and contains the *doublesex* (*dsx*) (BAKER and WOLFNER 1988; BAKER *et al.* 1991) and *lodestar* (*lds*) (GIRDHAM and GLOVER 1991) genes. The restriction map of BAKER and WOLFNER (1988) aligned with that of the λ clones isolated in the *Prat* screen. A physical map of the region containing *Prat*, *dsx* and *lds* is shown in Figure 4A. The positions of the distal breakpoints of the deficiencies dsx^{M+R2} (*R2*) and $Antp^{Ns+R17}$ (*R17*) (BAKER and WOLFNER 1988) are also shown. Note that the 3.5-kb *Eco*RI fragment containing the *Prat* open reading frame lies completely within the approximately 30-kb interval defined by the *R17* and *R2* deficiency breakpoints. Although many lethal complementation groups have been identified in the *dsx* region (Figure 4B) (BAKER *et al.* 1991), none is a candidate for alleles of *Prat* because none maps to the interval defined by the *R17* and *R2* breakpoints. The only mutationally identified locus that lies within this interval is *lds*, which encodes a protein important for chromosome segregation (GIRDHAM and GLOVER 1991).

Screen for *Prat* mutations: A screen for EMS-induced mutations that fail to complement the deficiency dsx^{M+R43} (R43; see Figure 4B) is outlined in Figure 5. The features of the purine syndrome phenotype chosen for the screen were wing venation defects and/or a decrease in red eye pigments. A *vermilion* (*v*) mutant background, which lacks brown eye pigments (LINDSLEY and ZIMM 1992), was chosen to facilitate the detection of a decrease in the red eye pigments. Such flies with decreased red eye pigments display an orange eye color. The parental third chromosome used for mutagenesis carried the *ebony* mutation e^{11}.

Approximately 21,000 F_1 individuals were screened and five independent mutations were isolated that complement *R17* and fail to complement *R2*, and thus map to the approximately 30 kb region containing *Prat* (Figure 4). These mutations also formed a single complementation group (data not shown) and were thus all candidate alleles of *Prat*. Generally, the mutant hemizygotes and heteroallelic combinations (*Prat⁻/R43*) have wing, leg and eye pigment defects of varying degrees. Examples of the wing phenotype are shown in Figure 6. The wing phenotype has variable penetrance and expressivity, with the most common characteristics of gaps in the posterior crossveins, incomplete L2 longitudinal veins, and irregular bristles along the posterior margin. The distal portions of legs are occasionally deformed (not shown). The bristle phenotype was not assessed. The red pigmentation in the eyes appears slightly reduced; however, it is not detectable in photographs. Four aspects of the mutations were characterized: sequence, enzyme activity, viability, and wing phenotype.

Sequence analysis of *Prat* mutations: Although the five mutations have the predicted phenotype and map genetically to the correct region, the possibility remained that these are mutations in another gene in the region. Indeed, at least two other transcribed regions of unknown function have been mapped in the interval between the R17 and R2 breakpoints (BAKER and WOLFNER 1988; GIRDHAM and GLOVER 1991). Direct proof that at least some of the mutations

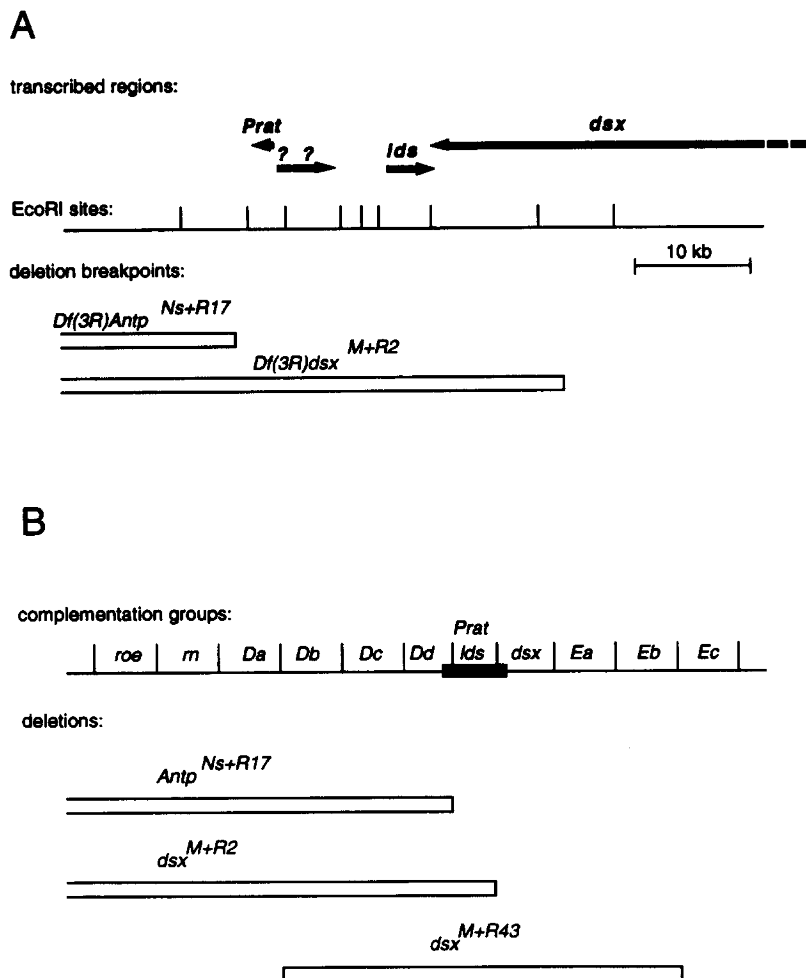

FIGURE 4.—(A) Physical map of the region around *Prat*. Transcript map taken from GIRDHAM and GLOVER (1991). Arrows indicate direction of transcription, if known. *Eco*RI site map and deletion breakpoints taken from BAKER and WOLFNER (1988). (B) Genetic map of the region around *Prat*. Black bar indicates the region shown in A. Complementation group and deficiency map taken from BAKER *et al.* (1991).

are alleles of *Prat* was found from sequence analysis. The sequence of the open reading frames of each allele was compared to that of the parental wild-type allele. Two out of five of the alleles (16A6 and 62B104) have point mutations resulting in amino acid substitutions at sites that are identical in the five species (Figure 3). These both lie in a region attributed with catalytic function and feedback regulation by purine nucleotides (ARGOS and HANEI 1983; MEI and ZALKIN 1990). 16A6 has an arginine for glycine substitution at a site that in *E. coli* has been shown to be important for feedback inhibition of the enzyme by guanine nucleotides (ZHOU *et al.* 1993). 62B104 has a substitution of phenylalanine for serine within the highly conserved region that contains a purine/pyrimidine phosphoribosyltransferase motif (BAIROCH 1992), which is presumably a site important for PRPP binding. A third allele, 12A19, has a leucine in place of serine or alanine. This site is within the domain necessary for glutamine-dependent activity as defined in *E. coli* (MEI and ZALKIN 1990). In this case, perhaps substitution with an amino acid with a larger side chain is the cause of the defect. No mutations were found in the coding regions of the other two alleles, 48A53 and 49A74. These presumably have lesions in transcriptional regulatory regions of the gene.

Enzyme assays of *Prat* mutants: To establish the relationship between enzyme activity and phenotype of three of the alleles, crude protein extracts were prepared from adult flies. Sufficient quantities of 12A19 and 48A53 hemizygotes were unavailable for the enzyme assays. PRAT activity of each extract, using either NH_3 or glutamine as a substrate, was assayed and calculated as a function of G6PDH activity to control for protein quantity and quality (see MATERIALS AND METHODS). PRAT activity in the mutant extracts is expressed relative to the activity in the wild-type extract so that correlations with viability data can be made (Table 1; see below). Using either NH_3 or glutamine as the substrate, the three mutations assayed yielded different PRAT activities, all lower than that of the wild-type allele.

Viability and the wing phenotype: The enzyme activities of FGARAT in $ade2^1$ flies and GART in $ade3^1$ flies are undetectable (HENIKOFF *et al.* 1986b), yet the visible phenotypes of these purine auxotrophic mutations are essentially wild-type (JOHNSTONE, NASH and NAGUIB 1985; KEIZER, NASH and TIONG 1989).

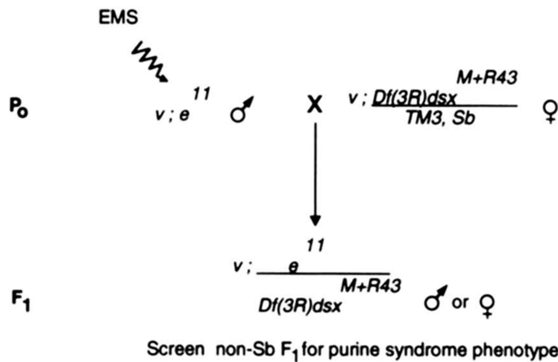

FIGURE 5.—Screen for *Prat* mutations.

To determine how activities of the *Prat* mutant enzymes correlate with the severity of their phenotype, the viability and wing phenotype of the mutants were scored.

Viability, or survival to adulthood, was measured as the ratio of mutant adults to wild-type siblings (nonbalancer/balancer sibs; see Table 1). This ratio was normalized to the viability of the parental e^{11} chromosome in a parallel cross to indicate the relative viability of *Prat* mutants. The wing phenotype was scored in the surviving mutant adult males and females with regard to whether or not there was a gap in the posterior cross vein of either one or both wings. Examples of mutant wings, including posterior cross vein gaps, are shown in Figure 6.

The data shown in Table 1 indicate that a reduction in PRAT activity to approximately 40% is sufficient to produce a purine syndrome phenotype, as measured by a reduction in viability and frequent wing defects. A comparison of the proportion of mutants with posterior cross-vein gaps to the enzyme activity and viability shows that 49A74 has a smaller proportion of cross vein gaps than would be expected, in comparison to the results for 16A6 and 12A19. The molecular basis for the 49A74 mutation was not determined and it is not a mutation in the amino acid coding region. Therefore, perhaps this disparity in the wing phenotype of 49A74 can be explained in terms of an alteration in either temporal or spatial regulation of expression.

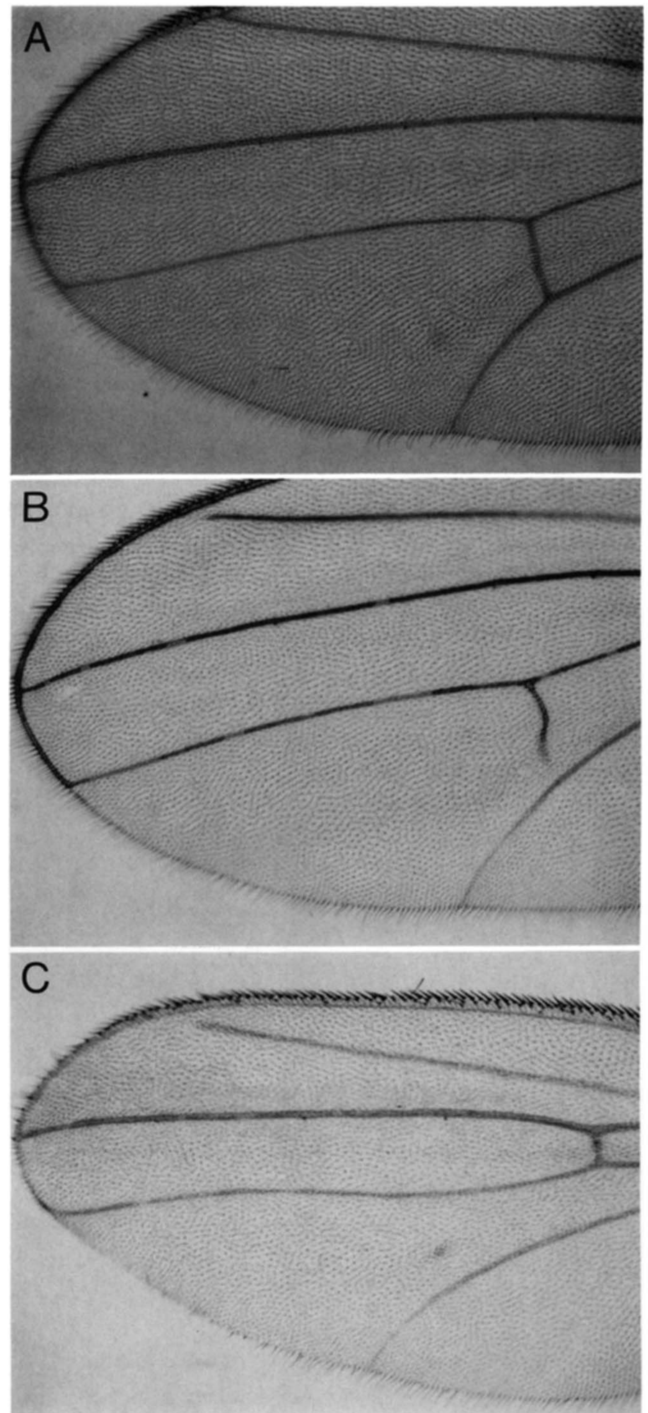

FIGURE 6.—Wing defects of two *Prat* mutants. (A) Wild-type (Amherst); (B) *v* ; *16A6* e^{11}/*R43*; (C) *v* ; *12A19* e^{11}/*R43*. The gap in the posterior cross-vein is the feature that was scored in Table 1.

DISCUSSION

By utilizing identities between the PRAT amino acid sequences from bacteria and yeast, Drosophila *Prat* was cloned. The PRAT amino acid sequence is most similar to the chicken, rat, human and *B. subtilis* sequences, which have a propeptide and iron-binding region. The organization of the gene is simple, with a coding region that is uninterrupted by introns. Extensive genetic and physical mapping in the region around *Prat* (BAKER and WOLFNER 1988; BAKER *et al.* 1991) and the phenotypic analysis of two other purine pathway genes *ade2* and *ade3* (TIONG and NASH 1990; TIONG *et al.* 1989) facilitated the isolation of five mutations in *Prat*. Analysis of these mutations supports the prediction that the *Prat* mutant phenotype is similar to those for *ade2* and *ade3*, genes encoding enzymes downstream in the pathway. A reduction in PRAT enzyme activity to about 40% of the wild-type activity is sufficient to produce a mutant pheno-

TABLE 1

Phenotypes of *Prat* mutants heterozygous with the deficiency dsx^{M+R43}

Allele	Relative enzyme activity[a]			Non-balancer/balancer sibs[b]		Relative viability[c]		Proportion of mutants with posterior cross vein gaps[d]
	1	2	3	1	2	1	2	
16A6	0.46	0.38	0.41	285/339	87/217	0.84	0.69	240/285 = 0.84
12A19	—[e]	—	—	21/482	46/1587	0.076	0.067	21/21 = 1.0
48A53	—	—	—	—	36/626	—	0.13	—
49A74	0.14	0.22	0.27	92/285	—	0.44	—	44/182 = 0.24
62B104	0.44	0.40	0.42	—	86/500	—	0.36	—
+[f]		1	1	463/377	241/339	1	1	4/463 = 0.009

[a] Enzyme activity was determined in three experiments as described in MATERIALS AND METHODS and is expressed as a proportion of the activity of the wild-type parental allele in each experiment. Experiments 1 and 2 were carried out with NH_3 as the substrate and experiment with L-glutamine as the substrate.

[b] Viability of *Prat* mutant hemizygotes was determined in two ways (crosses 1 and 2) which are described in detail in MATERIALS AND METHODS. Crosses 1 and 2 were between *Prat* mutant heterozygotes and dsx^{M+R43} heterozygotes, where both heterozygotes were carrying a dominantly marked chromosome *3* balancer. The progeny were scored with respect to the presence of the dominant balancer marker.

[c] The relative viability is the score for each *Prat* mutant chromosome (non-balancer/balancer sibs) normalized to the score for the parental e^{11} chromosome.

[d] While scoring the viability of mutants in cross 1, the presence of gaps in one or both posterior cross veins was scored.

[e] — = not determined.

[f] "+" indicates the wild-type e^{11} chromosome that was used in the mutant screen.

type, indicating that PRAT activity is limiting in Drosophila.

In contrast to the Drosophila gene which has no introns, the chicken PRAT gene has 10 introns (GAVALAS *et al.* 1993). In addition, the chicken PRAT gene shares a bidirectional promoter with the gene encoding aminoimidazole ribonucleotide carboxylase (AIRC) which performs steps 6 and 7 of the *de novo* purine pathway. The transcription start sites for these two genes are just 229 bases apart. No open reading frames in 2 kb of the sequence upstream of the Drosophila *Prat* open reading frame showed any apparent similarity with AIRC amino acid sequences (D. CLARK, unpublished results). Therefore, the Drosophila gene appears to be quite different from the chicken gene in its organization, possibly reflecting differences in the way expression is regulated.

The multiple sequence alignment (Figure 3) showed that Drosophila PRAT has a putative 18-amino acid amino-terminal propeptide. The 11-amino acid propeptides of *B. subtilis* and chicken PRAT are proteolytically processed to yield an enzyme with an amino-terminal cysteine (SOUCIET, HERMODSON and ZALKIN 1988; ZHOU *et al.* 1992). This cysteine together with aspartate at position 29 and a histidine at position 101 of the mature *E. coli* enzyme (boxed in Figure 2) form a catalytic triad motif that is typical of the *purF*-type of amidotransferases (WENG and ZALKIN 1987) and is essential for removal of the amide group from glutamine (MEI and ZALKIN 1989). These residues are conserved in Drosophila. As with *B. subtilis*, chicken, and rat PRAT, there is a conservative change from the aspartate to a glutamate residue in this motif.

In *B. subtilis*, the two glutamate residues in the propeptide upstream from the conserved cysteine, in addition to the cysteine itself, are necessary for propeptide processing as shown by site-directed mutagenesis experiments (SOUCIET *et al.* 1988). When the glutamate at position 10 was replaced by a valine, processing was abolished. These two glutamate residues are conserved in rat and chicken PRAT, suggesting that they are also important in these species for processing. If indeed Drosophila PRAT has a propeptide that is proteolytically processed, the fact that a histidine replaces one of these two glutamic acid residues and that the propeptide is longer suggest different requirements for propeptide processing in Drosophila.

Sequence analysis of the five *Prat* mutations identified three missense mutations. Two mutations, 16A6 and 62B104, lie in completely conserved regions in the catalytic portion of the enzyme, yet they produce a relatively mild mutant phenotype. On the other hand, the mutation 12A19 is a missense mutation that falls in a relatively less conserved region in the glutamine amide transfer portion of the protein. Even if this mutation eliminates the ability of PRAT to use glutamine as a substrate, one would predict that the 12A19 mutant enzyme would still be able to use NH_3 as a substrate and thus the phenotype should not be severe. Indeed, in *E. coli*, the first 237 amino acids (Figure 3) can be deleted and the enzyme is still functional with NH_3 as a substrate (MEI and ZALKIN 1990). Three explanations for this disparity are: (1) the 12A19 enzyme is unstable or unable to fold properly, (2) Drosophila PRAT cannot use NH_3 as a substrate *in vivo*, and (3) there is a second site mutation outside of the coding region that affects expression.

A prediction was made that mutations in *Prat* would have a similar phenotype as mutations in genes encod-

ing enzymes downstream in the pathway. This is a reasonable prediction since the pathway is essentially universal and there are no other known functions for the enzymes that perform steps 1 through 5. This prediction was correct in that *Prat* mutations have a purine syndrome phenotype characterized by pupal lethality, wing and leg defects, and a reduction in red eye pigments as is found for the semilethal *ade2* and *ade3* mutations (TIONG and NASH 1990; TIONG *et al.* 1989). Enzyme assays of protein extracts from three of the mutant lines showed that they had only a partial loss of function of PRAT, yet they all had reduced viability and wing defects. In contrast, GART activity is undetectable in $ade3^1$ mutants (HENIKOFF *et al.* 1986b), yet the flies are essentially wild-type save for purine auxotrophy. The molecular basis for the $ade2^1$ mutation is not known but, in any case, its FGARAT activity is undetectable (HENIKOFF *et al.* 1986b), and the flies are similar to $ade3^1$ mutants except for a reduction in eye pigments (JOHNSTONE *et al.* 1985). This correlation of the reduction in enzyme activity with the severity of phenotype in a given mutant leads to the implication that PRAT activity is present in limiting quantities in flies relative to GART and FGARAT. This is consistent with the view of PRAT as the rate-determining step and a regulatory point of *de novo* purine biosynthesis (WYNGAARDEN and KELLEY 1983).

In conclusion, the molecular cloning of *Prat* and mutagenesis of the endogenous gene provide a foundation for studying regulation of the gene and enzyme *in vivo*. An investigation into the regulation of this rate-limiting step in the pathway will contribute to understanding how the pathway is regulated as a whole in multicellular eukaryotes.

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LITERATURE CITED

ARGOS, P. A., and M. HANEI, 1983 A possible nucleotide-binding domain in the tertiary fold of phosphoribosyltransferases. J. Biol. Chem. **258:** 6450–6457.

ASHBURNER, M. (Editor), 1989 *Drosophila: A Laboratory Manual*, Ed. 1, Vol. 2, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

BAIROCH, A., 1992 PROSITE: a dictionary of sites and patterns in proteins. Nucleic Acids Res. **20** (Suppl.): 2013–2018.

BAKER, B. S., and M. F. WOLFNER, 1988 A molecular analysis of *doublesex*, a bifunctional gene that controls both male and female sexual differentiation in *Drosophila melanogaster*. Genes Dev. **2:** 477–489.

BAKER, B., G. HOFF, T. C. KAUFMAN, M. WOLFNER and T. HAZELRIGG, 1991 The *doublesex* locus of *Drosophila melanogaster* and its flanking regions: a cytogenetic analysis. Genetics **127:** 125–138.

BECKER, M. A., and M. KIM, 1987 Regulation of purine synthesis *de novo* in human fibroblasts by purine nucleotides and phosphoribosylpyrophosphate. J. Biol. Chem. **262:** 14531–14537.

CABOT, E. L., and A. T. BECKENBACH, 1989 Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comput. Appl. Biosci. **5:** 233–234.

CAVENER, D. R., and S. C. RAY, 1991 Eukaryotic start and stop translation sites. Nucleic Acids Res. **19:** 3185–3192.

CORPET, F., 1988 Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. **16:** 10881–10890.

DAIGNAN-FORNIER, B., and G. R. FINK, 1992 Coregulation of purine and histidine biosynthesis by the transcriptional activators BAS1 and BAS2. Proc. Natl. Acad. Sci. USA **89:** 6746–6750.

GAVALAS, A., J. E. DIXON, K. A. BRAYTON and H. ZALKIN, 1993 Coexpression of two closely linked avian genes for purine nucleotide synthesis from a bidirectional promoter. Mol. Cell. Biol. **13:** 4784–4792.

GIRDHAM, C. H., and D. M. GLOVER, 1991 Chromosome tangling and breakage at anaphase result from mutations in *lodestar*, a *Drosophila* gene encoding a putative nucleoside triphosphate-binding protein. Genes Dev. **5:** 1786–1799.

GLESNE, D. A., F. R. COLLART and E. HUBERMAN, 1991 Regulation of IMP dehydrogenase gene expression by its end products, guanine nucleotides. Mol. Cell. Biol. **11:** 5417–5425.

GRANDONI, J. A., R. L. SWITZER, C. A. MAKAROFF and H. ZALKIN, 1989 Evidence that the iron-sulfur cluster of *Bacillus subtilis* glutamine phosphoribosylpyrophosphate amidotransferase determines stability of the enzyme to degradation *in vivo*. J. Biol. Chem. **264:** 6058–6064.

HANS-ULRICK, M., B. SCHEIER, R. LAHTI, P. MÄNTSÄLÄ and G. H. BRAUS, 1991 Transcriptional activation of yeast nucleotide biosynthetic gene ADE4 by GCN4. J. Biol. Chem. **266:** 20453–20456.

HENIKOFF, S., 1987 Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155:** 156–165.

HENIKOFF, S., and M. EGHTEDARZADEH, 1987 Conserved arrangement of nested genes at the Drosophila *Gart* locus. Genetics **117:** 711–725.

HENIKOFF, S., M. A. KEENE, J. S. SLOAN, J. BLESKAN, R. HARDS and D. PATTERSON, 1986a Multiple purine pathway enzyme activities are encoded at a single genetic locus in *Drosophila*. Proc. Natl. Acad. Sci. USA **83:** 720–724.

HENIKOFF, S., D. NASH, R. HARDS, J. BLESKAN, J. F. WOOLFORD, F. NAGUIB and D. PATTERSON, 1986b Two *Drosophila melanogaster* mutations block successive steps of *de novo* purine synthesis. Proc. Natl. Acad. Sci. USA **83:** 3919–3923.

HULTMARK, D., R. KLEMENZ and W. GEHRING, 1986 Translational and transcriptional control elements in the untranslated leader of the heat-shock gene *hsp22*. Cell **44:** 429–438.

HUMPHREY, T., and N. PROUDFOOT, 1988 A beginning to the biochemistry of polyadenylation. Trends Genet. **4:** 243–245.

IWAHANA, H., J. OKA, N. MIZUSAWA, E. KUDO, S., II, K. YOSHIMOTO, E. W. HOLMES and M. ITAKURA, 1993a Molecular cloning of human amidophosphoribosyltransferase. Biochem. Biophys. Res. Commun. **190:** 192–200.

IWAHANA, H., T. YAMAOKA, M. MIZUTANI, N. MIZUSAWA, S., II, K. YOSHIMOTO and M. ITAKURA, 1993b Molecular cloning of rat amidophosphoribosyltransferase. J. Biol. Chem. **268:** 7225–7237.

JOHNSTONE, M. E., D. NASH and F. N. M. NAGUIB, 1985 Three purine auxotrophic loci on the second chromosome of *Drosophila melanogaster*. Biochem. Genet. **23:** 539–555.

KEIZER, C., D. NASH and S. Y. K. TIONG, 1989 The *adenosine2* locus of *Drosophila melanogaster*: clarification of the map position and eye phenotype of the *ade2-1* mutant. Biochem. Genet. **27:** 349.

KUBY, S. A., and E. A. NOLTMANN, 1966 Glucose 6-phosphate

dehydrogenase (crystalline) from brewers' yeast. Methods Enzymol. **9:** 116–125.

LINDSLEY, D. L., and G. G. ZIMM, EDS., 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego, Calif.

MAKAROFF, C. A., H. ZALKIN, R. L. SWITZER and S. J. VOLLMER, 1982 Cloning of the *Bacillus subtilis* glutamine phosphoribosylpyrophosphate amidotransferase gene in *Escherichia coli*: nucleotide sequence determination and properties of the plasmid-encoded enzyme. J. Biol. Chem. **258:** 10586–10593.

MANIATIS, T., R. C. HARDISON, E. LACY, J. LAUER, C. O'CONNELL and D. QUON, 1978 The isolation of structural genes from libraries of eukaryotic DNA. Cell **15:** 687–701.

MÄNTSÄLÄ, P., and H. ZALKIN, 1984 Glutamine nucleotide sequence of *Saccharomyces cerevisiae ADE4* encoding phosphoribosylpyrophosphate amidotransferase. J. Biol. Chem. **259:** 8478–8484.

MARTIN, D. W., JR., 1972 Radioassay for enzymic production of glutamate from glutamine. Anal. Biochem. **46:** 239–243.

MEI, B., and H. ZALKIN, 1989 A cysteine-histidine-aspartate catalytic triad is involved in glutamine amide transfer function in *purF*-type glutamine amidotransferases. J. Biol. Chem. **264:** 16613–16619.

MEI, B., and H. ZALKIN, 1990 Amino-terminal deletions define a glutamine amide transfer domain in glutamine phosphoribosylpyrophosphate amidotransferase and other PurF-type amidotransferases. J. Bacteriol. **172:** 3512–3514.

MESSENGER, L. J., and H. ZALKIN, 1979 Glutamine phosphoribosylpyrophosphate amidotransferase from *Escherichia coli*. J. Biol. Chem. **254:** 3382–3392.

MEYER, E., and R. L. SWITZER, 1979 Regulation of *Bacillus subtilis* glutamine phosphoribosylpyrophosphate amidotransferase activity by end products. J. Biol. Chem. **254:** 5397–5402.

OATES, D. C., and D. PATTERSON, 1977 Biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism: Characterization of Chinese hamster cell mutants defective in phosphoribosylpyrophosphate amidotransferase and phosphoribosylglycinamide synthetase and an examination of alternatives to the first step of purine biosynthesis. Somatic Cell Genet. **3:** 561–577.

ROLFES, R. J., and A. G. HINNEBUSCH, 1993 Translation of the yeast transcriptional activator GCN4 is stimulated by purine limitation: implications for activation of the protein kinase GCN2. Mol. Cell. Biol. **13:** 5099–5111.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SOUCIET, J.-L., M. A. HERMODSON and H. ZALKIN, 1988 Mutational analysis of the glutamine phosphoribosylpyrophosphate amidotransferase pro-peptide. J. Biol. Chem. **263:** 3323–3327.

TIONG, S. Y. K., and D. NASH, 1990 Genetic analysis of the *adenosine3* (*Gart*) region of the second chromosome of *Drosophila melanogaster*. Genetics **124:** 889–897.

TIONG, S. Y. K., C. KEIZER, D. NASH and D. PATTERSON, 1989 *Drosophila* purine auxotrophy: new alleles of *adenosine2* exhibiting a complex visible phenotype. Biochem. Genet. **27:** 333–348.

TSO, J. Y., H. ZALKIN, M. VAN CLEEMPUT, C. YANOFSKY and J. SMITH, 1982 Nucleotide sequence of *Escherichia coli purF* and deduced amino acid sequence of glutamine phosphoribosylpyrophosphate amidotransferase. J. Biol. Chem. **257:** 3525–3531.

WADA, K., S. AOTA, R. TSUCHIYA, F. ISHIBASHI, T. GOJOBORI and T. IKEMURA, 1990 Codon usage tabulated from the GenBank genetic sequence data. Nucleic Acids Res. **18** (Suppl.): 2367–2411.

WENG, M., and H. ZALKIN, 1987 Structural role for a conserved region in the CTP synthetase glutamine amide transfer domain. J. Bacteriol. **169:** 3023–3028.

WICKENS, M., 1990 How the messenger got its tail: addition of poly(A) in the nucleus. Trends Biochem. Sci. **15:** 277–281.

WYNGAARDEN, J. B., and W. N. KELLEY, 1983 Gout, pp. 1064–1114 in *Metabolic Basis of Inherited Disease*, edited by J. B. STANBURY, J. B. WYNGAARDEN and D. S. FREDRICKSON. McGraw-Hill, New York.

ZALKIN, H., and J. E. DIXON, 1992 *De novo* purine nucleotide biosynthesis. Prog. Nucleic Acid Res. Mol. Biol. **42:** 259–287.

ZHOU, G., J. E. DIXON and H. ZALKIN, 1990 Cloning and expression of avian glutamine phosphoribosylpyrophosphate amidotransferase: conservation of a bacterial propeptide sequence supports a role for posttranslational processing. J. Biol. Chem. **265:** 21152–21159.

ZHOU, G., S. S. BROYLES, J. E. DIXON and H. ZALKIN, 1992 Avian glutamine phosphoribosylpyrophosphate amidotransferase propeptide processing and activity are dependent upon essential cysteine residues. J. Biol. Chem. **267:** 7936–7942.

ZHOU, G., H. CHARBONNEAU, R. F. COLMAN and H. ZALKIN, 1993 Identification of sites for feedback regulation of glutamine 5-phosphoribosylpyrophosphate amidotransferase by nucleotides and relationship to residues important for catalysis. J. Biol. Chem. **268:** 10471–10481.

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