Genetic Interactions Between the Drosophila Abelson (Abl) Tyrosine Kinase and Failed Axon Connections (Fax), a Novel Protein in Axon Bundles

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ABSTRACT

Mutations in the *failed axon connections* (*fax*) gene have been identified as dominant genetic enhancers of the *Abl* mutant phenotype. These mutations in *fax* all result in defective or absent protein product. In a genetic background with wild-type Abl function, the *fax* loss-of-function alleles are homozygous viable, demonstrating that *fax* is not an essential gene unless the animal is also mutant for *Abl*. The *fax* gene encodes a novel 47-kD protein expressed in a developmental pattern similar to that of Abl in the embryonic mesoderm and axons of the central nervous system. The conditional, extragenic noncomplementation between *fax* and another *Abl* modifier gene, *disabled*, reveal that the two proteins are likely to function together in a process downstream or parallel to the Abl protein tyrosine kinase.

THE *Abl* gene was originally identified as the cellular homolog of the retroviral oncogene, *v-abl* (GOFF *et al.* 1980). Another oncogenic form of *Abl*, *Bcr-Abl*, is produced by a reciprocal translocation, termed the Philadelphia chromosome, present in patients with chronic myelogenous leukemia (SHTIVELMAN *et al.* 1985). Despite the consequences of the oncogenic forms of Abl, little is known about its normal function. There has been progress recently in the identification of several other signal transduction proteins interacting with Bcr-Abl or Abl including GRB2 (PENDERGAST *et al.* 1993), Crk (REN *et al.* 1994), Crk-l (TEN HOEVE *et al.* 1994) and Fes (ERNST *et al.* 1994). Targeted disruption of *c-Abl* in the mouse results in homozygous mutant progeny with lymphopenia and perinatal lethality but no striking morphological defects (SCHWARTZBERG *et al.* 1991; TYBULEWICZ *et al.* 1991). The absence of embryonic lethality, despite the general expression pattern, may indicate the existence of genetic redundancy, either with another kinase or by some other molecule(s).

A similar situation exists for the Drosophila homolog of *Abl*. In flies, Abl protein is present in most tissues throughout the early stages of embryogenesis (BENNETT and HOFFMANN 1992). At later stages, Abl is detected primarily in the longitudinal axons of the central nervous system and the muscle attachment sites of the somatic muscles. Despite the predominant expression in the CNS axons, Abl^- embryos produce an apparently wild-type nervous system. Those mutant animals that survive to adulthood have roughened eyes, are sterile and short-lived.

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Given the mild phenotypes of *Abl* mutant animals, a genetic screen was designed to identify genes that interact with *Abl* by enhancing the *Abl* mutant phenotypes (GERTLER *et al.* 1993). The genetic strategy of the screen predicted that in the sensitized, *Abl* mutant background, a reduction in the normal levels of a protein that functions in the Abl pathway would shift the mutant lethal phase from pharate adults to an embryonic or early larval stage. We termed this effect haploinsufficiency dependent upon an *Abl* mutant background, or HDA. The genes identified are not haploinsufficient themselves but only when the fly is also mutant for *Abl*. From this type of screen, we recovered dominant genetic enhancer mutations in five different complementation groups, with multiple alleles of three genes: *disabled* (*dab*), *prospero* (*pros*) and *failed axon connections* (*fax*) (GERTLER *et al.* 1989, 1993). We find that *fax* encodes a novel protein that is not necessary for viability in an otherwise wildtype genetic background. However, a requirement for *fax* is observed upon reduction or loss of *Abl* or *disabled*.

MATERIALS AND METHODS

Screen for genetic enhancers of the *Abl* mutant phenotype: The enhancer mutations were induced on the *Df(3L)stJ7, Ki roe* p^p and Abl^1 chromosomes using X-rays or ethyl methanesulfonate (EMS) (GERTLER *et al.* 1989). For each mutagenesis, third chromosomes were balanced with *TM6B, Tb*. Mutagenized males were pair mated to Abl^-/*TM6B, Tb* virgins and the ratio of *Tb vs.* Tb^+ pupae in the next generation was scored. Those vials that produced <10% of the expected *Abl* mutant progeny class were kept as putative enhancers. To date, ~16,500 chromosomes have been screened. We have identified five HDA genes, some with multiple alleles: three alleles of *prospero* ($pros^{M4}$, $pros^{M14}$ and $pros^{M44}$); five alleles of *disabled* (dab^{M2}, dab^{M29}, dab^{M54}, dab^{M100} and $dab^{M2}21$); and four alleles of *failed axon connections* (fax^{M7},

fax^{M12}, fax^{M34} and fax^{M42}). fax^{M12} and fax^{M42} are both cytologically visible inversions. Their cytology is as follows: *In(3L)fax*^{M12} (72F1,2;75A1) and *In(3L)fax*^{M42} (72F1,2;74A). Two additional HDA mutations on chromosome *3* are M89, which lies distal to *hairy*, and M109, which lies between *scarlet* and *curled*. Flies were maintained on the standard Drosophila cornmeal-yeast medium at 25°C.

Genetic analysis of *fax* mutants: The viability of *fax* mutant flies was determined by mating males of each *fax* mutant allele, balanced with *TM6B, Tb*, to females of the following genotypes: *Df(3L)stJ7, Ki roe* p^p*/TM6B, Tb; Df(3L)stG24, Ki roe* p^p*/TM6B, Tb; Df(3L)std11/TM6B, Tb; or Df(3L)st100.62/TM6B, Tb*. The flies were brooded onto new food every other day, and the progeny from three broods were scored. The percentage observed of the expected progeny class was determined by dividing the number of Tb^+, *fax* mutant progeny by one half of the total balanced (Tb), heterozygous flies. *Abl* transposons used in the genetic analysis (HENKEMEYER *et al.* 1990) are present on the second chromosome and encode either a kinase-impaired (*P{Abl*$^{K\text{-}N}$*}*) or a wild-type Abl protein (*P{Abl*$^+$*}*).

For genetic comparison, the fax^{M34} allele was recombined from the *Df(3L)stJ7* chromosome onto the Abl^1 chromosome. To generate *fax Abl dab* triple mutant chromosomes, the *faxM7* allele was recombined onto the Abl^1 dab^{M221} and *Df(3L)stJ7,* dab^{M2} chromosomes. The enhancer trap AA142 (gift of Dr. CHRISTIAN KLÄMBT, University of Köln, Germany) was recombined onto each of these triple mutant chromosomes. PCR primers (No. 548, 5′-CCTGGACTCGGGACTCAC-3′ and No. 1089, 5′-CACATTCCCAAGCCAGAG-3′) that flank the first intron of *fax* were used to detect the internal deletion present in the fax^{M7} allele. Potential recombinants were screened in pools of 10 flies by PCR. Candidate recombinants were then tested individually by PCR.

Southern analysis: Genomic DNA was obtained from mutant flies according to published procedures (ASHBURNER 1989). The *Df(3L)stG24, Ki roe* p^p*/TM6B, Tb* stock was crossed to following mutant chromosomes: Abl^1; fax^{M7} Abl^1; fax^{M12} Abl^1; fax^{M34} Abl^1; 1(3L)M32 Abl^1; HDA-M89 Abl^1; and HDA-M109 Abl^1. Because the fax^{M42} allele does not survive over the *Df(3L)stG24* chromosome, DNA was isolated from fax^{M42} Abl^1/*iso-1* flies (BRIZUELA *et al.* 1994). The DNA of 20 flies was digested with *Hin*dIII, electrophoresed through an 0.8% agarose gel and transferred to Hybond N^+ membrane (Amersham) in 20× SSC. The genomic blots were hybridized with ^{32}P-radiolabeled *fax* cDNA No. 837 (BEDIAN *et al.* 1991) for 24 hr at 60° in 250 mM sodium phosphate, 7% SDS and 30% polyethylene glycol. Blots were rinsed three times in 2× SSC, 1% SDS for 30 min each at 65°, followed by three times in 0.1× SSC, 1% SDS for 30 min each at 65°. Autoradiographs were exposed 16 hr at −70°, then developed.

Sequencing of *fax* cDNAs and mutants: The *fax* cDNAs were sequenced by standard dideoxy chain termination protocols (Sequenase, U.S. Biochemicals). The *fax* alleles were outcrossed to the *Df(3L)stG24, Ki/TM6B, Tb* stock. Mutant flies were collected and their DNA was extracted according to published procedures (ASHBURNER 1989). For each allele, PCR was performed on DNA from one fly per reaction in 2.5 mM $MgCl_2$, 1× Taq buffer, 0.2 mM each dNTP, 0.2 μmol of each oligonucleotide primer and one unit of Taq polymerase (Promega). Typical reaction conditions were 30 cycles of 1 min at 95°, 2 min at 58° and 2 min at 72°. The amplified genomic DNA fragment was gel isolated, purified and sequenced directly, using the *femto*-mole DNA Sequencing Kit (Promega) following manufacturer's instructions.

Some regions of the mutant *fax* gene were obtained by RT-PCR (FROHMAN 1990). Total RNA was prepared from flies in which the mutant *fax* allele was placed over *Df(3L)stG24*. Flies were frozen in liquid nitrogen and homogenized in 4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% sarkosyl and 0.1 M 2-mercaptoethanol (CHOMCZYNSKI and SACCHI 1987). Lysates were phenol-chloroform extracted multiple times and precipitated in 2 volumes of isopropanol. RNA pellets were resuspended in water. First strand cDNA synthesis was obtained using 5 μg total RNA, 0.5 μg random primers, 10 units RNasin (Promega), 25 units AMV reverse transcriptase, and 4 μl of 5× AMV-RT Buffer (Boehringer Mannheim) in 20 μl reaction volume. The reactions were incubated for 1 hr at 42°, then 30 min at 55°. cDNA samples were diluted to 300 μl in water for storage at −20°. The *fax* cDNA was amplified with primers directed to the 5′ (No. 169, 5′-TCGCAGTGGTGGTTTCTC-3′) and 3′ (No. 1524, 5′-TGCCTGCTTTTGCTTTAC-3′) untranslated sequences. The amplified products were sequenced directly, as described above with the appropriate primers.

Western analysis: Mutant pupae were obtained by crossing the various *fax* alleles to *Df(3L)stG24, Ki/TM6B, Tb*. Two pupae were lysed in 40 μl of 2× Laemmli sample buffer (preheated to 100°) (LAEMMLI 1970). Pupae were homogenized immediately on the hot block, boiled for 10 min, then centrifuged at 4° for 10 min. One pupa equivalent was electrophoresed in a 7.5% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane. The filter was blocked with 1× PBS, 5% nonfat dry milk and 0.1% Tween-20. The Mab F7D6, anti-Fax, primary antibody (BEDIAN *et al.* 1986) was diluted 1:2000 and incubated with the filter overnight at 4°. The filter was washed with 1× PBS and 0.1% Tween-20. The secondary antibody, goat anti-mouse:horseradish peroxidase conjugate (Boehringer Mannheim), was used at a 1:3000 dilution. Immune complexes were detected using the Enhanced Chemiluminescence Kit (Amersham), following manufacturer's instructions.

Antibody and RNA staining reactions: For embryonic phenotypic analysis, all mutant chromosomes were balanced with a TM3, Sb Ser chromosome that carries a P{w^+, *Ubx-lacZ*} transposon (gift of Y. HIROMI, Princeton University). Embryos stained with the Mab BP102 were fixed 20 min in 2 ml heptane and 2 ml of 8% formaldehyde, 100 mM PIPES, pH 7.0, 1 mM $MgSO_4$ and 2 mM EGTA. For immunohistochemical detection of Fax, using undiluted Mab F7D6 directly, embryos were fixed for 5 min in 2 ml 37% paraformaldehyde, 0.05 M EGTA and 2 ml heptane. After fixation, vitelline membranes were removed with 1 part heptane:1 part methanol and embryos were rehydrated in 1× PBS, 0.1% Triton X-100 and 0.5% BSA. The BP102 antisera was used at a dilution of 1:10 (gift of N. PATEL and C. GOODMAN, University of California, Berkeley). Anti-betagalactosidase (Promega) was used at a dilution of 1:300. The secondary antibody, goat anti-mouse IgG, and streptavidin-horseradish peroxidase conjugate (Boehringer Mannheim) were used at a final dilution of 1:300. Secondary and tertiary antibodies were preabsorbed against fixed, wild-type embryos before usage. Stained embryos were stored in 90% glycerol. For double staining embryos carrying the AA142 enhancer trap, a rabbit anti-betagalactosidase antibody (Rockland) was used at 1:1000 to detect midline glial cell *lacZ* expression and a monoclonal mouse BP102 antibody was used at 1:10 to detect CNS axons. Secondary antibodies were biotinylated goat anti-rabbit and horseradish peroxidase conjugated anti-mouse, both at 1:300 (Vector Laboratory). Streptavidin-alkaline phosphatase (1:300, Boehinger) was used as the tertiary antibody to detect *lacZ* expression. For embryonic *in situ* hybridizations, the *fax* cDNA No. 1-2, obtained from the NICK BROWN cDNA library (BROWN and KAFATOS 1988), was used as a template for (+) and (−) strand synthesis of digoxygenin-labeled RNA. Published hybridization conditions were fol-

TABLE 1

The *fax* HDA effect and mutant viability

Genotypes	n^a	Percent viability[b]
A. The *fax* HDA effect		
Abl^1/Df(3L)stJ7	3997	66
fax^{M7} Abl^1/Df(3L)stJ7	2671	0
fax^{M12} Abl^1/Df(3L)stJ7	1248	0
fax^{M34} Abl^1/Df(3L)stJ7	1877	0
B. Rescue of lethality by P{*Abl*}		
P{Abl$^{K\text{-}N}$}/+; Abl^1/Df(3L)stJ7	3624	117
P{Abl$^{K\text{-}N}$}/+; fax^{M7} Abl^1/Df(3L)stJ7	2568	49
P{Abl$^{K\text{-}N}$}/+; fax^{M12} Abl^1/Df(3L)stJ7	2756	51
P{Abl$^{K\text{-}N}$}/+; fax^{M34} Abl^1/Df(3L)stJ7	1722	65
P{Abl$^+$}/+; Abl^1/Df(3L)stJ7	3306	105
P{Abl$^+$}/+; fax^{M7} Abl^1/Df(3L)stJ7	2282	57
P{Abl$^+$}/+; fax^{M12} Abl^1/Df(3L)stJ7	2138	118
P{Abl$^+$}/+; fax^{M34} Abl^1/Df(3L)stJ7	2211	125
C. Effect of *Abl$^+$* on *fax* mutant viability		
Abl^1/Df(3L)stG24	2803	120
fax^{M7} Abl^1/Df(3L)stG24	2559	39
fax^{M12} Abl^1/Df(3L)stG24	1688	23
fax^{M34} Abl^1/Df(3L)stG24	2522	19
P{Abl$^+$}/+; fax^{M7} Abl^1/ Df(3L)stG24	2682	81
P{Abl$^+$}/+; fax^{M12} Abl^1/ Df(3L)stG24	2161	85
P{Abl$^+$}/+; fax^{M34} Abl^1/ Df(3L)stG24	2567	78

[a] The entire progeny was scored for each cross; *n*, the total number counted.

[b] The percent viability was calculated by dividing the number of mutant progeny observed by one half the number of the balanced, heterozygous siblings.

lowed (TAUTZ and PFEIFLE 1989). Whole-mount embryos and nerve cords were photographed using Nomarski optics.

RESULTS

fax alleles are dominant enhancers of *Abl* mutants: The *fax* gene was identified by the ability of heterozygous mutations in *fax* to enhance dominantly the *Abl* mutant phenotype, shifting the lethal phase to a prepupal stage in development (Table 1A,). To show that the *fax* mutations are not haploinsufficient themselves, two different *Abl* transgenes were used to rescue the *fax$^{-/+}$ Abl$^-$* lethality. The first transgene encodes wild-type Abl (*P{Abl$^+$}*) and the second encodes a kinase-impaired Abl protein (*P{Abl$^{K\text{-}N}$}*) (HENKEMEYER *et al.* 1990). The kinase-impaired Abl is sufficient to provide moderate rescue of the *fax$^{-/+}$ Abl$^-$* genotype, but wild-type Abl protein provides complete rescue of this mutant genotype (Table 1B). The deficiency *Df(3L)stG24* uncovers *fax* and is *Abl$^+$*. *fax$^-$ Abl$^{-/+}$* animals are weakly viable (Table 1C). This semilethality can be alleviated by increasing the dosage of *Abl*, via the *Abl* transposons, demonstrating that the *fax* gene is not necessary for viability in an *Abl$^+$* background. The *fax^{M7}* allele has been recombined onto an *Abl$^+$* chromosome and remains a homozygous viable stock. *fax$^-$* adults show no obvious mutant phenotypes.

Dosage-sensitive interactions between the enhancers *fax* and *disabled*: Both *fax* and *disabled* (*dab*) behave as dominant genetic enhancers of, and show a synergistic interaction with, *Abl* (GERTLER *et al.* 1989). To determine if *fax* and *dab* act on the same or parallel pathways, their genetic and phenotypic interactions were examined (Table 2). The pattern of genetic behavior observed with different *fax* and *dab* alleles has been consistent. For comparison, genetic data from the *fax^{M12}* hypomorphic allele and the *fax^{M7}* internally deleted protein allele are shown. Like the *fax$^{-/+}$ Abl$^-$* mutant phenotypes shown in Figure 1 (see below), *Abl$^-$ dab$^{-/+}$* embryos contain similar disruptions in the CNS longitudinal and commissural axons and are rescued by *P{Abl$^+$}* and *P{Abl$^{K\text{-}N}$}* (GERTLER *et al.* 1989; HENKEMEYER *et al.* 1990). *Df(3L)std11* and *Df(3L)stJ7, dab^{M2}* chromosomes (*Abl$^-$ dab$^-$*) were used to test for any genetic interaction between the two modifier genes. As seen with *fax* mutants, animals mutant for *dab* are weakly viable in a genetic background with wild-type Abl function (Table 2A). Mutations in *fax* and *dab* fully complement each other; however, animals heterozygous for *fax* and *dab*, in an *Abl* mutant background, are embryonic lethal and the resulting axonal phenotype is similar to the *Abl$^-$ dab$^-$* and *fax$^-$ Abl$^-$* double mutant phenotypes (Table 2B). This heterozygous, *Abl*-dependent lethality, for example *fax^{M7} Abl^1/Df(3L)std11* or *faxM7* Abl^1*/Df(3L)stJ7, dab^{M2}*, can be rescued by increasing the amounts of wildtype (*P{Abl$^+$}*), but not kinase-impaired (*P{Abl$^{K\text{-}N}$}*) Abl, indicating a requirement for Abl kinase activity when Fax and Dab functions are compromised. Nonallelic noncomplementation has been reported in several cases (REGAN and FULLER 1988; RANCOURT *et al.* 1995). The noncomplementation between *fax* and *dab* is conditional on reduced or absent Abl function.

To examine the *fax$^-$ dab$^-$* double mutant phenotypes, triple mutant chromosomes were generated by recombining the homozygous viable *fax^{M7}* allele onto the *abl^1 dab^{M221}* and *Df(3L)stJ7, dab^{M2}* chromosomes. The multigenic deficiency *Df(3L)st100.62*, which uncovers *fax, Abl* and *dab*, was also used for comparison. *P{Abl}* transposons were introduced on the second chromosome to compensate for the *Abl* mutations on the recombinant triple mutant chromosomes. Decreasing the dosage of *dab* in the *fax$^-$* mutant background results in a large decrease in the mutant viability, near 1% of the expected mutant progeny class (Table 2C). This enhancement of *fax* mutant semilethality is greater than the enhancement seen with *Abl* mutations (Table 1C). Similarly, *fax$^{-/+}$ dab$^-$* animals have equally low values of

TABLE 2

Genetic interactions between *fax* and *dab*

Genotypes	Wild-type gene dose			n^a	Percent viability[b]
	fax	*Abl*	*dab*		
A. Reduced viability of *dab* mutants					
P{Abl⁺}/P{Abl⁺}; Abl¹ dab^M221/Df(3L)std11	2	2	0	1135	17
P{Abl⁺}/P{Abl⁺}; Abl¹ dab^M221/Df(3L)stJ7, dab^M2	2	2	0	1101	27
B. *Abl⁺* rescue of *fax, dab trans*-heterozygotes					
Abl¹/Df(3L)std11	2	0	1	2070	0
fax^M7 Abl¹/Df(3L)std11	1	0	1	1741	0
fax^M12 Abl¹/Df(3L)std11	1	0	1	3866	0
P{Abl^K-N}/+; fax^M7 Abl¹/Df(3L)std11	1	1[c]	1	378	0
P{Abl^K-N}/+; fax^M12 Abl¹/Df(3L)std11	1	1[c]	1	430	0
P{Abl^K-N}/+; fax^M7 Abl¹/Df(3L)stJ7, dab^M2	1	1[c]	1	389	0
P{Abl^K-N}/+; fax^M12 Abl¹/Df(3L)stJ7, dab^M2	1	1[c]	1	173	3
P{Abl⁺}/+; fax^M7 Abl¹/Df(3L)std11	1	1	1	620	121
P{Abl⁺}/+; fax^M12 Abl¹/Df(3L)std11	1	1	1	638	103
P{Abl⁺}/+; fax^M7 Abl¹/Df(3L)stJ7, dab^M2	1	1	1	432	91
P{Abl⁺}/+; fax^M12 Abl¹/Df(3L)stJ7, dab^M2	1	1	1	476	85
C. Dosage sensitivity between enhancer mutations					
P{Abl⁺}/P{Abl⁺}; fax^M7 Abl¹/fax^M7 Df(3L)stJ7, dab^M2	0	2	1	1147	1
P{Abl⁺}/P{Abl⁺}; fax^M12 Abl¹/fax^M7 Df(3L)stJ7, dab^M2	0	2	1	1155	1
P{Abl⁺}/P{Abl⁺}; Df(3L)std11/fax^M7 Abl¹ dab^M221	1	2	0	1309	4
P{Abl⁺}/P{Abl⁺}; Df(3L)stJ7, dab^M2/fax^M7 Abl¹ dab^M221	1	2	0	1062	4
D. Enhancer viability over a multigenic deficiency					
P{Abl⁺}/+; Abl⁴/Df(3L)st100.62	1	1	1	3504	115
P{Abl⁺}/+; fax^M7 Abl¹/Df(3L)st100.62	0	1	1	3420	45
P{Abl⁺}/+; fax^M12 Abl¹/Df(3L)st100.62	0	1	1	4252	58
P{Abl⁺}/P{Abl⁺}; fax^M12 Abl¹/Df(3L)st100.62	0	2	1	1526	56
P{Abl⁺}/P{Abl⁺}; fax^M12 Abl¹/Df(3L)st100.62	0	2	1	1365	68
E. Failure of *Abl⁺* to rescue fax⁻ *dab⁻*					
P{Abl⁺}/P{Abl⁺}; fax^M7 Abl¹ dab^M221/Df(3L)st100.62	0	2	0	1171	0
P{Abl⁺}/P{Abl⁺}; fax^M7 Abl¹ dab^M221/fax^M7 Df(3L)stJ7, dab^M2	0	2	0	1211	0

[a] Total number counted in all progeny classes.

[b] The percent viability was calculated by dividing the number of mutant progeny observed by one half the number of balanced, heterozygous siblings.

[c] The Abl protein encoded by this mini-gene has impaired kinase activity (HENKEMEYER *et al.* 1990).

mutant viability. The few escaping flies show no cuticular defects beyond their smaller size and thinner abdomen when compared with their siblings. Animals doubly mutant for *fax* and *dab* are lethal and exhibit axonal defects in the CNS (Table 2E).

The genetic interaction between *fax* and *dab* alleles is more severe than when *Df(3L)st100.62* is used as a *fax⁻ Abl⁻ dab⁻* test chromosome (Table 2D). The molecular lesions in the *dab* alleles are unknown. One interpretation of the data is that the *dab* alleles are not null, but make mutant proteins. The interaction of multiple mutant proteins may be poisonous, resulting in increased phenotypic consequences and lethality. The axonal disruptions, though less severe, are still manifest when the multiply mutant chromosomes are placed over *Df(3L)st100.62* (data not shown). A difference in the genetic background of the multigenic deficiency is an alternate explanation. However, the interpretation of an interaction between *fax* and *dab* remains valid, as increasing the dosage of *Abl⁺*, in the *Df(3L)st100.62* background, has little effect on *fax⁻ dab⁻/+* viability. The lethality of *fax⁻ dab⁻* mutants is the same with all combinations of mutant chromosomes used. Therefore, although the absence of one of the redundant *Abl* pathways is not lethal, the animal becomes sensitized to any further perturbations in the other pathway(s).

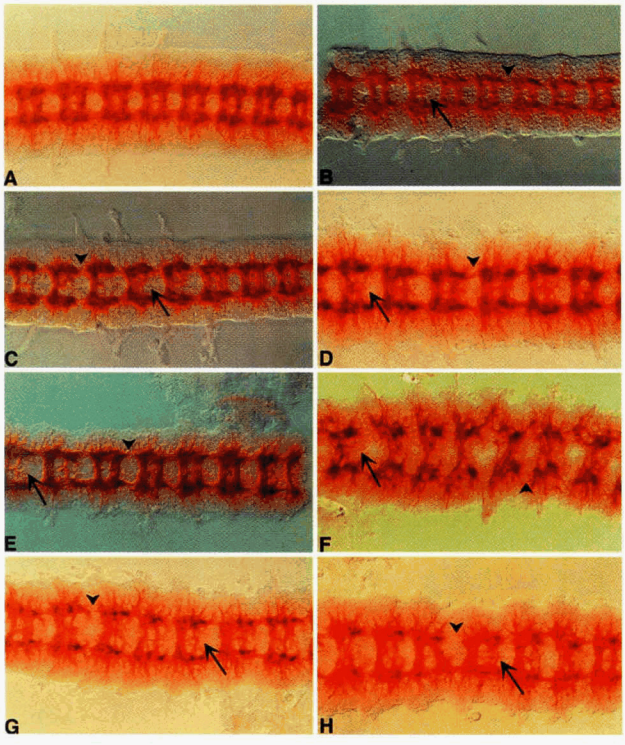


FIGURE 1.—The CNS axonal defects associated with *fax*, *Abl* and *dab* mutants. All embryos were stained with the antibody BP102. Embryos were mounted in 90% glycerol and the nerve cords were dissected away from the embryo. All nerve cords were obtained from late stage 15 to stage 16 embryos. (A) A wild-type CNS architecture. Animals mutant for *Abl* or *fax* also display a wildtype phenotype. (B) The phenotype of an animal haploinsufficient for *fax*, in an *Abl* mutant background (*fax*^{M34} *Abl*^{1}/*Df(3L)stJ7*). The longitudinal axon bundles (arrowheads) appear to be less dense than seen in wild type and a gap in the posterior commissure (arrows) is present infrequently. (C) The phenotype of a *fax*$^{-}$ *Abl*$^{-}$ double mutant (*fax*^{M12} *Abl*^{1}/*fax*^{M34} *Df(3L)stJ7*). The manifestation of the synergistic phenotype is the thinning of and gaps in the longitudinal connectives and the absent posterior commissural bundles. (D) The mutant phenotype of an *Abl*$^{-}$ *dab*$^{-}$ (*Abl*^{1} *dab*^{M221}/*Df(3L)stJ7, dab*^{M2}) embryo. The longitudinal and posterior commissural bundles are similarly disrupted as in the *fax*$^{-}$ *Abl*$^{-}$ animals. (E) The mutant phenotype of a *fax*$^{+}$/$^{-}$*Abl*$^{-}$ *dab*$^{-/+}$ *trans*-heterozygous animal, whose axonal phenotype resembles the double mutant phenotypes seen in *fax*$^{-}$ *Abl*$^{-}$ or *Abl*$^{-}$ *dab*$^{-}$ embryos above. (F) The increased severity associated with the loss of *fax*, *Abl* and *dab* (*fax*^{M7} *Abl*^{1} *dab*^{M221}/*fax*^{M7} *Df(3L)stJ7 dab*^{M2}) (G and H) The ability of increasing dosage of Abl to rescue the commissural and longitudinal phenotypes in the background of the same third chromosome mutant genotype. One copy of *Abl* (P{*Abl*$^{+}$}/+; G) results in partial restoration, but longitudinal and commissural defects remain even when two copies of *Abl* are present (P{*Abl*$^{+}$}/P{*Abl*$^{+}$}; H).

CNS axonal phenotypes of *Abl* modifier mutations: *Abl* is expressed in many tissues during embryogenesis, including the somatic and visceral mesoderm and the central nervous system (BENNETT and HOFFMANN 1992; GERTLER *et al.* 1989). Immunohistochemistry has shown that Abl is concentrated in the axon bundles of the CNS, but no gross defects are seen in *Abl* mutants (data not shown). Similarly, animals mutant for *fax* show no aberrant phenotypes in the central or peripheral nervous systems (data not shown). However, in animals mutant for *Abl* and heterozygous for an HDA mutation, such as *fax*, one observes minor differences in the longitudinal and posterior commissural axon bundles, as visualized with the antibody BP102 (Figure 1B). The longitudinal axon bundles appear less dense between segments and infrequent gapping is seen in the longitudinal connective and posterior commissural axons. The extent of severity in the axonal disruptions is variable from segment to segment. Animals double mutant for *fax* and *Abl* (Figure 1C) show a synergistic phenotype, in which the axonal disruptions are more pronounced than in the *fax*$^{-/+}$ *Abl*$^{-}$ embryos.

The commissural and longitudinal phenotypes of the *Abl*$^{-}$ *fax*, *dab trans*-heterozygotes are nearly identical to the *fax*$^{-}$ *Abl*$^{-}$ and *Abl*$^{-}$ *dab*$^{-}$ mutants (Figure 1, E, C and D, respectively). Pathfinding and fasciculation become extremely compromised when the embryo is triply mutant for *Abl*, *fax* and *dab* (Figure 1F). As the dosage of *Abl*$^{+}$ is increased in the *fax*$^{-}$ *dab*$^{-}$ background, commissure formation is partially restored, indicating an additional pathway for Abl function in the absence of *fax* and *dab* (Figure 1G). It appears that pioneering of both commissures occurs in these mutant backgrounds, but subsequent axons are unable to properly fasciculate or show aberrant pathfinding (Figure 1H). Formation of the longitudinal connectives remains affected in the *fax*$^{-}$ *dab*$^{-}$ mutants. Although this phenotype may be a secondary, developmental consequence due to the earlier difficulties of axons to properly fasciculate across the midline, it indicates that Abl function is not simply redundant for Fax and Dab, but that Fax and Dab may have unique functions during axonogenesis.

Defects in the development of axonal pathways can also be elicited by the absence or improper differentiation of the CNS midline cells (MENNE and KLÄMBT 1994). To verify whether midline defects occurred in the mutant backgrounds we used the AA142 enhancer trap line that is expressed in a subset of midline glial cells. These cells are affected by mutations, *e.g.*, Notch, that affect development of midline cells (MENNE and KLÄMBT 1994). The AA142 insertion was recombined onto a chromosome with mutations in *fax*, *Abl* and *dab*. As shown in Figure 2, midline glia expressing betagalactosidase were observed in embryos mutant for all three genes. At earlier stages, the pattern and number cell staining were similar to wild type (Figure 2, A and C). At later stages, the arrangement of the glia in the mutants was less ordered than in wildtype embryos (Figure 2, B and D). This may be because of the complete absence of normal axonal scaffolding. Although this experiment does not rule out defects associated with the mutations in other midline cell types, it does indicate that some midline cells are differentiating properly in the mutant background and therefore supports the hypothesis that the defects in the axonal architecture

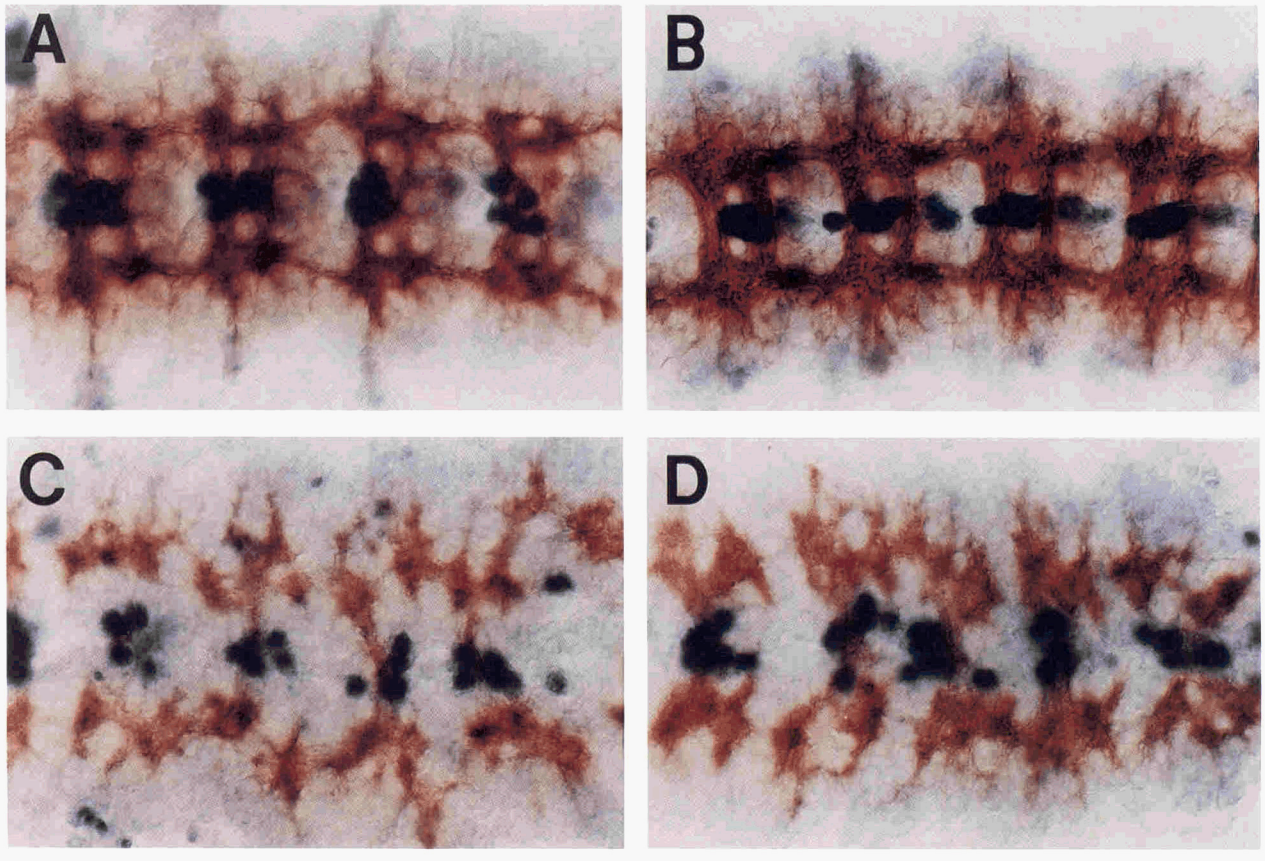
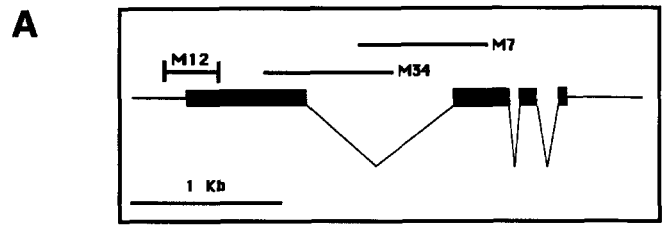


FIGURE 2.—Midline glia are present in *fax Abl dab* triple mutant embryos. The AA142 enhancer trap *P* element insertion was recombined onto the triple mutant chromosome. Antibodies to BP102 and betagalactosidase were used to detect axons and midline glia respectively. The BP102 staining is indicated in brown and the betagalactosidase staining is shown in blue. (A and B) Wild-type embryos at late stage 13 and stage 15, respectively; (C and D) mutant embryos at late stage 13 and stage 15, respectively. The midline glia expressing betagalactosidase (blue) are present in wild-type and mutant embryos. The number and pattern of cells are similar in mutant and wild-type embryos at late stage 13 (A and C) but in the stage 15 mutant embryos, the pattern of glial cells is different than in wild type (B and D). The axonal architecture (brown) is disrupted in the mutant embryos (C and D).

are associated with defects in axonal outgrowth or pathfinding and are not due to a total absence of midline cells.

The cloning of *fax*: The *fax* mutations were mapped meiotically to the region between *th* and *st* on chromosome *3* (GERTLER *et al.* 1989). Deficiency complementation tests indicated that *fax* is uncovered by the deficiencies *Df(3L)stG24* and *Df(3L)st100.62,* positioning the gene in the region of 72F2-7. Additionally, two X-ray alleles, fax^{M12} and fax^{M42}, contain cytologically visible inversions with breakpoints at 72F1,2 (data not shown). The cDNA No. 837 (BEDIAN *et al.* 1991), which was mapped by *in situ* hybridization to 72F4-7 on the polytene chromosomes, was used to begin a chromosome walk. DNA was prepared from flies in which the *fax* mutations had been placed over the deficiency chromosome *Df(3L)stG24.* Using the cDNA as a probe on genomic Southerns, several restriction fragment length polymorphisms (RFLPs) were observed (HILL 1995). fax^{M7} and fax^{M34} each contain an internal deletion (Figure 3A). Two novel restriction fragments were seen in the inversion allele fax^{M42}, indicating that the inversion breaks within the gene. An increase in fragment mobility was seen with the other inversion allele fax^{M12}, indicating that this inversion breaks near the 5′ end of the gene. These results indicated that mutant lesions in *fax* affected the DNA encoding the cDNA No. 837. No RFLPs were detected in two other HDA mutations, M89 and M109, which map meiotically to other regions of chromosome *3.*

The No. 837 cDNA was incomplete. We therefore screened the NICK BROWN 12–24-hr embryonic cDNA library to obtain a full-length cDNA (BROWN and KAFATOS 1988). The largest cDNA recovered (No. 1-2) was sequenced. Similarly, the *iso-1* genomic cosmid library was screened (TAMKUN *et al.* 1992). The location of introns was determined by comparing PCR products from the cosmid clone *vs.* the No. 1-2 cDNA, followed by sequence analysis. The presumptive start of translation (Figure 3B) exists 355 nucleotides from the 5′ end of the cDNA. The open reading frame encodes a novel 418 amino acid protein with a predicted molecular weight of 47 kD. A difference exists in the No. 837 and No. 1-2 cDNAs and the cDNA products obtained by



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ATGGCAAGCGAAGTGGCCCAAATACCCGCCGAGGAAACGCCCGCAGTGGCGGCGGCGGAAAAATCAGAGGAGCCG  (75)
 M  A  S  E  V  A  Q  I  P  A  E  E  T  P  A  V  A  A  A  A  E  K  S  E  E  P
GAAAAGTCAGCGGCCCCGCCAGCGGACTCAGCGGCCGCTCCAGCTGCCGCCCCCGCAGTGGAGAAGGCTGAGGAT  (150)
 E  K  S  A  A  P  P  A  D  S  A  A  A  P  A  A  A  P  A  V  E  K  A  E  D
GCCGATGGCGAGAAGAAGGACGGCGAGGCCGGAAAGCAGGACAAGCAGCAGGATGGCGAGGAGCCCAAAAAGGAC  (225)
 A  D  G  E  K  K  D  G  E  A  G  K  Q  D  K  Q  Q  D  G  E  E  P  K  K  D
GAGGCGGTGGCAGCACCCGTGGCGACCAAATCGGAAGCCCCGCCCGCCCAGAAATTCAATGTGCACAAGACCAAC  (300)
 E  A  V  A  A  P  V  A  T  K  S  E  A  P  P  A  Q  K  F  N  V  H  K  T  N
TTCGAGAAGGACATCATCTATCTGTACCAGTTCTCGCGCACCCCACTGCTGCCCTCCCTGTCGCCCTACTGCCTG  (375)
 F  E  K  D  I  I  Y  L  Y  Q  F  S  R  T  P  L  L  P  S  L  S  P  Y  C  L
AAGGTGGAGACCTGGCTGCGTCTTGTGGGCCTGAAATACGAGAATGTCGATCATAAGATGCGTTTCCGCTCCAAG  (450)
 K  V  E  T  W  L  R  L  V  G  L  K  Y  E  N  V  D  H  K  M  R  F  R  S  K
AAGGGTCAGCTGCCGTTCATCGAGCTGAATGGGGAGGAAATCGCCGATTCGGCCATCATCATCAAGGAACTGTCG  (525)
 K  G  Q  L  P  F  I  E  L  N  G  E  E  I  A  D  S  A  I  I  I  K  E  L  S
TCCAAATACGAGAAGTACCTGGACTCGGGACTCACCGCCGAGCAGAGGAATGTCTCGTACGCCACGATTGCCATG  (600)
 S  K  Y  E  K  Y  L  D  S  G  L  T  A  E  Q  R  N  V  S  Y  A  T  I  A  M
CTGGAGAACCATCTCATCTGGATCATCTTCTACTGGCGCGCCAAGTATCCGGACAATGTGCTCAAGGGCTACAAG  (675)
 L  E  N  H  L  I  W  I  I  F  Y  W  R  A  K  Y  P  D  N  V  L  K  G  Y  K
GTTAACTTGCAGCACGCCCTCGGCCTGCGGCTGCCCAACTCGATTCTGAACTTCTTCTTTAAGATCACCTTTGGT  (750)
 V  N  L  Q  H  A  L  G  L  R  L  P  N  S  I  L  N  F  F  F  K  I  T  F  G
    ✕
CGCAAGTGGTTCCAGGGCACGAAGAAGCTGAAGGCGCACGGCATCGGTGTCCACAGCGCCGAGGAGATCGAGGAG  (825)
 R  K  W  F  Q  G  T  K  K  L  K  A  H  G  I  G  V  H  S  A  E  E  I  E  E
TTCGGCAAGGACGACCTGAAGGTGCTCAGCGAGATGCTCGACTGCAAGCCTTTCTTCTTCGGCGACGAGCCCACC  (900)
 F  G  K  D  D  L  K  V  L  S  E  M  L  D  C  K  P  F  F  F  G  D  E  P  T
ACCCTGGATGTGGTGGCCTTCGCTGTCCTCTCGCAGCTCCACTATCTGTCCAAGGACATTGCGTATCCGCTGCGC  (975)
 T  L  D  V  V  A  F  A  V  L  S  Q  L  H  Y  L  S  K  D  I  A  Y  P  L  R
GACTACATGACCGAGAAGTGCCCCAACTTGATTGGCCACGTTTCTCGCATGAAGGACAAGTGCTTCCCCGACTGG  (1050)
 D  Y  M  T  E  K  C  P  N  L  I  G  H  V  S  R  M  K  D  K  C  F  P  D  W
                                    ✕
GACGAGATCTGCACGAAGCTGGACCTCAATGCGCACATTCCCAAGCCAGAGCCCGAGACCAAGGAGGGCAAGGAG  (1125)
 D  E  I  C  T  K  L  D  L  N  A  H  I  P  K  P  E  P  E  T  K  E  G  K  E
GGTGGCGAGCAGGAGAAATCAAACGAACAGGAGGGCACTGAGGGCGACAAGATCGAGAAGGAGTTGGAGAAGGAC  (1200)
 G  G  E  Q  E  K  S  N  E  Q  E  G  T  E  G  D  K  I  E  K  E  L  E  K  D
    ✕
AAGTCAAACGAGAAGGAGTCGACCGAGGAGAACAAAGAGAAGGAGGAAACAAAGTAAATT
 K  S  N  E  K  E  S  T  E  E  N  K  E  K  E  E  T  K  *
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FIGURE 3.—Gene organization and sequence of *fax*. (A) An illustration of the location of three mutations in *fax*. Mutant lesions from the *fax* alleles were identified by PCR amplification of different segments of the gene followed by direct sequence analysis. fax^{M7} contains an internal deletion, breaking 278 bases within the first intron and rejoining at nucleotide 1004 in the second exon. An AG dinucleotide is present in the Lys343 codon acts as a splice acceptor and allows the open reading frame to resume at Asp344 (1029). fax^{M34} also contains an internal deletion. The 5′ breakpoint is after codon Glu139 (417) and the 3′ breakpoint is within the first intron. RNA splicing of intron 1 brings exons 2–4 into the proper reading frame to continue translation of the mRNA. The inversion breakpoint of the fax^{M12} allele is believed to reside between positions −211 and 246. All segments of *fax* from the fax^{M12} allele 3′ from primer No. 213, beginning at nucleotide 246, can be amplified by PCR; whereas, no part of the gene was amplified with primer No. 169, located at −211. (B) Nucleic and amino acid sequence of the *fax* cDNA is presented. The length of the *fax* ORF encoded by the cDNA is indicated by the numbers in parentheses to the left of the sequence. *fax* encodes a protein of 418 amino acids. Untranslated nucleotide sequence 5′ and 3′ to the open reading frame are not shown but are listed with the gene in the database (U21685). The location of exon boundaries are indicated by (×) above the nucleotide sequence. The three amino acids **WFQ** denote the alternate peptide encoded by the splice acceptor site present at the 5′ end of exon 2 (756) and absent if splicing resolves at (765).

RT-PCR. Genomic sequence has shown that two AG dinucleotides are separated by 7 bp at the 3′ end of Intron 1. If the first AG is used as the splice acceptor, as found in the library-derived cDNAs, then the amino acids tryptophan, phenylalanine and glutamine will be encoded (in bold, Figure 3B). However, cDNAs synthesized by RT-PCR from wild-type and mutant adult RNA contain transcripts that represent splicing at both the first and second AG dinucleotides. The relative abundance of one splicing form *vs.* the other has not been quantified and the importance of the splice variation is unknown.

Two domains of Fax demonstrate limited similarity to the neurofilaments. The amino terminus of Fax (Met1 to Ala91) is rich in alanine (28%), proline (12%) and glutamic acid (17%) and the carboxy terminus (Pro363 to Lys418) is rich in lysine (21%) and glutamic acid (33%). Serine and threonine residues are interspersed in these terminal domains. The tail domain of the NF-L and NF-M neurofilaments are also highly charged with glutamate and lysine residues (GEISLER *et al.* 1984). Consequently, the carboxy terminus of Rat NF-M shows an overall 38% identity and 60% similarity to the carboxy terminus of Fax, while the same terminus of Bovine NF-L, which has a greater content of alanines, shows an overall 29% identity and 52% similarity to the amino terminus of Fax.

Molecular characterization of the *fax* alleles M12, M7 and M34: To determine the location of the *fax* mutations, *fax* alleles were placed over *Df(3L)stG24* so that only the mutant gene would be amplified by PCR. For fax^{M7} and fax^{M34}, cDNA from the mutant alleles was synthesized by RT-PCR from mutant adult RNA. The inversion breakpoint for the fax^{M12} allele is believed to be near the 5′ end because all domains of the gene can be amplified by PCR from the fax^{M12} DNA except the 5′ most region, between nucleotide −211 (position of primer No. 169) of the 5′ untranslated sequences and nucleotide 246 (position of primer No. 213) in the first exon. This result is consistent with the Southern data, in that the cDNA probe did not detect any new restriction fragments in the fax^{M12} DNA, but an increase in length of the *Hin*dIII band, indicating the addition of novel DNA at the 5′ end of the gene. Based upon results from immunohistochemistry (see below), the fax^{M12} coding region remains intact. Because very little protein is de-

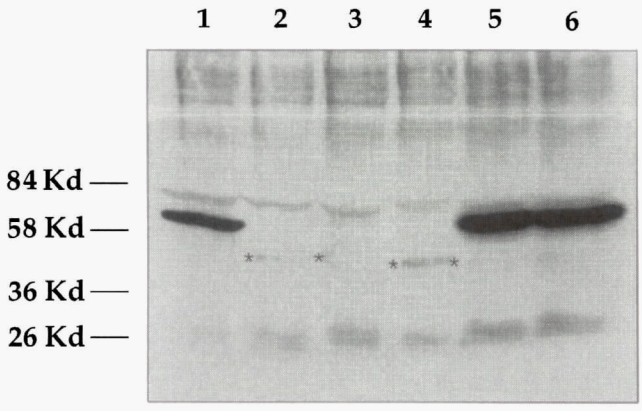

FIGURE 4.—Western analysis of mutant Fax proteins. *fax* mutant pupae were produced by mating *fax* mutant animals to *Df(3L)stG24*. Each lane represents the protein from a single pupa. Proteins were detected with the monoclonal antibody F7D6. Cross-reactive bands demonstrate relatively equal amounts of protein in each lane. Lane 1 is a positive control from the *Df(3L)stG24/TM6, B* stock. Lanes 2 and 4 show the truncated proteins (**) produced from the fax^{M7} and fax^{M34} alleles. The truncated proteins are present at considerably lower levels of detection. Lane 3 is from the fax^{M12} allele and no protein is detected in these pupae. Lanes 5 and 6 show normal Fax proteins produced in flies with the non-*fax* HDA mutations M89 and M109.

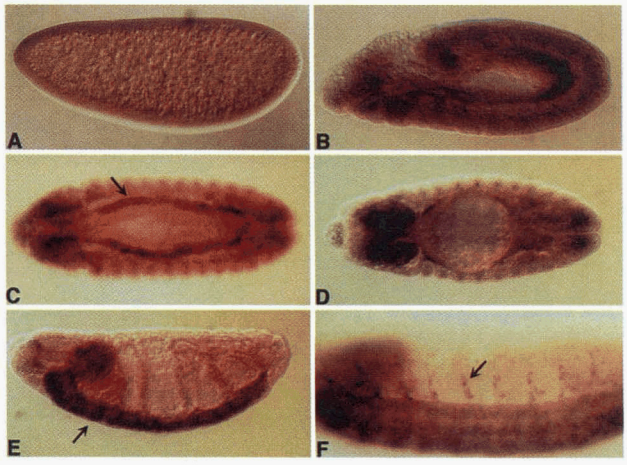

FIGURE 5.—Embryonic *fax* RNA *in situ* expression pattern. Sense and antisense *fax* RNA probes were labeled with digoxygenin, using the cDNA No. 837 as a template, and hybridized to wild-type embryos. Embryos are oriented with anterior to the left. No *fax* RNA is detected in the blastoderm embryo (A). The earliest detection of *fax* RNA is shortly after gastrulation (B). Dorsal views show increasing expression in the visceral mesodermal tissues (C and D). The pattern subsequently changes, decreasing in the mesoderm and increasing in the central nervous system (E). As shown in stage 16 embryos (F), *fax* RNA is also detected in the peripheral nervous system.

tected from this mutation, this allele is considered hypomorphic.

The fax^{M7} and fax^{M34} alleles contain internal deletions. As determined by RT-PCR and DNA sequencing, we detect mutant transcripts from both alleles that are spliced in-frame. For fax^{M7}, the 5′ breakpoint is 277 nucleotides within the first intron and the 3′ breakpoint is 239 nucleotides in exon 2, at Leu335 (Figure 3). The RNA transcript has the normal splice donor sequence of intron 1 and uses the next AG dinucleotide available within exon 2 for the splice acceptor, present in the Lys343 codon (AAG). The result is an in-frame, mRNA transcript encoding an internally deleted protein (Met1-Lys252; Asp344-Lys418) with a predicted molecular weight of 37 kD. Similarly, for the fax^{M34} allele, the 5′ breakpoint is located just after the Glu139 codon of exon 1 and the 3′ breakpoint residing in the first intron. Because RT-PCR has shown that this internal deletion can also be resolved by splicing in-frame, a novel splice donor site is presumed to be present by the fusion of intron sequence in exon 1. Both of the normal AG splice acceptor sites of exon 2 are used to complete the processing of the transcripts. The resulting protein (Met1-Glu139; Trp253-Lys418) has a predicted molecular weight of 33 kD.

Western analysis: The monoclonal antibody F7D6 (BEDIAN *et al.* 1986) was used for Western blots on *fax* mutant pupae. The pupae were lysed directly in sample buffer and subjected to SDS-PAGE. Wild-type Fax migrates greater than the predicted size of 47 kD (Figure 4, lane 1). This effect is more pronounced in the internally deleted proteins from the fax^{M7} and fax^{M34} alleles (Figure 4, lanes 2 and 4). Expression of the mutant proteins, though not quantitated, is considerably less than wild type. No Fax protein from fax^{M12} mutants was detected (Figure 4, lane 3). Fax protein of normal size and quantity is detected in extracts from pupa mutant for the two other HDA mutations, M89 and M109, generated on the same genetic background.

Embryonic expression pattern: The embryonic expression pattern of *fax* was examined using the No. 837 cDNA to generate digoxygenin-labeled RNA probes for *in situ* hybridizations (TAUTZ and PFEIFLE 1989). Sense and anti-sense probes were used to stain wild-type embryos. As shown in Figure 5, no *fax* message was detected in pregastrula embryos (Figure 5A). Upon gastrulation, RNA is detected in the epidermis and visceral mesoderm (Figure 5B). The expression pattern undergoes a pronounced change, such that epidermal expression decreases to background levels while mesodermal expression remains high (Figure 5C). Visceral mesoderm expression decreases once formation of the gut tube is complete (Figure 5D). Concurrently, central nervous system expression intensifies (Figure 5E). *fax* message is also detected in the peripheral nervous system (Figure 5F). The Fax protein expression pattern correlates well with the RNA pattern (HILL 1995).

DISCUSSION

Genetic redundancy and multiple Abl signaling pathways: Our goal was to discover molecules that interact with the Abl cytoplasmic tyrosine kinase using the strategy of modifier genetics. From the enhancer screens,

we expected to identify genes that function in the *Abl* pathway or an alternate, parallel pathway. Five complementation groups were identified by their dominant enhancement of the *Abl* mutant phenotype. The genetic interactions for two of these enhancers, *disabled* and *prospero*, were described previously (GERTLER *et al.* 1989, 1993). Genetic characterization of *dab* indicated the existence of a redundant, parallel pathway. In contrast, mutations in *prospero* demonstrated that the *Abl* pathway is also sensitive to more distant perturbations in neural development. In this report, the genetic behavior of a third modifier gene, *failed axon connections*, is described.

The nature of the interactions between Fax, Dab and Abl remains unclear. No phosphotyrosine was detected on the Fax protein, and although Dab contains phosphotyrosine, it has not been shown to be a direct substrate of Abl (GERTLER *et al.* 1993). Because a direct molecular association has not been observed between Abl and the enhancer molecules, we have used a genetic analysis to determine their relationships (GUARENTE 1993). Flies mutant for *fax*, *Abl* or *dab* individually are weakly viable. This may indicate either functional redundancy or subtle, individual, nonredundant roles yet to be identified (THOMAS 1993). Each gene is a dominant genetic enhancer of the other, resulting in embryonic lethality or a considerably reduced progeny class. This type of dosage sensitivity may reflect the redundancy of the related pathways.

Heterozygous interactions, also referred to as nonallelic noncomplementation (RANCOURT *et al.* 1995) or second-site noncomplementation (REGAN and FULLER 1988), usually indicate a proximity of two molecules that function in the same pathway (XU *et al.* 1990; SIMON *et al.* 1991; HEBERLEIN *et al.* 1993). With respect to *fax*, *Abl* and *dab*, heterozygous interactions between two genes are observed, but they are dependent upon the loss of the third gene. In each case, the consequence is lethality and the disruptions of the central nervous system axons are similar. As shown here, the *fax, dab* heterozygous interaction is dependent upon Abl kinase activity. When the comparison is extended to double mutant analysis, we continue to see synergistic interactions, resulting in nearly identical axonal phenotypes. The results are consistent with the existence of multiple, interdependent pathways, each required for the fidelity of axonal development.

The genetic comparison of nonnull alleles must be taken into consideration when interpreting the data. Epistasis tests and pathway analysis are best determined using null alleles and conditions of active/inactive states with no intermediate (GUARENTE 1993; AVERY and WASSERMAN 1992). None of the hypomorphic enhancer mutations show an allele-specific interaction. Certainly, the *Abl, dab* heterozygous condition, in a *fax* mutant background, is not as deleterious when the multigenic deficiency *Df(3L)st100.62* is used rather than a triple mutant chromosome. A *fax, Abl* heterozygous condition, in a *dab* mutant background, is also less deleterious when *Df(3L)st100.62* is used as a tester chromosome (data not shown). Although a difference in genetic background may account for some of the discrepancy between *Df(3L)st100.62* and the recombinant triple mutant chromosomes, the presence of mutant protein may also have adverse effects. The presence of multiple, nonnull mutations may be poisonous to the animal.

The *fax, dab* heterozygous interaction is *Abl*-dependent regardless of whether deficiencies or hypomorphic alleles are examined. For this reason, we believe that *fax* and *dab* both act in parallel pathways. Fax and Dab may be components of a protein complex whose formation or integrity may be sensitive to Abl kinase activity. By analogy with other cytoskeletal proteins, loss of any one molecule compromises the animal, but is not lethal (WITKE *et al.* 1992). Only when the other components are mutated or absent do the cells manifest cytoskeletal defects. *Abl* may also function via additional pathways, for example ones affected by the HDA mutations M89 and M109, and the *Abl* suppressor gene *enabled* (GERTLER *et al.* 1990, 1995), although the genetic relationships between these candidate mutations and the known modifier genes remain to be tested.

Genetic redundancy of tyrosine kinases: Mutations in cytoplasmic tyrosine kinases in the mouse system also demonstrate genetic redundancy. Mice mutant for members of the widely expressed Src family of kinases, *src*, *fyn* and *yes*, show restricted or no phenotypes in the single mutant animals. *src* mutant mice develop the bone disease osteopetrosis (SORIANO *et al.* 1991). Animals mutant for *fyn* show some defects in T-cell receptor signaling and in the hippocampus, but no other overt phenotypes (APPLEBY *et al.* 1992; STEIN *et al.* 1994). Production of the double mutant combinations between *src*, *fyn* and *yes* results in an increase in lethality, demonstrating loss of compensation for one kinase by another (STEIN *et al.* 1994). However, some specificity is observed in the *in vitro* outgrowth of src^- neurites on a matrix of the cell-adhesion molecule L1 (IGNELZI *et al.* 1994). Another example of genetic redundancy is seen in hck^- and fgr^- animals (LOWELL *et al.* 1994). In this case, compensation by an increase in the kinase activity of Lck was detected in the *hck* mutant macrophages. However, this redundancy was insufficient when hck^- fgr^- mutants were challenged with *Listeria* infection (LOWELL *et al.* 1994).

Fax may have properties similar to neurofilaments: Fax protein is localized to the cellular membranes, indicating a potential role in cell-cell interactions. Immunohistochemical stains on embryonic tissue cross-sections show strong expression of Fax along the membrane furrows of the epidermis (BEDIAN *et al.* 1986). Electron microscopic images further demonstrate the localization of Fax with the plasma membrane (BEDIAN and

JUNGKLAUS 1987). The mechanism by which Fax is targeted to the membrane is unknown.

The amino and carboxy termini of Fax show limited similarity to the carboxy terminus of the neurofilaments. Neurofilaments are the major component of intermediate filaments in most mature neurons. They are composed of a large, central rod domain flanked by an amino terminal globular domain and a variable length, non-α-helical carboxy terminus (WEBER *et al.* 1983). Neurofilament assembly and disassembly is regulated by posttranslational modifications in the head domain (NIXON and SIHAG 1991). *O*-glycosylation and phosphorylation is observed on some amino-terminal serines and threonines (DONG *et al.* 1993; LEE and CLEVELAND 1994). Neurofilaments self-assemble via coiled-coil interactions in the rod domain. The carboxy tailpiece of NF-M, rich in charged residues, is important for the formation of cross-bridges to interact with other neurofilaments or microtubules (GEISLER *et al.* 1984; HISANAGA *et al.* 1991). The carboxy terminus contains serines and threonines whose phosphorylation state is regulated by multiple kinases. A cdc2-like kinase has been identified that can recognize and phosphorylate the KSP motifs in this domain (HELLMICH *et al.* 1992; SHETTY *et al.* 1993). The tau protein kinase has also been shown to phosphorylate the KSP motifs of NF-H and thereby regulate its ability to associate with microtubules (MIYASAKA *et al.* 1993). Additionally, the NAK115 kinase has been shown to associate with neurofilaments *in vitro* and *in vivo* (XIAO and MONTEIRO 1994).

The termini of Fax may provide domains for protein-protein interactions, similarly regulated as the neurofilaments, important for proper localization or response to Abl signaling. Although the serines and threonines in the amino and carboxy termini of Fax do not fit the KSP consensus for substrates of NF-kinases, these residues may be substrates for an unknown kinase. Serine/threonine kinase activity was detected in a Fax immunoprecipitation extract (BEDIAN *et al.* 1991), perhaps indicating an associated kinase. The proposed regulation of Fax by serine/threonine phosphorylation invokes an intermediate kinase that functions in the Abl signaling pathways. However, given the cloning of the *Caenorhabditis elegans* gene *unc-51*, which encodes a serine/threonine kinase (OGURA *et al.* 1994), such a molecule important for axonogenesis in Drosophila is not unexpected.

The *Abl* pathways and axonal development: Glial cell-axon interactions are important in the development of the Drosophila nervous system. An association of the midline glial cells and pioneering axons is necessary for the formation and separation of the anterior and posterior commissures (KLÄMBT *et al.* 1991). Homophilic cell-surface glycoproteins, such as FasI, FasII and FasIII, are expressed on different subsets of axons and are necessary for proper fasciculation of distinct axon bundles (PATEL *et al.* 1987; GRENNINGLOH *et al.* 1991; MCALLISTER *et al.* 1992). The method by which these adhesion molecules transmit information remains largely unclear. However, one possible mechanism is exemplified by the *Abl*$^-$ *fasI*$^-$ double mutant interaction, resulting in an absence of commissural axon bundles (ELKINS *et al.* 1990).

Although we have been using the axon architecture as a measure of gene interaction, the phenotypes observed can indicate changes inside the glia as well as the axon. *Abl* and the three characterized enhancer genes *fax*, *dab* and *pros* are each expressed in both neuronal and glial cells of the central ganglion. The only identified *Abl*-suppressor gene, *enabled*, is also expressed in the same pattern (GERTLER *et al.* 1995). Prospero may affect the Abl pathways through its expression in the longitudinal glia (DOE *et al.* 1991; VAESSIN *et al.* 1991); whereas, Fax, Dab and Ena may function inside the axons. Consequently, the response to signals passed between the two cell types may be compromised in these mutant backgrounds. It is hoped that the two remaining uncharacterized HDA mutations, M89 and M109, will shed more light on these pathways. Both mutations show genetic interactions with *fax* and *Abl*, similar to those described here between *fax*, *Abl* and *dab* (HILL 1995). Further analysis of their role in the Abl signaling pathways may link the intracellular responses to the extracellular signals.

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