Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10395–10399, November 1993
Genetics

Further evidence for function of the *Drosophila* Notch protein as a transmembrane receptor

(cell-fate regulation/neurogenesis/dominant gain of function)

DANIEL LYMAN* AND MICHAEL W. YOUNG*†

*Laboratory of Genetics and †Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021

Communicated by Norton D. Zinder, August 5, 1993 (received for review June 24, 1993)

ABSTRACT ***N* locus mutations associated with unusual mutant phenotypes were found to alter the structure of the encoded protein. Two mutations, N^{Co} and N^{60g11}, eliminate much of the cytoplasmic domain. N^{Co} can act as a null allele or as a competitive inhibitor of N^+ function, whereas N^{60g11} produces dominant gain of function in some cell types. This difference in function can be attributed to retention of cdc10/SWI6 repeats in the Notch^{60g11} protein. The results suggest a role for these repeats in intracellular signaling and are consistent with action of Notch as a receptor. nd^3 and $l(1)N^B$ alter extracellular epidermal growth factor-like and lin-12/Notch elements, respectively. nd^3 eliminates a conserved cysteine residue, so the mutation may result in complete loss of function for a single Notch epidermal growth factor element. N^{60g11} and $l(1)N^B$ produce related gain-of-function phenotypes. It is proposed that $l(1)N^B$ produces an extracellular modification of the protein that stimulates aberrant intracellular signaling by the Notch cytoplasmic domain.**

The *N* locus of *Drosophila* encodes a transmembrane protein that mediates cell-fate specifications during ectodermal and mesodermal development (1–6). In the ectoderm this protein (referred to as Notch) regulates a choice between neural and epidermal cell fates. Genetic mosaic studies have shown that higher levels of *N* activity are associated with dermoblast specification, whereas lower activity stimulates neuroblast differentiation (7–11). Thirty-six epidermal growth factor (EGF)-like elements and three cysteine-rich lin-12/Notch repeats compose most of the extracellular domain (≈1700 aa) of Notch (12–15). The cytoplasmic domain, ≈900 aa, includes six tandem cdc10/SWI6 or ankyrin repeats, sequences involved in protein–protein interactions in several systems (see ref. 16). Notch is similar in structure to proteins identified in *Xenopus* (17), human (18, 19), mouse (20), rat (21), and *Caenorhabditis elegans* (22, 23). Genetic studies of two *N*-related *C. elegans* genes, *lin-12* and *glp-1*, also indicate a role in cell-fate specification (24, 25).

Two models for Notch function have been proposed. (*i*) On the one hand, it has been suggested that Notch acts as a signal-transducing receptor that responds to interaction with a second transmembrane protein Delta (8, 10). (*ii*) An alternative suggestion also allows for extracellular interaction with a second protein such as Delta but envisions a more limited role as a cell adhesion molecule (for review, see refs. 9–11). A choice between these alternative roles should be informed by a functional study of segments of the Notch protein composing its extracellular and intracellular domains.

Null mutations and deletions of the *N* locus typically produce hypertrophy of the embryonic nervous system (a "neurogenic" phenotype) in hemizygotes and homozygotes and incision (notching) of the wing tips in *N*/+ heterozygous adults. Heterozygous adults also tend to overproduce some thoracic bristles (microchaetae), which reflects hypertrophy of the peripheral nervous system. These effects are suppressed by duplication of the *N* locus [i.e., *N*/+; *Dp*(*1*)N^+] (26). Some *N* point mutations are associated with atypical phenotypes. For example, in earlier work distinctive gain-of-function alleles, the split (*spl*) and Abruptex (*Ax*) mutations, were found to be caused by single-amino acid substitutions in certain EGF-like elements of Notch (27, 28). Further analysis has shown that these mutations can affect the molecular interaction of the extracellular domains of Notch and Delta (29).

Four mutations are characterized in this study, the dominant mutation Notch-Confluens (N^{Co}), two gain-of-function mutations N^{60g11} and $l(1)N^B$, and a hypomorphic mutant nd^3. A comparison of the structures of these altered proteins and the different mutant phenotypes they produce indicates that Notch functions as a receptor. Surprisingly, signal transduction appears to require little more than the repeated cdc10 elements of the cytoplasmic Notch domain. As these sequences are involved in protein–protein interactions in many systems (16), activation of Notch presumably involves a specific protein association with the cdc10 repeats.

MATERIALS AND METHODS

DNA Cloning and Sequencing. Genomic DNA fragments were isolated for N^{Co} (*Sac* I, nt 12,160–17,808; ref. 13) and $l(1)N^B$ (*Eco*RI/*Xho* I, nt 8,983–14,966; ref. 13) and subcloned as sets of overlapping deletion derivatives for direct sequencing in pBluescript SK (Stratagene) or M13mp18/19 vectors (New England Biolabs). The complete sequence was obtained for nt 12,160–17,520 in the case of N^{Co} and for nt 10,747–14,966 [$l(1)N^B$]. To obtain the N^{60g11} sequence, genomic DNA from N^{60g11}/Ax^{E2} heterozygotes was amplified by PCR and sequenced with methods suggested by C. Wesley, Rockefeller University, New York (C. Wesley and W. F. Eanes, unpublished work) or the CircumVent (New England Biolabs) method. Regions of sequence divergence for N^{60g11} and Ax^{E2} were further analyzed by cloning PCR-amplified N^{60g11} DNA for direct sequencing. A *Xho* I–*Cla* I fragment (nt 1994–2918), which includes exons 3 and 4 (13), was cloned from nd^3-carrying flies. The cloned nd^3 DNA was amplified by PCR and sequenced by using the chain-termination method, as described (29). The C^{105} substitution was confirmed by also sequencing cloned genomic nd^3 DNA (nt 1994–2526).

In addition to the N^{Co} mutation depicted in Fig. 1, this allele differs from the Canton S sequence (13) at three positions. Thr-1561 (ACG) has been changed to serine (AGC), Ser-2257 (AGT) has been changed to glycine (GGT), and a glutamine (CAG) insertion was found between Gln-2567 and Gln-2568 in the strep/opa repeat (12, 13). All three

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: EGF, epidermal growth factor.

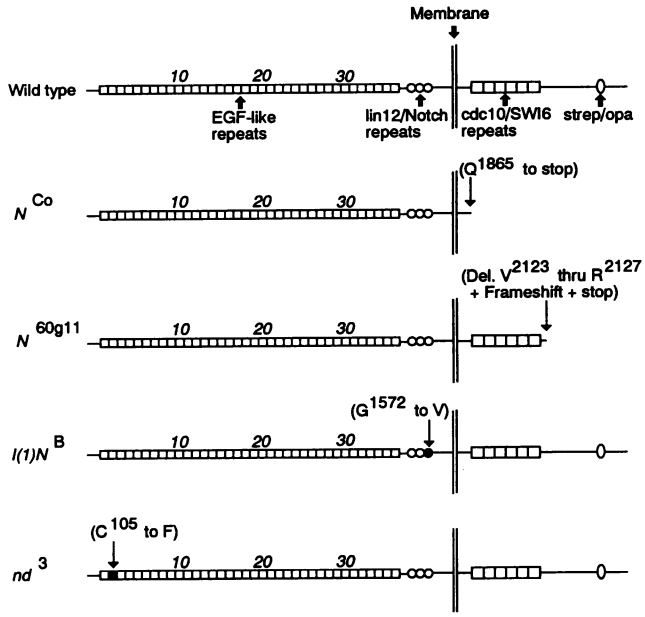

FIG. 1. Predicted structures of mutant Notch proteins encoded by N^{Co}, N^{60g11}, $l(1)N^{B}$, and nd^{3}. Map at top depicts structure of wild-type Notch protein. Notch crosses the cell membrane once at the location indicated (Membrane). Extracellular sequences (including the N terminus) of the transmembrane protein are at left. For the extracellular domain, 36 tandemly repeated EGF-like sequences are indicated by numbered boxes. Three cysteine-rich repeat sequences characteristically found in Notch-related proteins from other species are indicated for the extracellular domain as lin-12/Notch repeats. The intracellular domain includes six cdc10/SWI6 repeats (boxes). A polyglutamine sequence referred to as strep/opa is indicated by an oval. Maps below show changes associated with each of the mutations; maps are not drawn to scale. For example, cytoplasmic cdc10/SWI6 repeats comprise ≈200 amino acids of the 900-aa intracellular domain. In N^{Co} the codon for glutamine at aa 1865 has been converted to a stop codon. In N^{60g11} a 16-bp deletion (see text) would remove sequences coding for 5 amino acids (Val-2123–Arg-2127) and introduce a frameshift resulting in translation of 19 different amino acids before termination of the protein. In $l(1)N^{B}$ the Val-1572 substitution affects the third lin-12/Notch repeat. In nd^{3} Cys-105 (TGC) has been changed to phenylalanine (TTC) in the second Notch EGF-like repeat. Del., deletion.

changes appear to be silent polymorphisms: Ser-1561 also has been found in nd^{3} and $l(1)N^{B}$ (indicated below and in text) and in fa^{s88} and nd^{1} (D.L., unpublished observation), and Gly-2257 was previously observed in several *Ax* mutants and in the *spl* mutant (27). In each case the substitutions have been shown not to be responsible for the mutant phenotype (refs. 27, 30 and 31). The glutamine insertion after Gln-2567 in N^{Co} also was found in association with the nd^{1} allele (D.L., unpublished observation). The nd^{1} mutation has been mapped to another region of the gene (30). A silent Ser-2257 (AGT) → Gly (GGT) substitution was detected in the N^{60g11} allele (see text). Two amino acid substitutions distinguish $l(1)N^{B}$ from Canton S. Notch. Thr-1561 (AGC) has been changed to serine (ACG) in $l(1)N^{B}$, and Gly-1572 (GGC) has been changed to valine (GTC). As described above for N^{Co}, Ser-1561 has been detected as a silent polymorphism in several *Drosophila melanogaster* strains.

Developmental Studies. Wing and bristle phenotype were examined in adult flies reared at 25°C on standard cornmeal agar, unless otherwise indicated. For bristle counts in Table 1, adult flies were examined live by using a stereo dissecting microscope, and all bristles on the mesonotum were counted and recorded in the table (counts are for microchaetae). All *Drosophila* strains are described in Lindsley and Zimm (26) or in the text.

Table 1. Suppression or enhancement of mesonotal microchaetae formation in *N* mutant heteroallelic combinations

Genotype	$\bar{x}$ ± SD	*n*	% WT
$N^{60g11}/+$	283 ± 29	10	—
$N^{60g11}/+$ (29°C)	252 ± 21	10	—
$N^{60g11}/+$ (21°C)	210 ± 14	10	83
N^{60g11}/Ax^{9B2}	173 ± 25	7	68
Ax^{9B2}/Ax^{9B2}	275 ± 28	9	—
$l(1)N^{B}/+$	145 ± 18	7	57
$l(1)N^{B}/Y;Dp(N^{+})$	70 ± 31	8	27
$l(1)N^{B}/Ax^{9B2}$	66 ± 34	10	26
$N^{Co}/+$	353 ± 29	8	138
N^{Co}/Ax^{9B2}	338 ± 36	7	133
$N^{264\text{-}40}/+$	286 ± 35	9	—
$N^{264\text{-}40}/Ax^{9B2}$	283 ± 32	9	—
Canton S (WT)	260 ± 21	8	—
Oregon R (WT)	247 ± 14	8	—

Data summarize the mean number ($\bar{x}$) of small bristles (microchaetae) on the mesonotum of adult flies with the indicated genotypes. Unless otherwise noted, all crosses were made at room temperature (25°C) to obtain the reported phenotypes. *n*, Number of adult flies examined; % WT, value of $\bar{x}$ from mutant expressed as percentage of a combined $\bar{x}$ value of the Canton S and Oregon R means [—, no significant difference from wild type (WT)]. Gain-of-function phenotypes associated with $l(1)N^{B}$ and N^{60g11} also are enhanced in trans by Ax^{16172}, Ax^{E2}, Ax^{28a}, Ax^{71d} (data not shown).

RESULTS

Notch-Confluens. N^{Co} arose spontaneously (26). Homozygous and hemizygous embryos exhibit a strong neurogenic phenotype (ref. 26 and D.L., unpublished observation). Heterozygous adults ($N^{Co}/+$) produce a wing phenotype resembling the dominant Confluens (*Co*) phenotype associated with *N* locus duplication: Wings are seldom notched; instead wing veins are abnormally thickened and expand to form deltas at the junction with the wing tip (26). This phenotype also is similar to that seen upon heterozygous deletion of Delta (*Dl*). Like *Dl* mutants, N^{Co} heterozygotes markedly overproduce thoracic microchaetae (Table 1). Overproduction of microchaetae in $N^{Co}/+$ flies is more severe than that generally observed for $N/+$ heterozygotes (e.g., $N^{264\text{-}40}/+$, Table 1). Unlike other N^{-} mutations, an extra dose of N^{+} enhances rather than suppresses the N^{Co} wing phenotype (26). We have not studied the effects of such N^{+} duplications on the thoracic bristle phenotype of N^{Co} flies.

Gene-dosage interactions occur between *N* and *Dl* (32, 33). For example, the heterozygous loss-of-function phenotype for *Dl* is enhanced by a duplication of *N* and is suppressed by heterozygous *N* null mutation or deletion. This behavior does not hold for N^{Co}. Rather, N^{Co} enhances the phenotype of hypomorphic and null alleles of *Dl* (ref. 34 and D.L., unpublished observation). Thus, in homozygotes or hemizygotes, N^{Co} acts like a null mutant, but in heterozygotes it behaves neither as a null nor as a hypomorphic *N* allele, in response to either *N* duplication or *Dl* deletion.

N^{Co} has been mapped by genetic recombination to the interval separating two previously sequenced mutations Ax^{9B2} and nd^{2} (27, 30, 35, 36). N^{Co} DNA encompassing the sites of these mutations was sequenced (see Fig. 1, legend), and several nucleotide substitutions were detected that would distinguish the protein from that of the wild-type strain, Canton S (13). All but one of the substitutions have been observed in other *D. melanogaster* strains and cannot, therefore, be responsible for the N^{Co} phenotype (see *Materials and Methods*). The remaining substitution, a nonsense mutation at Gln-1865 (CAG → TAG; Fig. 1), lies midway between the Notch transmembrane domain and the cdc10/SWI6 repeats. This stop codon would delete all but 98 amino acids of the intracellular domain of the Notch protein (Fig. 1).

As this is the only unique sequence change at *N*, it should be responsible for the N^{Co} phenotype.

Notch-60g11. N^{60g11} is an x-ray-induced, embryonic lethal (26). The wing phenotype of $N^{60g11}/+$ heterozygotes is temperature sensitive. Strong notching is produced by development at 29°C, but the wings are close to wild type at 21°C. Heterozygotes also show a temperature-dependent, spl-like, dominant eye phenotype with aberrantly arranged ommatidia at 21°C but show normal eye morphology at 29°C (26). This roughened-eye phenotype is enhanced by Enhancer of split-Dominant [$E(spl)^D$, S. Kidd, personal communication]. Homozygous N^{60g11} females carrying a duplication of N^+ are viable at 29°C but, unlike *N* deletions, are lethal at 21°C. Thus, at low temperature, females with one wild-type *N* locus can survive with one copy but not with two copies of N^{60g11} (26).

As mentioned earlier, null mutation or heterozygous deletion of *N* causes some overproduction of adult thoracic bristles, and this adult neurogenic phenotype results from mild hypertrophy of some elements of the peripheral nervous system (26). In contrast, Table 1 shows that N^{60g11} suppresses bristle development in a temperature-dependent fashion. Microchaetae are underproduced in $N^{60g11}/+$ heterozygotes at 21°C but are found in normal numbers when development occurs at 29°C. This antineurogenic activity at lower temperatures suggests that N^{60g11} produces a dominant gain rather than loss of function. At higher temperatures the protein appears to lose function and so resembles other null or N^- alleles. Fig. 2 and Table 1 show that both the dominant eye and bristle phenotypes associated with N^{60g11} are enhanced in trans by *Ax* mutations at low temperature (21–25°C). As earlier work has indicated that the *Ax* mutations are gain-of-function alleles at *N* (27, 37, 38), these interactions further suggest that N^{60g11} is associated with hypermorphic or neomorphic *N* function at low temperature and loss of function at 29°C.

N^{60g11} has been genetically mapped between N^{Co} and nd^2 (36). Because N^{Co} was physically placed in this study and nd^2 was previously located (30), N^{60g11} was mapped by sequencing genomic DNA corresponding to the N^{Co}–nd^2 interval of the N^{60g11} allele (see Fig. 1). Comparison of the N^{60g11} sequence and the sequence of Canton S *N* revealed changes at two locations. A nucleotide substitution was found that would change Ser-2257 (AGT) to glycine (GGT). This substitution was previously found on the Ax^{E2}-containing chromosome, where the substitution was not associated with the mutant phenotype (27). The second alteration in N^{60g11}, a 16-bp deletion removing nt T^{15442}–A^{15457} inclusive (5′-TGTGGCCAGCGAGCGA-3′), is shown in Fig. 1. The deletion affects the *N* coding sequence beginning seven amino acids after the cdc10/SWI6 repeats and produces a frameshift of the remaining coding sequence. Nineteen different amino acids should be translated before the termination codon is reached ($TGA^{15516-18}$). Thus, most of the Notch intracellular domain would be deleted in N^{60g11} (Fig. 1).

lethal-1-Notch-B. $l(1)N^B$ was generated by x-ray mutagenesis. Mutant males and females die as embryos or during the first larval instar. Embryos do not show a neurogenic phenotype (refs. 26 and 39; D.L., unpublished observation). $l(1)N^B/N^+$ females do not have notched wings but have reduced eyes and underproduce thoracic microchaetae (Table 1; ref. 26). $l(1)N^B$ males can be rescued by a duplication of N^+ but still underproduce thoracic bristles. In fact, underproduction of microchaetae in $l(1)N^B$; $Dp(1)N^+$ males is more severe than in heterozygous females (Table 1; ref. 26). We have not studied the bristle phenotype associated with a comparable N^+ duplication in females (i.e., $N^+/l(1)N^B$;$Dp(1)N^+$). A further indication that $l(1)N^B$ is associated with hypermorphic or neomorphic *N* function comes from genetic complementation studies with hypomorphic *N* alleles nd^1 and nd^3. $l(1)N^B/nd^1$ and $l(1)N^B/nd^3$ heterozygotes continue to underproduce microchaetae as in $l(1)N^B/+$, and the wing nicking observed for nd^1 and nd^3 homozygotes is suppressed in these heterozygotes (D.L., unpublished observation). Also, a genetic interaction is seen between $l(1)N^B$ and the *Ax* alleles (Table 1). Underproduction of macrochaetae and microchaetae is strongly enhanced in $l(1)N^B/Ax^{9B2}$ heterozygotes (Fig. 2 and Table 1), and the heterozygotes produce an exaggerated *Ax* wing phenotype (gapping of wing veins L2, L4, and L5; data not shown). Thus, $l(1)N^B$ enhances rather than suppresses gain-of-function phenotypes previously associated with *Ax*.

Genetic recombination has placed $l(1)N^B$ between *spl* and N^{Co} (47). $l(1)N^B$ was physically located by sequencing the *spl*–N^{Co} interval on the $l(1)N^B$ chromosome (Fig. 1). Two substitutions were detected, only one [Gly-1572 (GGC) → Val (GTC)] of which could cause the $l(1)N^B$ phenotype (Fig. 1 and *Materials and Methods*). This substitution would alter the structure of the third lin-12/Notch repeat of the extracellular domain of the protein (Fig. 1).

notchoid-3. nd^3 was x-ray induced (26). Homozygous and hemizygous nd^3 flies are viable and fertile and exhibit thickened wing veins with deltas and variably notched wings. The wing phenotype resembles that seen in N^+/N^- heterozygotes (26). A further indication that nd^3 is a hypomorphic *N* allele comes from complementation tests with *N* locus deficiencies and null alleles. Heterozygous nd^3/N^- flies are usually inviable; rare escapers have an exaggerated N phenotype and are sterile (26). The hypomorphic *N* alleles, nd^1 and nd^2, produce mutant phenotypes resembling nd^3. nd^1 and nd^2 have been physically mapped, and both affect the structure of the intracellular domain of the Notch protein (30). Heterozygous combinations of these alleles and nd^3 produce the nd^3 phenotype (26).

No evidence for DNA insertion or deletion at the *N* locus has come from restriction mapping of nd^3 DNA (40). Genetic recombination mapping placed nd^3 very close to the mutation N^{264-40} (one recombinant in 75,100 tested chromosomes, or 0.003 map unit distal to N^{264-40}) (41). N^{264-40} is associated with an insertion at the proximal edge of two clustered microexons of *N* (exons 3 and 4) (13, 40). As physical and genetic maps have been shown to be well correlated at *N* (≈275 kb per map unit) (27, 31, 40), we supposed that nd^3 might be found in the vicinity of exons 3 and 4. Therefore, a genomic, *Xho* I–*Cla* I fragment (nt 1994–2918), which includes exons 3 and 4, was recovered and sequenced from nd^3 flies (Fig. 1). The restriction fragment also includes 375 nt of intron DNA upstream of exon 3 and 22 nt downstream of exon 4 (13). A single coding sequence change was found in exon 3; Cys-105 (TGC) → Phe (TTC). Cys-105 corresponds to the second cysteine residue in the second EGF-like repeat of the Notch protein (Fig. 1; refs. 12 and 13). Sequence analyses of *N*-like genes from *C. elegans*, *Xenopus*, rat, mouse, and human indicate that among widely divergent species, homologous cysteine residues are absolutely conserved (12, 13, 17–23), and in EGF itself all cysteines are known to be involved in disulfide bonding critical to hormone function (42, 43). The results suggest that the Cys-105 substitution is the basis of the nd^3 mutation.

DISCUSSION

Earlier work has shown that different EGF-like elements of the Notch protein have different functions (27, 28). Each of six Abruptex (*Ax*) alleles, all of which cause dominant underproduction of wing sensillae and have related, dominant wing-vein-gapping phenotypes, correspond to amino acid substitutions in 6 neighboring EGF elements of 36 elements comprising the extracellular domain of Notch (elements 24–29) (27, 28). *spl*, which causes a roughened-

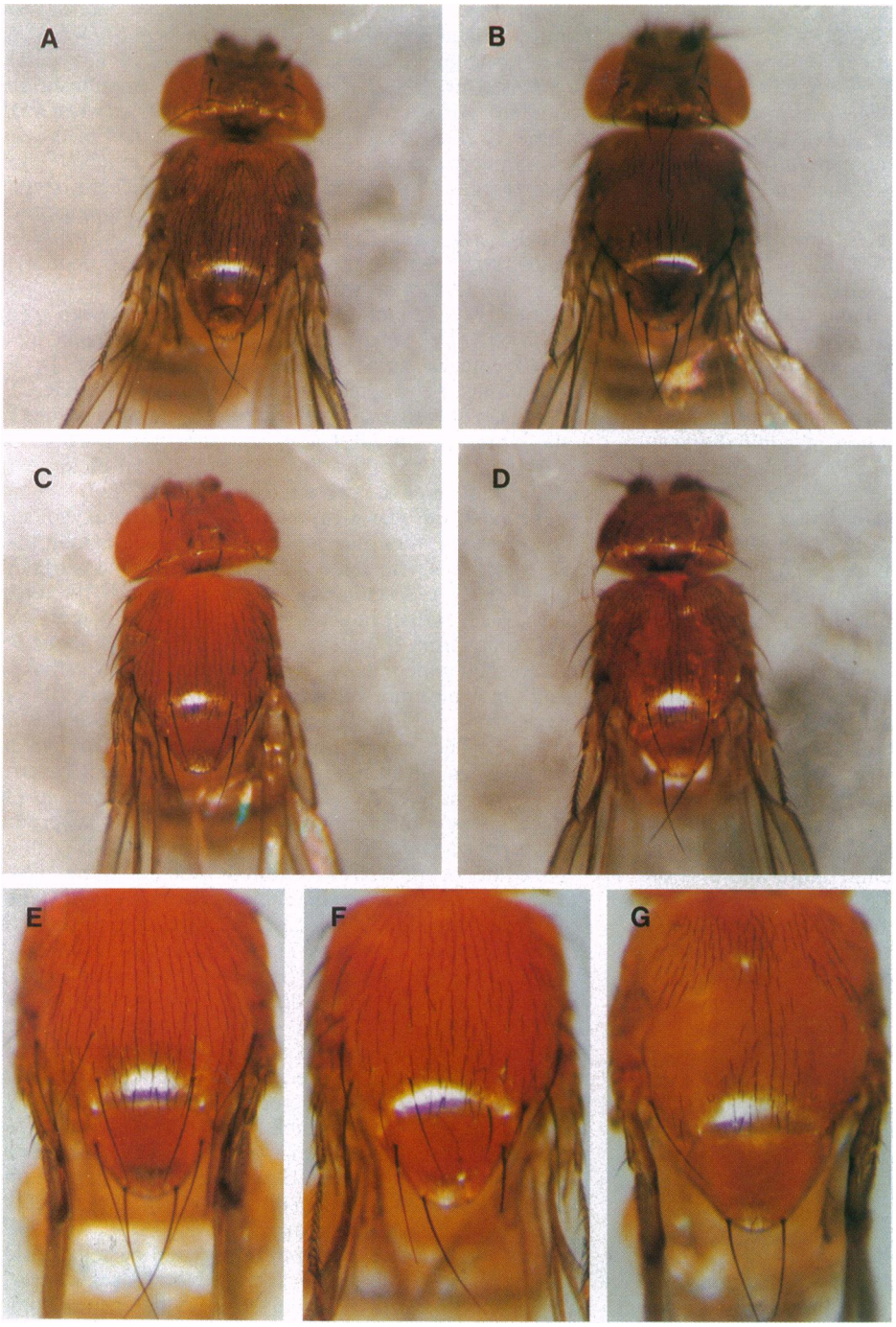

FIG. 2. Abruptex enhancement of N^{60g11} and $l(1)N^{B}$ phenotypes. (*A*) Ax^{9B2} homozygote. (*B*) $l(1)N^{B}/Ax^{9B2}$. (*C*) $N^{60g11}/+$. (*D*) N^{60g11}/Ax^{9B2}. (*E–G*) Higher magnification views of thoracic bristle patterns from wild type (Canton S), $N^{60g11}/+$, and N^{60g11}/Ax^{9B2}, respectively. Note also differences in eye phenotypes for *C* and *D*.

eye phenotype, is associated with an amino acid substitution in the 14th Notch EGF element, some distance from the Abruptex mutable region of Notch (27, 28). The analysis of nd^{3} provides further evidence for this differentiation of function. The hypomorphic, notched-wing phenotype of nd^{3} is distinct from *spl* and *Ax*, and the mutation appears associated with a cysteine substitution in the second Notch EGF-like element. It is difficult to imagine how the structure of an EGF-like element could be more severely affected by amino acid substitution. Cysteine substitutions were previously associated with two *Ax* mutations, Ax^{59b} and Ax^{59d} (27), and although most *Ax* alleles are viable as homozygotes, Ax^{59b} and Ax^{59d} are both lethal (26). Possibly the nd^{3}, Ax^{59b} and Ax^{59d} phenotypes correspond to complete loss of function for each of the affected EGF-like elements of the Notch array.

N^{Co} is associated with a deletion of most of the intracellular domain of Notch. Although superficially similar in phenotype to N^{+} duplication, genetic analyses indicate that this allele cannot suppress the neurogenic phenotype associated with loss of *N* function, but, like N^{+}, N^{Co} can enhance the Delta phenotype of Dl^{-}/Dl^{+} (i.e., $N^{Co}/N^{+};Dl^{-}/Dl^{+}$) heterozygotes. This unexpected behavior appears linked to retention of the extracellular sequences in the Notch-Confluens protein. Notch and Delta proteins can interact *in vitro* and *in vivo* through sequences composing their extracellular domains (29, 44, 45), and evidence presented elsewhere suggests a role for such extracellular interactions in intracellular signaling by Notch (10, 11, 29). A Notch-Confluens protein capable of interaction with Delta but unable to produce an intracellular response in the absence of a cytoplasmic Notch domain might behave as a competitive inhibitor of Delta. This interpretation

would be consistent with the dominant Confluens wing phenotype and the substantial overproduction of microchaetae observed in $N^{Co}/+$ flies. In fact, a Confluens (Delta-like) phenotype might be expected for a mutant that interferes with wild-type Delta protein function.

Physical mapping of the N^{60g11} mutation predicts a protein that resembles N^{Co};N^{60g11} also retains the wild-type extracellular Notch sequence and has lost most of the cytoplasmic domain. However, for N^{60g11} loss of intracellular sequence occurs after the Notch cdc10/SWI6 repeats, so that all of these repeated sequence elements are retained in the mutant. Unlike N^{Co}, at low temperature N^{60g11} shows a dominant gain of function. The rough-eye phenotype of $N^{60g11}/+$ flies resembles the gain-of-function mutant split (46) and is enhanced by the dominant third chromosome mutation Enhancer of split-Dominant, $E(spl)^D$. $N^{60g11}/+$ flies also underproduce bristles (microchaetae), in contrast to the overproduction of these bristles that would be associated with heterozygous deletion or null mutation of N (Table 1). As shown in this study, both the underproduction of thoracic bristles and the spl-like eye phenotype are enhanced by N gain-of-function mutations such as the Ax alleles. The distinctly different phenotypes produced by N^{Co} and N^{60g11} suggest that the extended cytoplasmic domain of the Notch^{60g11} protein retains some intracellular activity lost in Notch^{Co} and that in some cell types [e.g., bristle mother cells (4, 37)] this cytoplasmic function might be inappropriately regulated to give the dominant gain-of-function phenotypes. It seems likely from a comparison of the structures and activities of the Notch^{Co} and Notch^{60g11} proteins that the Notch cdc10/SWI6 repeat sequences play a role in intracellular signaling by the Notch protein. Recent studies, involving transgenic *Drosophila* embryos expressing truncated forms of Notch under heat shock control, also are concordant with this view. For example, while transgenes encoding full-length Notch proteins restore embryonic cuticle and central nervous system development, Notch proteins deleted only for the cdc10 sequences are functionless in a N^- genetic background and provide dominant loss of function in N^+ embryos (48). In any case, the results stemming from analysis of N^{Co} and N^{60g11} are consistent with earlier suggestions that Notch functions in cell-fate specification as a signal-transducing protein. Of course, a role as receptor, as indicated by the data, does not rule out additional involvement in processes of cell adhesion (for reviews, see refs. 9–11).

Physical mapping of $l(1)N^B$ indicates that subtle alteration of the lin-12/Notch repeats also can produce a dominant gain of function for the Notch protein. This Notch hyperactivity may be "ligand independent", as the phenotype is unaffected by mutations of the *Dl* locus (e.g., $l(1)N^B/+;Dl^-/+$ resembles $l(1)N^B/+;+/+$, D.L., unpublished observation). This interpretation is reinforced by results from a separate study of transgenic *Drosophila*—deletion of all lin-12/Notch repeats can produce a dominant gain-of-function phenotype in Dl^- embryos (48). The phenotypes produced by $l(1)N^B$ and N^{60g11} are similar, as are the phenotypes generated by both mutations in combination with Ax alleles. Particularly as $l(1)N^B$ hyperactivity appears to be Delta independent, possibly extracellular lin-12/Notch element mutation stimulates an aberrant intracellular Notch function that is comparable to that provided by N^{60g11}.

We thank Toby Lieber and Simon Kidd for comments on the manuscript. This work was supported by the National Institutes of Health (Grant GM25103). M.W.Y. is an Investigator of The Howard Hughes Medical Institute.

1. Lehman, R., Jimenez, F., Dietrich, U. & Campos-Ortega, J. A. (1983) *Wilhelm Roux Arch. Dev. Biol.* **192,** 62–74.
2. Dietrich, U. & Campos-Ortega, J. A. (1984) *J. Neurogenet.* **1,** 315–322.
3. Cagan, R. L. & Ready, D. F. (1989) *Genes Dev.* **3,** 1099–1112.
4. Hartenstein, V. & Posakony, J. W. (1990) *Dev. Biol.* **142,** 13–30.
5. Corbin, V., Michelson, A. M., Abmayr, S. M., Neel, V., Alcamo, E., Maniatis, T. & Young, M. W. (1991) *Cell* **67,** 311–323.
6. Ruhola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L. Y. & Jan, Y. N. (1991) *Cell* **66,** 433–449.
7. Hoppe, P. E. & Greenspan, R. J. (1990) *Development* **109,** 875–885.
8. Heitzler, P. & Simpson, P. (1991) *Cell* **64,** 1083–1092.
9. Greenspan, R. J. (1990) *New Biol.* **2,** 595–600.
10. Greenwald, I. & Rubin, G. M. (1992) *Cell* **68,** 271–281.
11. Ghysen, A., Dambly-Chaudiere, C., Jan, L. Y. & Jan, Y. N. (1993) *Genes Dev.* **7,** 723–733.
12. Wharton, K. A., Johansen, K. M., Xu, T. & Artavanis-Tsakonas, S. (1985) *Cell* **43,** 567–581.
13. Kidd, S., Kelly, M. R. & Young, M. W. (1986) *Mol. Cell. Biol.* **6,** 3094–3108.
14. Kidd, S., Baylies, M. K., Gasic, G. P. & Young, M. W. (1989) *Genes Dev.* **3,** 1113–1129.
15. Johansen, K. M., Fehon, R. G. & Artavanis-Tsakonas, S. J. (1989) *Cell Biol.* **109,** 2427–2440.
16. Michaely, P. & Bennet, V. (1992) *Trends Cell Biol.* **2,** 127–129.
17. Coffman, C., Harris, W. & Kintner, C. (1990) *Science* **249,** 1438–1441.
18. Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S. D. & Sklar, J. (1991) *Cell* **66,** 649–661.
19. Stifani, S., Blaumueller, C. M., Redhead, N. J., Hill, R. E. & Artavanis-Tsakonas, S. (1992) *Nature Genet.* **2,** 119–127.
20. Franco Del Amo, F., Smith, D. E., Swiatek, P. J., Gendron-Maguire, M., Greenspan, R. J., McMahon, A. P. & Gridley, T. (1992) *Development* **115,** 737–744.
21. Weinmaster, G., Roberts, V. J. & Lemke, G. (1991) *Development* **113,** 199–205.
22. Yochem, J., Weston, K. & Greenwald, I. (1988) *Nature (London)* **335,** 547–550.
23. Yochem, J. & Greenwald, I. (1989) *Cell* **58,** 552–563.
24. Greenwald, I. & Seydoux, G. (1990) *Nature (London)* **346,** 197–199.
25. Mango, S. E., Maine, E. M. & Kimble, J. (1991) *Nature (London)* **352,** 811–815.
26. Lindsley, D. L. & Zimm, G. G. (1992) *The Genome of Drosophila melanogaster* (Academic, New York).
27. Kelly, M. R., Kidd, S., Deutsch, W. A. & Young, M. W. (1987) *Cell* **51,** 539–548.
28. Hartley, D. A., Xu, T. & Artavanis-Tsakonas, S. (1987) *EMBO J.* **6,** 3407–3417.
29. Lieber, T., Wesley, C. S., Alcamo, E., Hassel, B., Krane, J. F., Campos-Ortega, J. A. & Young, M. W. (1992) *Neuron* **9,** 847–859.
30. Xu, T., Rebay, I., Fleming, R. J., Scottgale, T. N. & Artavanis-Tsakonas, S. (1990) *Genes Dev.* **4,** 464–475.
31. Kidd, S. & Young, M. W. (1986) *Nature (London)* **323,** 89–91.
32. Vassin, H., Vielmetter, J. & Campos-Ortega, J. A. (1985) *J. Neurogenet.* **2,** 291–308.
33. De la Concha, A., Dietrich, U., Weigel, D. & Campos-Ortega, J. A. (1988) *Genetics* **118,** 499–508.
34. Alton, A. K., Fechtel, K., Kopczynski, C. C., Shepard, S. B., Kooh, P. J. & Muskavitch, M. A. T. (1989) *Dev. Genet.* **10,** 261–272.
35. Foster, G. G. (1975) *Genetics* **81,** 99–120.
36. Welshons, W. J. (1974) *Genetics* **76,** 775–794.
37. Palka, J., Schubiger, M. & Schwaninger, H. (1990) *Development* **109,** 167–175.
38. De Celis, J. F., Mari'-Beffa, M. & Garcia-Bellido, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 632–636.
39. Poulson, D. (1967) *Drosoph. Inf. Serv.* **42,** 81.
40. Kidd, S., Lockett, T. J. & Young, M. W. (1983) *Cell* **34,** 421–433.
41. Welshons, W. J. (1958) *Cold Spring Harbor Symp. Quant. Biol.* **23,** 171–176.
42. Cooke, R. M., Wilkinson, A. J., Baron, M., Pastore, A., Tappin, M. J., Campbell, I. D., Gregory, H. & Sheard, B. (1987) *Nature (London)* **327,** 339–341.
43. Montelione, G. T., Wuthrich, K., Nice, E. C., Burgess, A. W. & Scheraga, H. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5226–5230.
44. Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muscavitch, M. A. T. & Artavanis-Tsakonas, S. (1990) *Cell* **61,** 523–534.
45. Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, L., Cherbas, P. & Artavanis-Tsakonas, S. (1991) *Cell* **67,** 687–699.
46. Baker, N. E., Mlodzik, M. & Rubin, G. M. (1990) *Science* **250,** 1370–1377.
47. Welshons, W. J. (1965) *Science* **150,** 1122–1129.
48. Lieber, T., Kidd, S., Alcamo, E., Corbin, V. & Young, M. W. (1993) *Genes Dev.* **7,** 1949–1965.