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29. We thank P. Ponte for I-22 and J-24 RNA probe construction; D. Morgan, P. Ponte, and J. Day for manuscript critique; and C. Zarow for helpful discussions. Tissue for this study was obtained from J. Rogers, Institute for Biogerontology Research, Sun City, AZ, and from the Alzheimer's Disease Research Center of Southern California Autopsy Core. Supported by grants from the National Institute on Aging to C.E.F. (AG05142 and AG07909), and by California Biotechnology. S.A.J. was supported by a grant from the Alzheimer's Disease and Related Disorders Association.

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Genetic Suppression of Mutations in the *Drosophila abl* Proto-Oncogene Homolog

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The *Drosophila abelson* (*abl*) gene encodes the homolog of the mammalian *c-abl* cytoplasmic tyrosine kinase and is an essential gene for the development of viable adult flies. Three second-site mutations that suppress the lethality caused by the absence of *abl* function have been isolated, and all three map to the gene *enabled* (*ena*). The mutations are recessive embryonic lethal mutations but act as dominant mutations to compensate for the neural defects of *abl* mutants. Thus, mutations in a specific gene can compensate for the absence of a tyrosine kinase.

THE CYTOPLASMIC TYROSINE KINASES were originally discovered as the products of oncogenes encoded by transforming retroviruses (*1*). The proto-oncogenic forms of cytoplasmic tyrosine kinases are expressed in normal cells and have been implicated in essential cellular processes, including cell proliferation and differentiation (*2*). To further understand the roles of cytoplasmic tyrosine kinases in cellular functions, we have isolated mutations in the *abelson* (*abl*) cytoplasmic tyrosine kinase of *Drosophila* (*3*) and examined the consequences to cell growth and development. Mutations in *abl* result in death of the animal during metamorphosis or in appearance of adults exhibiting a variety of mutant phenotypes, including roughened eyes, reduced longevity, and reduced fecundity. The *abl* protein can be detected by immunohistochemical methods in differentiating neural cells of the embryonic central nervous system (CNS) and the retinal cells of the eye.

We have isolated mutations that act as genetic enhancers and suppressors of the mutant phenotypes of *abl*. These mutations can be used to identify genes whose products act in the same biological processes as the *abl* cytoplasmic tyrosine kinase, and in some cases might identify genes whose products directly interact with the tyrosine kinase either as regulators of *abl* activity or as substrates for *abl*. One of the genetic enhancers is the *disabled* (*dab*) gene (*4*). Heterozygous mutations in *dab* act as dominant genetic enhancers of the mutant phenotypes caused by homozygous *abl* mutations: a *dab* mutation shifts the mutant phenotype from pupal lethality to embryonic and early larval lethality. In animals homozygous mutant for both *abl* and *dab*, severe disruptions are observed in the axonal connections of the developing CNS (*4*).

We used a screening procedure to recover second-site mutations that would shift the lethal phase of animals homozygous mutant for *abl* and heterozygous mutant for *dab* from embryonic or larval stages back to the pupal stage. The strategy would detect dominant mutations on the second or third chromosomes, which account for 80% of the *Drosophila* genome (*5*). From 4000 mutagenized chromosomes, three independent mutations were recovered; each of the mutations not only compensated for the embryonic or larval lethality of the mutant animals, but also allowed recovery of fertile adult flies. All three mutations segregate with the second chromosome and map to chromosome arm 2R between the dominant visible mutations *Black cell* (*Bc*) and *Punch* (*Pu*) (*6*). Genetic analysis indicates that all three mutations are allelic (see below); we have named this complementation group *enabled* (*ena*).

The characteristics of the *ena* mutations were tested in a number of different genetic backgrounds (Table 1). In animals mutant for *abl*, which would otherwise die as pupae, each of the three *ena* mutations acts in a dominant fashion to compensate for the absence of *abl* and allow recovery of fertile adults. Even the defective pattern of retinal

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Table 1. Suppression of *abl* and *dab* mutant phenotypes by mutations at the *ena* locus. The percentages given represent the fraction of the theoretical mutant progeny classes that survive to adulthood. Genetic crosses were set up in vials between five males and five females. The flies were transferred to fresh food daily for 7 days. At least 400 progeny were scored for each experiment. To generate the $ena^{210}/+$; abl^{-} dab^{-}/abl^{-} dab^{+} genotype [Df(3L)*stE36 e*]/(TM6B) males were crossed to ena^{210}/CyO; [Df(3L)*std11, Ki roe* p^{p}]/(TM6B) females. The $ena^{210}/+$; abl^{-} dab^{-} genotype was generated by crossing [Df(3L)*stj7* dab^{M2} *Ki roe* p^{p}]/(TM6B) males to ena^{210}/CyO; [Df(3L)*std11 Ki roe* p^{p}]/(TM6B) females. The $ena^{210}/+$; abl^{+} dab^{-}/abl^{-} dab^{-} stock was generated by crossing [Df(3L)*stj7* dab^{M2} *Ki roe* p^{p} Tn*abl*]/(TM6B) males to ena^{210}/CyO; [Df(3L)*std11 Ki roe* p^{p}]/(TM6B) females. The theoretical expected progency class was calculated as the number of Cy^{+} *Tb* adults observed divided by 2. The dab^{M2} mutation, Tn*abl*, and the normal lethal phase of the crosses between the deficiencies used are described in (*3, 4*). Equivalent levels of suppression were observed in reciprocal crosses.

Genotype	ena^{+} (%)	ena^{210} (%)
abl^{-} dab^{-}/abl^{-} dab^{+}	10	100
abl^{-} dab^{-}/abl^{-} dab^{-}	0	0
abl^{-} dab^{-}/abl^{+} dab^{-}	0	35

cells in the abl^{-} adult eye is largely rescued by the *ena* mutations (Fig. 1). In animals mutant for *abl* and hemizygous for *dab*, which would otherwise die during embryogenesis or during early larval development, each of the three *ena* alleles permits recovery of normal fertile adults. In view of the independent derivation of the three mutations, it is not surprising that each of the three *ena* alleles has a quantitatively distinct effect on the mutant backgrounds examined. The ena^{210} allele consistently permits the highest recovery of adult progeny in *abl* mutant genetic backgrounds (Table 1). The fraction of the mutant progeny class restored to adult viability is 10 to 30% lower in the presence of either ena^{62} or ena^{18}.

In the presence of wild-type *abl* function, mutations in *dab* result in lethality at the embryonic and early larval stages (*4*). These mutant animals have some gaps in the axonal bundles of the embryonic CNS. The presence of any of the three *ena* alleles allows some *dab* mutant animals to survive to adult stages (Table 1), although the suppression of the *dab* lethal phenotype is not as complete as that observed for the *abl* mutant animals.

The double mutation $abl^{-}dab^{-}$ results in embryonic lethality and a complete disruption of axonal bundles in the embryonic CNS (*4*). The presence of the *ena* mutations did not permit these mutant animals to survive to pupal or adult stages. However, examination of the embryonic CNS in these animals demonstrates that the *ena* mutations alleviate the defects such that the axonal bundles appear much more intact (Fig. 2). The improvement in the $abl^{-}dab^{-}$ double mutant phenotype caused by the presence of an *ena* mutant allele is similar to the improvement in the double mutant phenotype achieved when a copy of abl^{+} or dab^{+} is added to the genetic background. That is, the phenotype of the suppressed $abl^{-}dab^{-}$ animals is similar to the phenotypes of (abl^{-} dab^{+})/($abl^{-}dab^{-}$) and ($abl^{+}dab^{-}$)/($abl^{-}dab^{-}$) animals.

Another gene that shows genetic interactions with *abl* is *fasciclin I* (*fas I*) (*7*). The fasciclins are membrane-bound proteins associated with growing axons in grasshoppers and *Drosophila* (*8*). Embryos doubly mutant for *abl* and *fas I* show defects in the axons of the CNS that are more severe than the defects observed when either gene is singly mutant (*7*). The *ena* mutations allow complete recovery of the abl^{-} fas^{-} double mutant animals as fertile adults.

To determine whether the *ena* mutations interact with other genes important for neural development in *Drosophila*, we examined their effects on the lethal phenotype of two mutant genes that disrupt the embryonic CNS. The presence of *ena* mutations did not compensate for the lethal effects of mutations in *embryonic lethal, abnormal visual system* (*elav*) (*9*), or *ventral nervous system condensation defective* (*vnd*) (*10*). Because the *ena* mutations also compensate for the abl^{-} defects in eye imaginal disk neural development, we examined the effect of the *ena* mutations on the mutant phenotype of *sevenless*. The *sevenless* gene encodes a transmembrane tyrosine kinase which is required for the determination of the R7 photoreceptor cell in the ommatidia (*11*). The presence of *ena* mutations had no effect on the *sevenless* phenotype. From these results we conclude that mutations in *ena* have some specificity for alleviating the neural defects caused by mutations in *abl* and *dab*.

Any combination of *ena* mutations leads to embryonic lethality, that is, the mutations are allelic. This was determined by counting embryos derived from crosses between any two mutations and establishing that approximately 25% of the embryos fail to hatch (*12*). In cuticular structures elaborated by *ena* mutant embryos, although the majority of the mutant embryos have no major cuticular aberrations (Fig. 3A), a small proportion (approximately 10 to 20%) do exhibit cuticular defects. Some *ena* mutant embryos have severe defects in head structures (Fig. 3B). Most extreme are embryos that secrete larval cuticle that is largely devoid of many structures characteristic of wild-type cuticles (Fig. 3C). Further examination of *ena* mutant embryos by DAPI staining and immunostaining with antibody to horseradish peroxidase did not reveal any gross abnormalities in the nuclear content or in the structure of the nervous system of developing embryos, respectively (*13*).

It is likely that the observed recessive phenotypes of the *ena* mutations do not represent the null phenotype caused by a complete loss of *ena* gene product. The fact that the three alleles differ in the degree to which they suppress abl^{-} mutant phenotypes indicates that they differ in the amount of residual *ena* function, therefore no combination of two alleles would completely eliminate *ena* function. The available chromosomal deficiencies in the region between *Bc* and *Pu* do not completely span this interval, and those that we have tested complement the *ena* mutations. Therefore, we currently

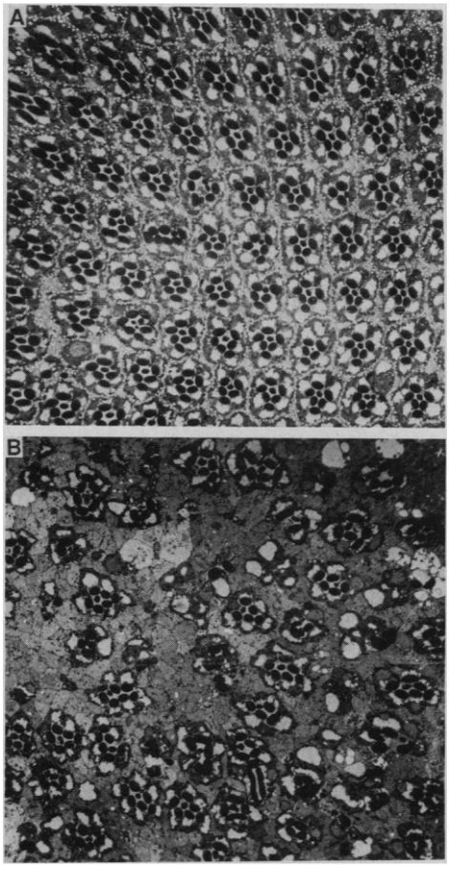

Fig. 1. Effect of *ena* mutations on the *abl* mutant phenotype in the adult eye. (**A**) Electron micrograph of an eye section prepared from an abl^{-} adult in the presence of an *ena* mutation [$ena^{210}/+$; abl^{1}/Df(3L)*stE36*]. The overall structural arrangement of the ommatidial arrays is normal. Only a few abnormalities of the rhabdomeres are observed. (**B**) Electron micrograph of an eye section from an abl^{-} adult [abl^{1}/Df(3L)*stE36*]. The phenotype includes disruption or loss of rhabdomeres within the ommatidia along with disruption of the ommatidial array (*3, 19*). Final magnification is ×1100.

have no chromosomal deletions with which to define further the null phenotype of *ena* (*14*). Such deletions would also be useful in demonstrating whether a simple reduction in the level of the *ena* gene product (Df *ena*/+) to one-half the wild-type amount is sufficient to compensate for the *abl* mutant phenotypes (*15*). Alternatively, some specific change in the structure or function of the *ena* gene product might be required that would not be mimicked by a deletion. The recovery of three alleles from 4000 mutagenized chromosomes, however, is most consistent with the generation of loss-of-function mutations.

We have now isolated three mutations of a single gene; these compensate for the absence of the *abl* tyrosine kinase during *Drosophila* development. The *ena* mutations suppress disruptions of neural development in both the embryo and the eye caused by *abl* mutations but do not affect the disruptions of neural development caused by three other mutations (*elav*, *vnd*, and *sev*). As in the case of *abl*, mutations in *ena* are lethal to the organism but do not reveal a spectacular phenotype. We have postulated on the basis of our studies on the phenotypic interactions between mutations in *abl* and *dab* (*4*) that there may be considerable redundancy in the regulatory pathways upon which the *abl* proto-oncogene homolog functions in *Drosophila*. In order to observe a mutant phenotype for *abl* in the embryonic CNS, it is necessary to reduce or eliminate the function of other genes like *dab*. Similarly, the role of *ena* in *Drosophila* development may be masked by redundant functions.

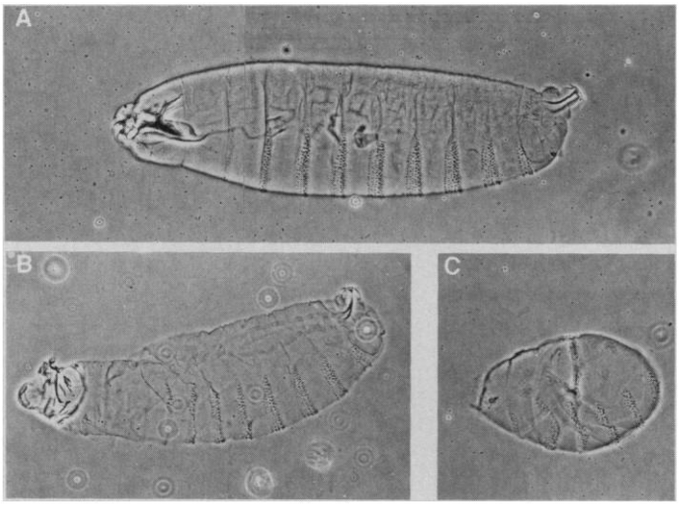

Fig. 3. Cuticular phenotype of the recessive lethal *ena* mutations. The majority of the dead mutant embryos have normal cuticular structures (**A**) whereas 10 to 20% show some abnormalities including defective head structures (**B**) or the absence of both anterior and posterior structures (**C**). Mutant embryos are derived from the same crosses used to establish the lethal phase of the *ena* mutations (*12*).

The phosphorylation of proteins on tyrosine residues influences post-translational regulation of many gene products. Phosphorylation on tyrosine can function to activate (*16*) or repress (*17*) the activities of target proteins. The genetic interaction discussed above indicates that the product of *ena* and the *abl* tyrosine kinase may act in opposition to one another, probably in the context of neural development in the embryonic CNS and the eye imaginal disk. For example, if the *abl* tyrosine kinase were normally required to negatively regulate the activity of the *ena* product, mutations in *abl* would result in abnormally high activity of *ena*. The detrimental effects caused by this activity might be alleviated by reducing the quantity of functional *ena* gene product. Identification of the *ena* gene product and characterization of its biochemical relation to the *abl* cytoplasmic tyrosine kinase is needed to understand the molecular basis of the genetic interaction.

The isolation of mutations in *ena* and *dab* demonstrates the potential of a genetically facile system such as *Drosophila* for analysis of proto-oncogene homolog functions. Recently, the construction of mice that are germline mutant for *c-abl* has been reported (*18*). It will be important to determine whether the genetic relationships that are being defined for *Drosophila abl* are recapitulated in the mouse system.

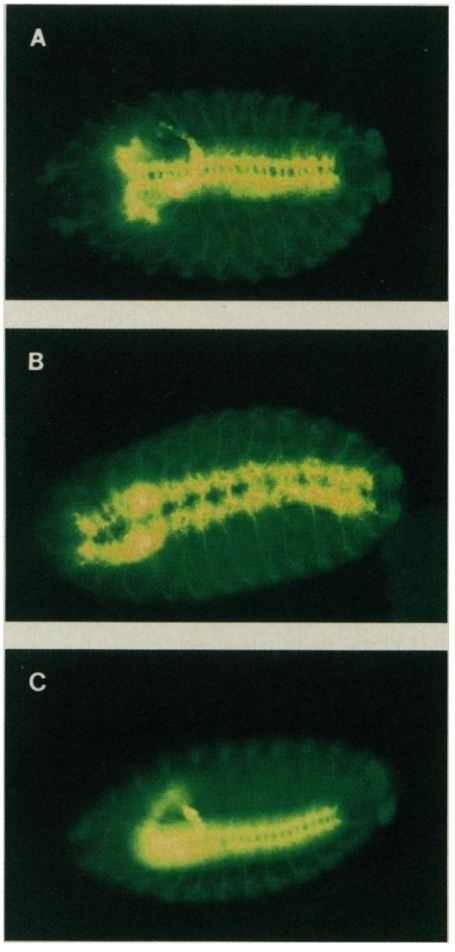

Fig. 2. Effect of *ena* mutations on the CNS disruption observed in abl^- dab^- embryos. Staining of the embryonic CNS was accomplished with fluorescene-conjugated antibody to horseradish peroxidase (*20*). (**A**) Wild-type embryo. (**B**) abl^- dab^- embryo. The double mutant shows disrupted axonal connections in every neuromere. (**C**) ena^{210}/+; abl^- dab^- embryo. In the presence of the *ena* mutation only minor defects are observed in the embryonic CNS, including a small number of gaps in axonal connectives and occasional thinning of axonal bundles. Fixation and staining were performed as described (*4*). A mating was set up between outcrossed stocks of the genotype [Df(3L)*stj7* dab^{M2} *Ki roe* p^p]/(Canton S) males and ena^{210}/Tokyo Wild; [Df(3L)*std11 Ki roe* p^p]/(Tokyo Wild) females. The Canton S/Tokyo Wild; abl^- dab^- progeny were easily observed by the presence of the abl^- dab^- double mutant phenotype. Animals showing subtle defects in the CNS were presumed to be carrying the ena^{210} allele in the abl^- dab^- background as such animals are not observed in similar crosses that do not contain the ena^{210} allele.

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5. Male flies carrying the deficiency Df(3L)*std11*, which breaks in the *abl* gene and extends proximally along the left arm of the third chromosome through *dab* (7), were treated with ethyl methane sulfonate [E. B. Lewis and F. Bacher, *Dros. Info. Serv.* **43**, 193 (1968)] and crossed to females carrying the balancer chromosomes TM3/TM6B. Male progeny bearing the TM6B balancer were then crossed individually to two (abl^1 *kar red e*)/(TM6B) females. Individual crosses were scored for the suppression of the embryonic or larval lethality of the abl^1/Df(3L)*std11* progeny class as indicated by the presence of pupal cases not carrying the dominant marker *Tubby* (on TM6B). Chromosomes carrying suppressor mutations were balanced over CyO. The stocks used are described in: D. L. Lindsley and E. H. Grell, *Genetic Variation of* Drosophila melanogaster, Carnegie Institution of Washington Publ. No. 627 (1968); D. L. Lindsley and G. Zimm, *The Genome of* Drosophila melanogaster, part 1 [*Dros. Info. Serv.*, vol. 62 (1985); part 2, vol. 64 (1986); part 3, vol. 65 (1987)].
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No Specific Recognition of Leader Peptide by SecB, a Chaperone Involved in Protein Export

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Most proteins destined for export from *Escherichia coli* are made as precursors containing amino-terminal leader sequences that are essential for export and that are removed during the process. The initial step in export of a subset of proteins, which includes maltose-binding protein, is binding of the precursor by the molecular chaperone SecB. This work shows directly that SecB binds with high affinity to unfolded maltose-binding protein but does not specifically recognize and bind the leader. Rather, the leader modulates folding to expose elements in the remainder of the polypeptide that are recognized by SecB.

PROTEINS DESTINED FOR EXPORT from the cytoplasm of *Escherichia coli* to the periplasm or outer membrane are made as precursors containing amino-terminal leader sequences that are essential for localization and that are removed during the process. The initial step during export of a subset of outer membrane and periplasmic proteins including the maltose-binding protein is recognition by SecB, an oligomeric cytosolic factor (monomer; M_r, 16,600) (*1–3*). Investigations carried out both in vivo and in vitro indicate that SecB binds to precursor maltose-binding protein at sites that are distinct from the leader (signal) sequence and blocks folding of the precursor, thus maintaining it in an export-competent state (*2–6*). An opposing view, that SecB specifically recognizes the leader sequence, has been presented on the basis of observations of a complex between SecB and precursor maltose-binding protein in vitro and the inability to demonstrate a similar complex between SecB and matured maltose-binding protein (*7*). The resolution of this issue is central to the understanding of initial events during protein localization in prokaryotes, and in eukaryotes as well, since it has been shown that SecB is functionally similar to the mammalian signal recognition particle (*7*).

In the investigations described here we have used purified proteins (*8*) so that the parameters of the system are well defined. Determination of the sequence of the first four amino acyl residues of the purified precursor and matured forms of maltose-binding protein established that each had the authentic amino terminus (*9*). The kinetics of the denaturant-induced, reversible unfolding of these polypeptides has been extensively characterized, allowing us to precisely control the rate of refolding (*6, 10, 11*). The free species of maltose-binding protein (M_r, ~40,000) were resolved from the oligomeric complexes of SecB and maltose-binding protein by gel-filtration chromatography. SecB formed a complex with precursor maltose-binding protein when refolding of the denatured precursor was initiated in the presence of an excess of SecB

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