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Restriction of P-Element Insertions at the Notch Locus of *Drosophila melanogaster*

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P elements move about the *Drosophila melanogaster* genome in a nonrandom fashion, preferring some chromosomal targets for insertion over others (J. C. J. Eeken, F. H. Sobels, V. Hyland, and A. P. Schalet, Mutat. Res. 150:261–275, 1985; W. R. Engels, Annu. Rev. Genet. 17:315–344, 1983; M. D. Golubovsky, Y. N. Ivanov, and M. M. Green, Proc. Natl. Acad. Sci. USA 74:2973–2975, 1977; M. J. Simmons and J. K. Lim, Proc. Natl. Acad. Sci. USA 77:6042–6046, 1980). Some of this specificity may be due to recognition of a particular DNA sequence in the target DNA; derivatives of an 8-base-pair consensus sequence are occupied by these transposable elements at many different chromosomal locations (K. O'Hare and G. M. Rubin, Cell 34:25–36, 1983). An additional level of specificity of P-element insertions is described in this paper. Of 14 mutations induced in the complex locus Notch by hybrid dysgenesis, 13 involved P-element insertions at or near the transcription start site of the gene. This clustering was not seen in other transposable element-induced mutations of Notch. DNA sequences homologous to the previously described consensus target for P-element insertion are not preferentially located in this region of the locus. The choice of a chromosomal site for integration appears to be based on more subtle variations in chromosome structure that are probably associated with activation or expression of the target gene.

Certain classes of transposable elements in *Drosophila melanogaster* are mobilized at high frequency by interstrain crosses. This makes them useful for laboratory production of new *Drosophila* mutants. The best studied of these transposable elements is the P element, which appears to be mobilized in the germ line, but not in the soma, of offspring derived from interstrain crosses. These dysgenic crosses generally involve mating flies that have the transposable element (P strains) with flies that do not (M strains) (12).

Several newly recovered mutant alleles of the X-linked gene Notch were inspected for evidence of insertional mutagenesis. Stocks N^{BH9}, N^{DTV}, N^{PI}, $l(1)N^{27-3}$, N^{30-4}, $l(1)N^{37-10}$, $l(1)N^{42-2}$, and $l(1)N^{57-4}$ were produced by P-M hybrid dysgenesis (12) with the P strains Harwich and Pi. N^{D13}, N^{D16}, N^{D30}, $N^{24/46A/1}$, and $l(1)N^{32/25/B}$ occurred in dysgenic crosses with Green's P strain MR-h12 (4, 8), and $nd^{3.1072}$ arose in P-M crosses with a Vermeil/Madison-76 P male. The scheme of genetic crosses used to generate most of the new mutants has been reported elsewhere (4, 24). Table 1 lists the 14 dysgenic mutants and their associated phenotypes. Of these, 13 are lethal when hemizygous or homozygous. The remaining mutant, $nd^{3.1072}$, is viable in these combinations, has an adult wing-nicking phenotype, and is lethal in heterozygous combination with Notch deficiencies, making it indistinguishable from existing notchoid alleles of the Notch locus (13). The lethal mutations, when heterozygous with a wild-type gene, show either no mutant phenotype or various degrees of wing nicking (Table 1). The heterozygous wing-nicking phenotypes are comparable to those produced by the *N* subclass of Notch locus mutations (13).

We constructed physical maps of DNAs from the mutants by hybridizing segments of cloned Notch DNA to restriction digests of total genomic DNA from each of the mutants (Fig. 1, legend). Notch DNAs from 13 of the mutants were found to contain insertions, and all insertions had restriction maps characteristic of P elements or partially deleted P elements. The P elements were inserted in both orientations with respect to Notch and ranged in size from 0.5 to 2.9 kilobases (kb). Maps of these are shown in Fig. 1. Of the 13 P-element-associated mutations, insertion sites for 12 were indistinguishable from each other by restriction mapping (Fig. 1). All were located within 0.1 kb of the Notch transcription start site at position 78 of Fig. 2. The remaining insertion, associated with the mutation N^{D30}, mapped close to but

TABLE 1. Dysgenic mutants

Strain	Wing nicking in het.[a]	Affected chromosome[b]	Source[c]
N^{30-4}	Yes	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	1
$l(1)N^{57-4}$	No	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	1
$l(1)N^{42-2}$	No	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	1
$l(1)N^{27-3}$	No	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	1
N^{PI}	Yes	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	1
$l(1)N^{37-10}$	No	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	1
N^{DTV}	Yes	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	1
N^{D30}	Yes	*y mei-9*^{a} *mei-41*^{D5}	2
N^{D16}	Yes	*y mei-9*^{a} *mei-41*^{D5}	2
N^{D13}	Yes	*y mei-9*^{a} *mei-41*^{D5}	2
$N^{24/46A/1}$	Yes	Berlin K	2
$l(1)N^{32/25/B}$	No	Berlin K	2
N^{BH9}	Yes	*In(1)sc*^{8}, *sc*^{8} *B*	1
$nd^{3.1072}$	No	Vermeil	3
$nd^{3.1072rev}$	NA	Vermeil	3
$N^{30-4rev}$	NA	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	3
$l(1)N^{27-3rev}$	NA	*In(1)sc*^{8}, y^{31d} *sc*^{8} w^a	3

[a] Wing nicking in het. indicates the phenotype in females heterozygous for the mutation and a wild-type X chromosome. For $nd^{3.1072}$, which is viable, wing nicking is recessive. NA, Not applicable.

[b] X chromosome in which the mutation or reverse mutation was recovered.

[c] Sources of the strains were 1, J. P. Gergen, Princeton University, N.J. (see also reference 24); 2, A. Schalet, J. Eeken, and F. Sobels, State University of Leiden, The Netherlands (see reference 4); and 3, R. L. Berg and M. R. Kelley, this study.

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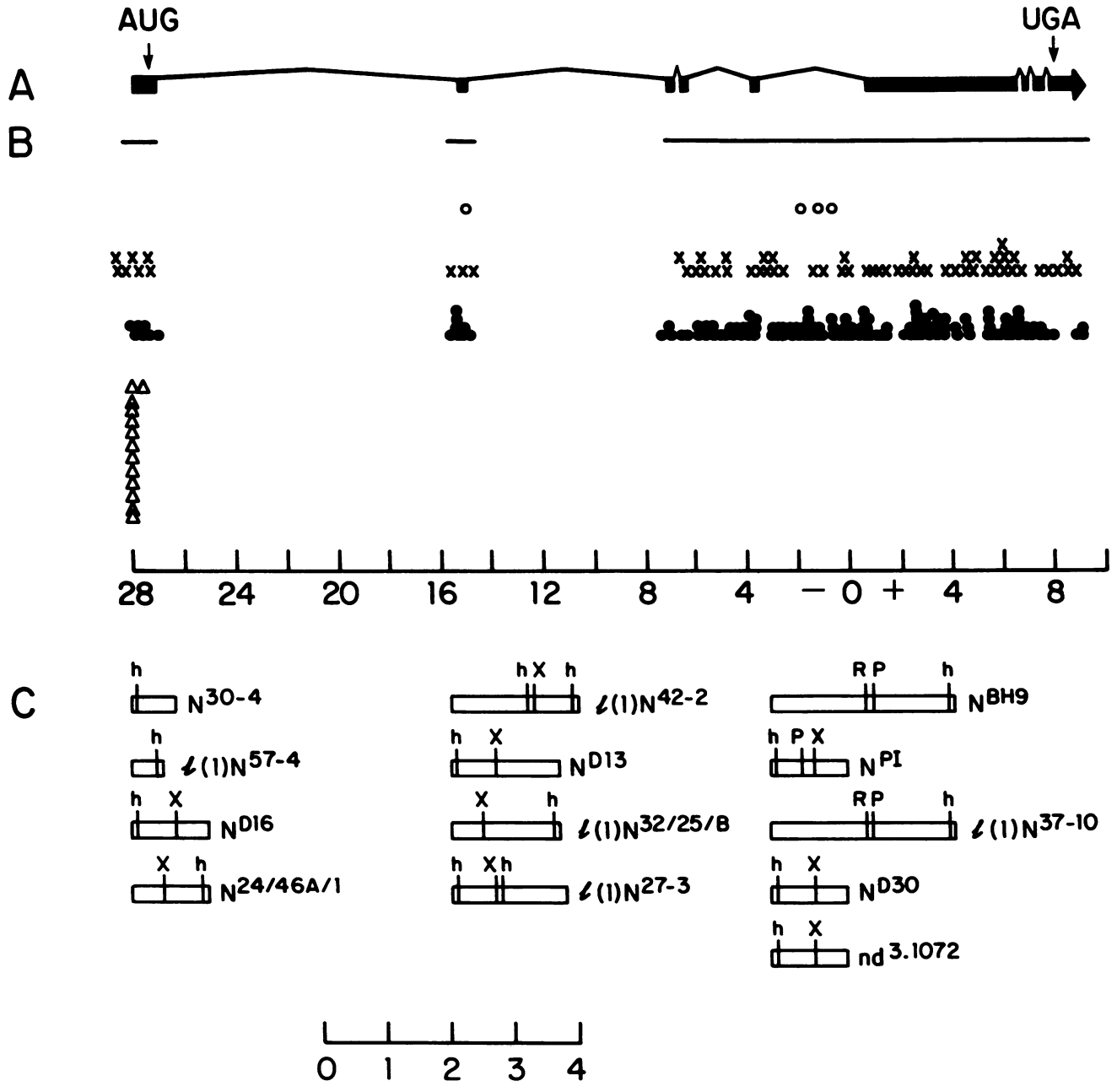

FIG. 1. Maps of P-element insertions at Notch. (A) Structure of the predominant 10.4-kb RNA transcribed from the Notch locus (from reference 10). (B) Horizontal lines indicate sequenced portions of the Notch locus (10). Beneath these are shown the distributions of sequences related to the P-element consensus target; Symbols: ○, a perfect match with an 8-bp target (see the text); ×, a match of 7 of 8 bp; ●, the 4-bp sequence CAGA. The search program has been described previously (10). The symbol △ represents a P-element insertion. The coordinate scale is in kilobases and is the same as that used earlier for reporting the restriction map and DNA sequence of the Notch locus (10, 11). In contrast to P-element insertions, copia-like insertion elements were found at locations throughout the Notch transcription unit (not shown). Mutations associated with copia-like elements include N^{55e11} (coordinate −26); *l(1)N* (−24); *fa* (−11); fa^{g} (−10); N^{264-40} (−6); N^{s} (−3); and $N^{264-109}$ (+4). Maps of these insertions at Notch have been presented elsewhere (11). (C) Open bars represent maps of P-element insertions at Notch. The scale below the maps is in kilobases. Abbreviations: h, *Hin*dIII; X, *Xho*I; R, *Eco*RI; P, *Pvu*II. In screening mutant genomic DNAs for insertion sequences, probes covered the 37-kb Notch transcription unit shown in A and 4.5 kb of the upstream sequence. All P-element insertions occurred at coordinate −28 as shown in B (see also the text).

downstream of that cluster, falling between positions 409 and 525 (Fig. 2 and legend). All insertions thus fell upstream of the Notch translation initiation codon at position 877.

Restriction mapping of DNA from each of the parental stocks used to produce the 13 insertion-associated Notch mutations indicated that P elements were not present in the upstream region of the gene before recovery of the new mutants. Spontaneous revertants of N^{30-4}, $l(1)N^{27-3}$, and $nd^{3.1072}$ ($N^{30-4rev}$, $l(1)N^{27-3rev}$, and $nd^{3.1072rev}$ in Table 1) also were isolated and mapped. Revertants of N^{30-4} and $l(1)N^{27-3}$ have lost the corresponding elements, resulting in restriction patterns indistinguishable from that of the wild type (data not

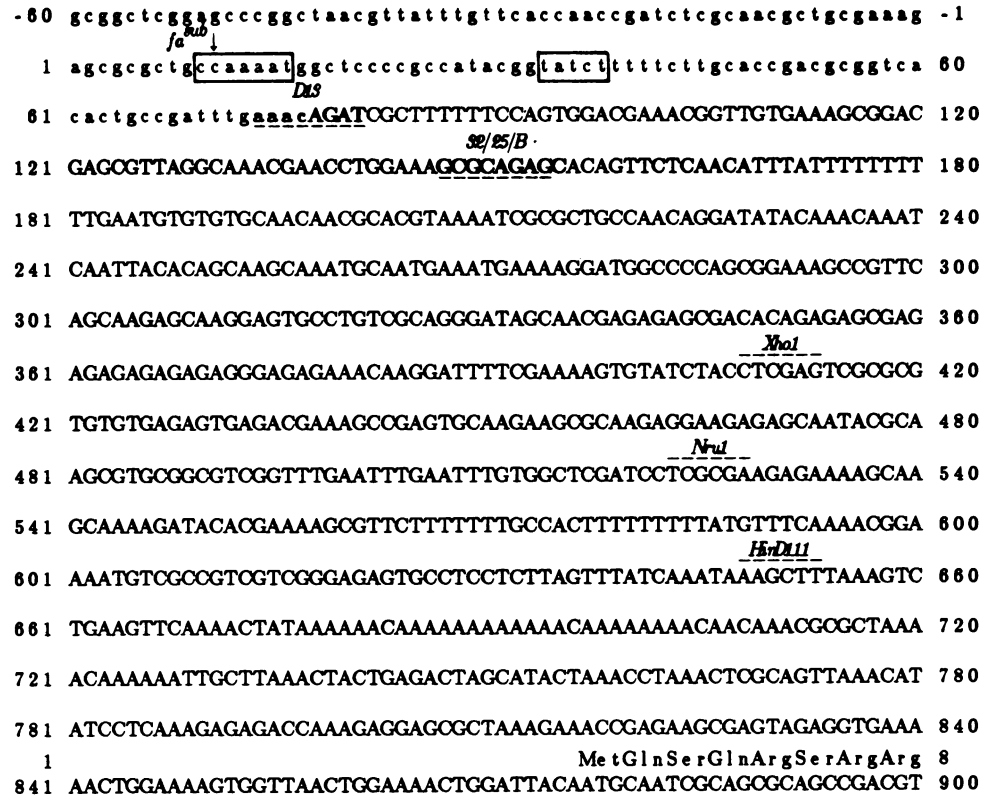

FIG. 2. Sequences of P-element insertion sites. Uppercase letters show the sequence of transcribed DNA (10). Lowercase letters show the sequence of the promoter region, and putative CAT and TATA elements are indicated in boxes (10). The location of the mutation fa^{swb} is shown at position 10 (10). Deletions that extend leftward from this position, through flanking genes *rst* and *vt*, fail to produce Notch-related phenotypes, so fa^{swb} should mark the 5′ limit of the locus (10, 21). DNAs from N^{D13} and $l(1)N^{32/25/B}$ were cloned in pEMBL19 (3, 10) and sequenced by conventional methods (2, 16). The 8-bp sequences that were duplicated by P-element insertions in the mutants are underlined. The *Xho*I, *Nru*I, and *Hin*dIII cleavage sites shown were used to locate most insertions. Twelve insertions (including those sequenced) mapped to the same interval, about 0.35 ± 0.1 kb left of *Xho*I. N^{D30} mapped between *Xho*I and *Nru*I. The first eight amino acids of the predicted Notch protein are indicated. Translation starts at nucleotide position 877.

shown). DNA from the revertant of $nd^{3.1072}$ retained about 200 base pairs (bp) of the original 1.2-kb insertion element (data not shown). Thus, the mutant phenotypes associated with all insertions should be caused by the integrated P elements.

DNAs from two of the mutants, $l(1)N^{32/25/B}$ and N^{D13}, were cloned so that sites of P-element insertions could be determined by DNA sequencing (Fig. 2, legend). Assuming that both P-element insertions created 8-bp duplications (15), the target sequence for $l(1)N^{32/25/B}$ was GCGCAGAG at position 147 and that for N^{D13} was AAACAGAT beginning at position 74 (Fig. 2). Nucleotide 5 of the N^{D13} target duplication corresponds to the site of Notch transcription initiation. Both target duplications share the sequence CAGA with the 8-bp consensus P-element target sequence GGCCAGAC (15). Since all exons and most introns at the Notch locus have been sequenced (10, 22), both DNA strands of the gene were searched for homology to the 8-bp consensus target sequence, the 8-bp target sequences of the two Notch mutants, and also CAGA. Perfect matches were found for two of the 8-bp sequences, GGCCAGAC (two matches) and AAACAGAT (two matches). A total of 61 homologies were found when a single mismatch was allowed for 8-bp sequences, and 136 perfect homologies to the CAGA sequence were seen. The locations of all of these homologies are shown in Fig. 1B. Clearly, clustering of P-element insertions is not due to restricted distribution of DNA sequences homologous to the consensus target.

The phenotypes generated by these P-element insertions are similar to those observed when Notch expression is reduced or the gene is deleted (13). Many reduced-function Notch mutations that do not involve P-element insertion were mapped in earlier molecular studies (11, 23). These included a number of putative point mutations and a variety of copia-like transposable-element insertions. No clustering of these mutations was observed; rather, they were distributed throughout the 37-kb Notch transcription unit in a fashion that appeared to follow regions of the gene that code for protein (10, 11, 23; see also Fig. 1, legend). Eight of the P-element-induced mutations described in this paper fall in the loss-of-function (*N*) subclass of Notch locus mutations (Table 1; reference 13). In earlier studies, *N* mutations mapped predominantly to protein-coding regions of the Notch locus, and none mapped to the 5′ regulatory region affected by the P-element insertions analyzed in this report (10, 11, 23).

Restriction of P-element insertions to the transcription initiation region of Notch is not strain specific. The mutations analyzed in this study were derived from three independent screens involving crosses with five P strains, and insertion mutations were recovered in X chromosomes from four distantly related laboratory stocks (Table 1).

Significant numbers of unselected P-element insertions recently have been reported at two other *Drosophila* loci for comparison to Notch. At *RpII215*, seven of nine independently derived P-element insertions map within 1 kb of the transcription start site (17), and each of five P-element insertions located thus far at rudimentary affects the 5′ end of the gene (20). Several classes of transposable elements (not related to P) have been mapped to the promoter regions of other *Drosophila* genes (9, 14, 19), but without the overwhelming degree of specificity seen for P elements. Thus, P elements appear to be atypical *Drosophila* transposons in their preference for transcription start sites.

As mentioned earlier, P-element insertion mutations occur with quite different frequencies at different genetic loci (4, 5, 7, 18), so there may be two levels of insertion specificity: (i) choice of a target gene and (ii) choice of a position within an affected gene. In trying to imagine how these specificities are achieved at Notch, rudimentary, and *RpII215*, it may be important to consider when the transposon and its targets are active during development. P elements are mobilized in germ cell precursors, and the ovaries of dysgenic flies develop abnormally (6, 12). Notch transcripts are found in early embryos and unfertilized eggs (1, 10, 11), and Notch RNA synthesis occurs at its highest levels, in adults, in ovaries (S. Kidd, unpublished data). *RpII215*, which codes for a subunit of RNA polymerase, and rudimentary, which produces three enzymes essential for pyrimidine synthesis and female fertility, also should be expressed in the germ line. Therefore, P elements, Notch, rudimentary, and *RpII215*, are probably active in the same cells. Clustering of insertions suggests that P-element integrations are facilitated by a chromosome structure, at the transcription start site, that accompanies gene activation. Affinity for a chromosome structure associated with genes active in the *Drosophila* germ line would explain both levels of insertion specificity.

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