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Activated *Drosophila* Ras1 is selectively suppressed by isoprenyl transferase inhibitors

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ABSTRACT **Ras CAAX (C = cysteine, A = aliphatic amino acid, and X = any amino acid) peptidomimetic inhibitors of farnesyl protein transferase suppress *Ras*-dependent cell transformation by preventing farnesylation of the Ras oncoprotein. These compounds are potential anticancer agents for tumors associated with *Ras* mutations. The peptidomimetic FTI-254 was tested for Ras1-inhibiting activity in whole animals by injection of activated *Ras1*^{val12} *Drosophila* larvae. FTI-254 decreased the ability of Ras1^{val12} to form supernumerary R7 photoreceptor cells in the compound eye of transformed flies. In contrast, it had no effect on the related supernumerary R7 phenotypes of flies transformed with either the activated sevenless receptor tyrosine kinase, Raf kinase, or a chimeric Ras1^{val12} protein that is membrane associated through myristylation instead of isoprenylation. Therefore, FTI-254 acts as an isoprenylation inhibitor to selectively inhibit Ras1^{val12} signaling activity in a whole-animal model system.**

Ras proteins are membrane bound GTP-binding proteins that transduce mitogenic signals from receptor tyrosine kinases (RTKs) to the nucleus (1). Certain mutations in *Ras* genes produce constitutively activated products resulting in deregulated growth control. Mutations in *RAS* genes are linked to 50% of human colon carcinomas and >90% of human pancreatic carcinomas (2). Association of Ras with the membrane is essential for Ras-induced cell transformation and is mediated through a covalently linked isoprenyl group (3–6). Isoprenylated proteins, such as Ras, share a carboxyl-terminal CAAX sequence (C = cysteine, A = aliphatic amino acid, and X = any amino acid), and the isoprenyl group is transferred to the sulfhydryl moiety of the cysteine residue from an isoprenyl diphosphate donor (7, 8). Three enzymes are known to catalyze protein isoprenylation: farnesyl protein transferase (FPTase), geranylgeranyl protein transferase I (GGPTase I), and geranylgeranyl protein transferase II (GGPTase II). The identity of the carboxyl amino acid of the CAAX sequence specifies enzyme recognition by FPTase or GGPTase I, whereas GGPTase II recognizes an XXCC or XCXC sequence motif. Posttranslational modification of mammalian Ras includes the attachment of a 15-carbon farnesyl moiety by the FPTase enzyme (9, 10). FPTase recognizes and farnesylates peptides as short as four amino acids provided they consist of the Ras CAAX consensus sequence (7). Thus, CAAX tetrapeptides serve as alternative substrates for farnesylation *in vitro* and can competitively inhibit farnesylation of Ras (9–14). CAAX-based peptidomimetics designed to inhibit FPTase block Ras modification in cell culture and have antiproliferative activities against *Ras*-transformed cells both in cell culture and in *nude* mice (15–21, 38). Furthermore, these compounds selectively inhibit *Ras*-induced phenotypes in several assays even though they reduce farnesylation of other proteins (14). However, the specificity of CAAX peptidomimetics for inhibiting oncogenic Ras signaling at the level of individual cells has not been characterized in whole-animal assays. We therefore investigated peptidomimetic effects on Ras and other components of the Ras signaling pathway *in vivo*.

We have used *Drosophila melanogaster* as the test model for peptidomimetic action for a variety of reasons. First, the mechanism by which RTKs transduce signals is highly conserved among eukaryotic species. Analysis of the *Drosophila* sevenless and torso RTK systems has defined a linear pathway involving Ras and members of the Raf/MAP kinase families that is homologous to the tyrosine kinase pathway in mammalian cells (22, 23). Gain-of-function alleles of the sevenless RTK, *Ras1*, or *Raf* genes in *Drosophila* resemble oncogenic forms of the corresponding mammalian genes and result in an uncoupling of signal transduction from extracellular signals (24–26). In particular, an activated phenotype is associated with *Ras1*^{val12}, a mutation equivalent to oncogenic [val^{12}]Ras in mammalian cells. Second, although the Ras1 pathway in *Drosophila* is required for cell proliferation, it is also involved in the RTK signaling that regulates cell fate decisions (23). In the developing eye, the specification of the R7 photoreceptor cell type depends on activation of the sevenless RTK and transduction through the Ras1 pathway. Constitutive activation of the receptor, Ras1, or Raf results in supernumerary R7 cell development. Consequently, the flies contain extra R7 cells, allowing for both rapid and quantitative measurement of signaling activities at the level of individual cells. R7 cell fate determination occurs at the third-instar larval stage when animals can undergo microinjection. Shortly thereafter, the larvae pupate, and eclose several days later into adults. Third, the profiles for isoprenyl group usage is closely related between whole-cell extracts prepared from mammalian and *Drosophila* cell lines (27). Several *Drosophila* proteins, such as nuclear lamin and Rab2, have the same isoprenyl modification as their mammalian homologs. Finally, strains carrying activating mutations in signaling genes are otherwise isogenic and are less likely to exhibit phenotypic differences because of other factors.

We report that injection of Ras CAAX peptidomimetics into *Ras1*^{val12} larvae suppresses formation of the supernumerary R7 cells in the compound eye. This suppression requires the CAAX element in Ras1^{val12} since substitution of the CAAX element rendered Ras1^{val12} insensitive to their suppressive effects. Moreover, injection of larvae carrying activating mutations in sevenless or Raf did not result in a suppression of their supernumerary R7 cell phenotypes, demonstrating the biological specificity of these peptidomimetics.

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Abbreviations: RTK, receptor tyrosine kinase; FPTase, farnesyl protein transferase; GGPTase I and II, geranylgeranyl protein transferase I and II, respectively; CAAX, C = cysteine, A = aliphatic amino acid, and X = any amino acid; DMSO, dimethyl sulfoxide; DTT, dithiothreitol.

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MATERIALS AND METHODS

Fly Stocks, Adult Eye Sectioning, and Scanning Electron Microscopy. The *Ras^{Myr}* fly stock containing the *sev-Dsrc90Ras1^{val12}ΔC186* construct was generated by Henry Chang (28). Sequences encoding the 90 N-terminal amino acids from Dsrc containing the myristylation signal sequence was added to the sequence encoding the N terminus of Ras1^{val12}, and the sequence encoding the C-terminal 4 amino acids (CAAX) were removed. The new coding sequence was placed into the pES3/DM30 sevenless expression vector. The activated rolled transgene was a gift from Felix Karim (28) who inserted the Sevenmaker coding sequence into the pES3/W8 sevenless expression vector to generate the *sev-Sem* stock. The construct was modeled after *sE-rl^{D334N}* from Brunner *et al.* (29). Histological sectioning of adult eyes and scanning electron microscopy were done as described in refs. 30 and 31, respectively.

Larval Microinjection. Wandering third-instar larvae were immobilized on a microscope slide with double-stick tape and partially anesthetized by exposure to Fly-Nap (Carolina Biological Supply) for 15 min. Before injection, the larvae were covered with Halocarbon oil (Halocarbon Products, Hackensack, NJ). Glass injection needles pulled to an average diameter of 10 μm delivered ≈3 nl of the peptidomimetic into the hemocoelic cavity at the third thoracic larval segment. A specific injection location is not essential since random injections along the larvae had no effect on the ability of the peptidomimetic to suppress the activated phenotype. The average injection volume was determined from micrometer measurements. After injection, larvae were removed from the tape and placed in standard food until eclosion. Working stock solutions of peptidomimetics were 100 mM peptidomimetic/10 mM dithiothreitol (DTT) in dimethyl sulfoxide (DMSO) and were further diluted with 1 mM DTT. Untreated third instar larvae remained in food vials until eclosion and did not undergo manipulation.

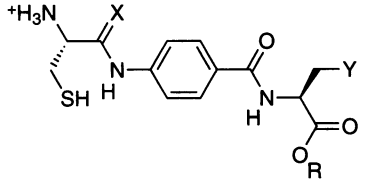
GGPTase I Assays. Geranylgeranyl modification of Ha-Ras-CVLL by mammalian GGPTase I *in vitro* was assayed as described (21). IC_{50} values for each peptidomimetic were determined by assaying GGPTase I activity at multiple concentrations of peptidomimetic.

RESULTS AND DISCUSSION

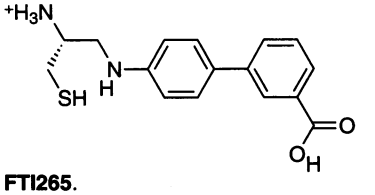
Ras CAAX Peptidomimetic Treatment Rescues the Ras1^{val12} Phenotype. *Drosophila* transgenic lines bearing the dominant activating *Ras1^{val12}* mutation (25) were used to evaluate the effect of a series of CAAX peptidomimetics (Fig. 1) on the transforming activity of *Ras1* in the compound eye. *Ras1^{val12}* gene activity causes a severe roughening of the external surface of the eye (compare Fig. 2 *A* and *B*), and sections through eyes reveal multiple R7 cells per facet or ommatidium (compare Fig. 2 *F* and *G*). We injected a single dose of peptidomimetic FTI-244 into *Ras1^{val12}* larvae and examined the eye phenotypes of treated individuals following pupation. FTI-244 is carboxylmethylated to facilitate cellular uptake and inhibits Ras processing in cell culture (33). There was significant suppression of the external eye phenotype, suggesting that this compound reduced Ras1^{val12} activity (Fig. 2*C*). We treated lines with a related compound, FTI-232, which is a potent FPTase inhibitor *in vitro* but is unable to inhibit FPTase in cell culture due to membrane impedance associated with a free carboxylate group (21, 33). We injected FTI-232 into *Ras1^{val12}* larvae and observed no suppression of the rough-eye phenotype (Fig. 2*D*), consistent with the observation that FTI-232 does not inhibit Ras activity in cell culture. FTI-254, a more potent analog of FTI-244 (21), was also tested by injection into *Ras1^{val12}* larvae. A suppression of the rough-eye phenotype similar to that seen with FTI-244 was observed (Fig. 2*E*). Sections through these eyes revealed an almost complete suppression of the Ras1^{val12} phenotype; most ommatidia contain one R7 cell rather than the two or three evident in untreated animals (Fig. 2*H*).

Since the Ras1^{val12} eye phenotype varies significantly between individuals, we quantitated our data by grouping individuals into phenotypic classes on the basis of the average number of R7 cells per ommatidium. Members within each group varied <10% from the mean. Uninjected *Ras1^{val12}* flies constitute four phenotypic classes, varying from 1.5 to 3.0 R7 cells per ommatidium (Fig. 3*A*). Flies injected with 20 mM FTI-254 (60 pmoles) exhibited a significant downward shift in their distribution among the four classes, and 30% of the flies created a new phenotypic class with an average of almost one R7 cell per ommatidium (Fig. 3*A*). The treated flies had a total average of 1.8 R7 cells per ommatidium, compared with the untreated flies with a total average of 2.6 R7 cells per ommatidium. The degree of suppression was dosage dependent since injection of 40 mM FTI-254 (120 pmoles) reduced the average R7 cell number to 1.3 R7 cells per ommatidium. In the ommatidia with one R7 cell, the position of the R7 cell indicated that it was a wild-type R7 cell and not one of the ectopic cells transformed by *Ras1^{val12}* (Fig. 2*H*). This suggests that the development of *Ras1^{val12}*-transformed cells is more sensitive to FTI-254 than the development of cells in which the sevenless RTK/Ras1 pathway operates normally. Moreover, fewer than 1% of ommatidia scored were completely lacking R7 cells, suggesting that normal Ras1 activity is not significantly inhibited by FTI-254, whereas Ras1^{val12} activity is profoundly inhibited.

Suppression of the Ras1^{val12} Phenotype Is Due to the Inhibition of Isoprenylation Modification. To demonstrate that the changes in the Ras1^{val12} phenotype correlated with an inability of the Ras1^{val12} protein to localize to the plasma membrane, we treated larvae transformed with a chimeric *Ras1* gene. *Ras1^{Myr}* contains the val-12 activating substitution, but the CAAX isoprenylation signal sequence was removed, and the myristylation signal sequence from Dsrc was added to the N terminus (28). Flies transformed with this gene exhibit an eye phenotype similar to that of flies transformed with *Ras1^{val12}* (Fig. 2*I*), suggesting that myristylation is sufficient to localize activated Ras1 to the plasma membrane. Injection of 40 mM FTI-254 did not rescue the Ras^{Myr} phenotype but did slightly reduce the average number of R7 cells per ommatid-



FTI232. X=O, Y=CH_2SCH_3, R=H
FTI244. X=O, Y=CH_2SCH_3, R=CH_3
FTI254. X=2H, Y=CH_2SCH_3, R=CH_3
FTI280. X=2H, Y=$CH(CH_3)_2$, R=CH_3



FTI265.

FIG. 1. Structure of the CAAX peptidomimetics. FTI-232, FTI-244, and FTI-265 were prepared as described in refs. 21 and 32. FTI-280 was prepared by substituting L-leucine methyl ester for L-methionine methyl ester in the synthesis of FTI-254 described in ref. 21.

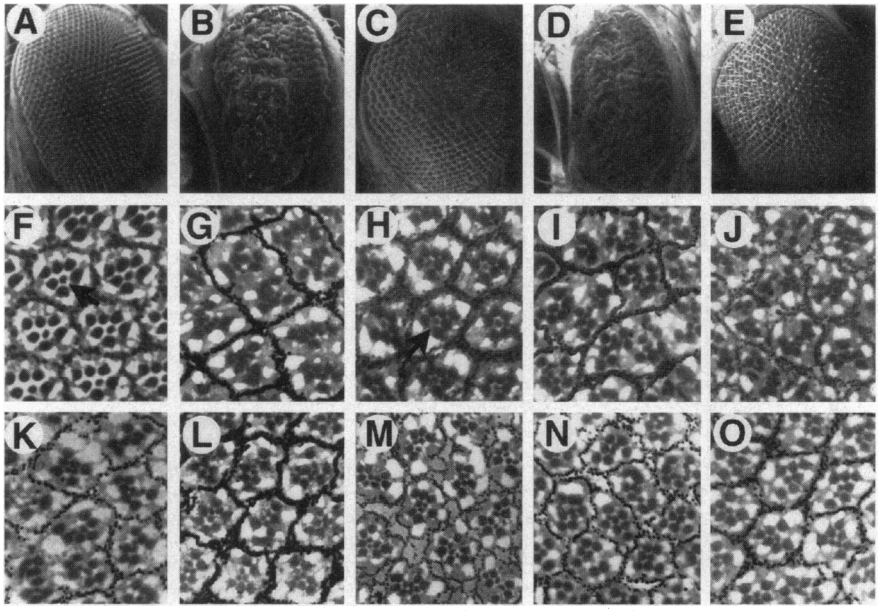


FIG. 2. Compound eye morphology of transformed flies after treatment with Ras CAAX peptidomimetics. (*A–E*) Scanning electron micrographs of eyes from a wild-type fly (*A*), *Ras1*^{val12} fly (*B*), *Ras1*^{val12} fly after treatment with 33 mM FTI-244 (*C*), *Ras1*^{val12} fly after treatment with 33 mM FTI-232 (*D*), and *Ras1*^{val12} fly after treatment with 40 mM FTI-254 (*E*). (*A–E*, ×45.) (*F–O*) Tangential plastic sections through the apical retina of a wild-type fly (*F*), *Ras1*^{val12} fly (*G*), *Ras1*^{val12} fly after treatment with 40 mM FTI-254 (*H*), *Ras*^{Myr} fly (*I*), *Sev*^{S11} fly (*J*), *sE-raf*^{tor4021} fly (*K*), *sev-Sem* fly (*L*), *Sev*^{S11} fly after treatment with 40 mM FTI-254 (*M*), *sE-raf*^{tor4021} fly after treatment with 40 mM FTI-254 (*N*), and *sev-Sem* fly after treatment with 40 mM FTI-254 (*O*). The R7 cell in a wild-type ommatidium is marked by an arrow in *F*, and a single R7 cell in an ommatidium from a *Ras1*^{val12} fly treated with FTI-254 is indicated with an arrow in *H*. Untreated animals served as a control for comparison because injection with the peptidomimetic carrier solvents alone, DMSO and DTT did not alter the eye phenotype. (*F–O*, ×450.)

ium from 2.1 to 2.0 (Fig. 3*B*). The reduction may be due to other isoprenylated proteins, possibly Ras1 itself, contributing to R7 cell differentiation. However, comparison of FTI-254 treatment of *Ras1*^{val12} and *Ras*^{Myr} flies demonstrates the specificity of FTI-254 for inhibiting isoprenyl modification of Ras1 in whole animals.

The Ras CAAX Peptidomimetics Specifically Suppress Ras1. To evaluate the selectivity of FTI-254 for Ras1 inhibition, we assayed the effect of FTI-254 on supernumerary R7 cell formation in flies transformed with either an activated sevenless, *Raf*, or rolled MAP kinase gene. None of these gene products are modified by isoprenylation. In addition, they function within the same signal transduction pathway as Ras1 to trigger R7 cell differentiation (23). The sevenless RTK functions upstream of Ras1 while Raf and rolled function downstream of Ras1 in the pathway. Activated forms of sevenless (*Sev*^{S11}) (24), Raf (*sE-raf*^{tor4021}) (26), and rolled (*sev-Sem*) produce a similar phenotype to Ras1^{val12} with supernumerary R7 cell formation (Fig. 2 *J–L*). Injection of 40 mM FTI-254 into *Sev*^{S11} and *sE-raf*^{tor4021} larvae had no effect on supernumerary R7 cell formation (Fig. 2 *M* and *N*; Fig. 3*C*). This suggests that FTI-254 is highly specific for *Ras1*^{val12}-transformed cells. It further suggests that normal Ras1 activity is not significantly affected by FTI-254 in the ectopic R7 cells transformed by *Sev*^{S11} since normal Ras1 activity is required for transformation by *Sev*^{S11} (R.W.C., unpublished data). Because the number of supernumerary R7 cells did not decrease after injection, it also rules out the possibility that transformed cells are more sensitive to cytotoxic effects of FTI-254 treatment. Injection of 40 mM FTI-254 into *sev-Sem* larvae partially rescued the eye phenotype (Fig. 2*O*). The average number of R7 cells per ommatidium decreased from 2.4 to 1.6 after treatment. Since *sev-Sem* is a weak gain-of-function mutation, it may be sensitive to upstream elements of the Ras1 pathway in cells where Sevenless is not activated by Boss. Indeed, *Ras1* loss-of-function mutations act as dominant suppressors of *Sem* (29, 34). Perhaps FTI-254 inhibits the activity of wild-type Ras1 enough to significantly reduce the ability of *sev-Sem* to transform cell fates.

***Drosophila* Ras1 Is Geranylgeranylated.** Although other proteins besides mammalian Ras are *in vivo* substrates for farnesylation, most isoprenylated proteins are modified by a geranylgeranyl group (7). Indeed, evidence suggests that *Drosophila* Ras1 is geranylgeranylated. The C-terminal amino acid of Ras1 is leucine, which normally serves as a substrate for GGPTase I (1, 7, 8). Moreover, null mutations in the *Drosophila* GGPTase I gene suppress the Ras1^{val12} phenotype in a dosage-dependent manner (28). Therefore, to confirm that the Ras1^{val12} protein is an *in vivo* substrate for GGPTase I, we injected *Ras1*^{val12} larvae with peptidomimetics that exhibit different potencies for inhibiting GGPTase I. The *in vitro* IC_{50} values for inhibition of GGPTase I by various peptidomimetics were determined (Table 1). FTI-254 was more potent than FTI-265 in inhibiting GGPTase I but was less effective than FTI-280. FTI-265 is a nonpeptide CAAX mimetic with the tripeptide AAX replaced by a biphenyl derivative (Fig. 1). FTI-280 is identical to FTI-254 except for a Leu residue at the carboxyl position (Fig. 1). Injection of 40 mM FTI-265 into *Ras1*^{val12} larvae was less effective than injection of 20 mM FTI-254 in suppressing supernumerary R7 cell formation (Fig. 3*D*). Injection of 20 mM FTI-280 into *Ras1*^{val12} larvae was more effective than injection of 40 mM FTI-254 at suppressing supernumerary R7 cell formation (Fig. 3 *A* and *D*). Moreover, FTI-254 suppressed the Ras1^{val12} phenotype when 120 pmoles was injected into larvae. Assuming uniform distribution of the compound in each larva, this corresponds to a whole-body concentration of 40 μM, above its *in vitro* IC_{50} value for GGPTase I. Taken together, these data support previous evidence that Ras1^{val12} is geranylgeranylated and correlates *in vitro* GGPTase I inhibitory activities of the peptidomimetics with their ability to suppress the Ras1^{val12} phenotype *in vivo*.

To investigate the effect of the peptidomimetics on other members of the Ras superfamily of GTPases, we treated Roughened larvae with FTI-280. Roughened is an activated mutant allele of the *Drosophila* homolog of Rap1A (35). The

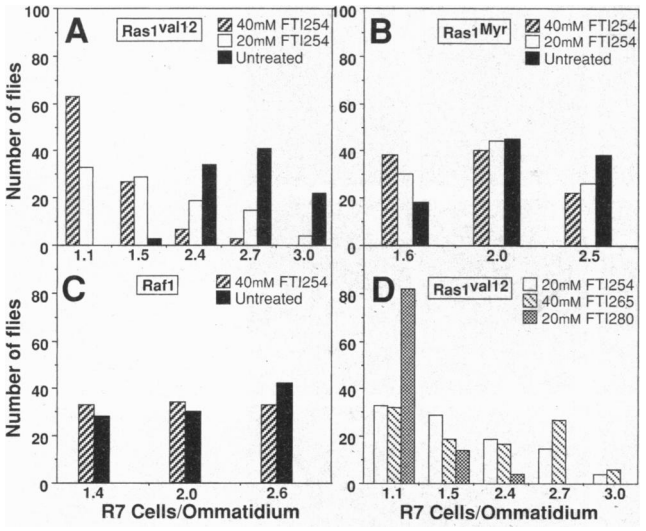


FIG. 3. Suppression of ectopic R7 cell transformation by injection of Ras CAAX peptidomimetics into $Ras1^{val12}$-transformed larvae (*A* and *D*), Ras^{Myr}-transformed larvae (*B*), or $sE\text{-}raf^{tor4021}$-transformed larvae (*C*). Larvae were either injected with the indicated concentration of peptidomimetic or received no treatment. For each transformed line, the flies that eclosed were classified into different groups according to the severity of their rough-eye phenotypes. Approximately 12 flies were randomly chosen from each group, and their eyes were sectioned tangentially. Only the anterior-most region of the eye was sectioned and analyzed because of the nonuniformity of the supernumerary R7 cell phenotype across the surface of the eye. For each group, a total of 400 ommatidia were scored to determine the average number of R7 cells per ommatidium. The mean values for each group are indicated along the horizontal axis and are plotted against the number of flies placed within that group. Groups were delineated such that the standard deviation from the mean number of R7 cells per ommatidium in each group was less than 10%. A total of 100 flies were examined for each peptidomimetic concentration injected.

Roughened protein is likely geranylgeranylated since its C-terminal residue is leucine, and its mammalian homolog is geranylgeranylated (35, 36). Injection of 20 mM FTI-280 suppressed the rough-eye phenotype of Roughened (R.C.K., unpublished data). Thus, other activated GTPases that are dependent upon isoprenylation can be inhibited by CAAX peptidomimetics *in vivo*.

The Ras CAAX Peptidomimetics Have a Low Level of Toxicity. Comparison of various peptidomimetics *in vitro* suggest they have a 10- to 100-fold increased specificity for inhibiting their cognate enzymes (21). Because the peptidomimetic concentrations injected in these experiments were probably sufficient to affect both farnesylated and geranylgeranylated proteins, we examined the general cytotoxicity of FTI-254 by measuring lethality of animals injected with various doses of the peptidomimetic (Fig. 4). Although increasing doses of FTI-254 resulted in lower viability, part of this effect was due to the presence of the carrier solvent, DMSO, which by itself caused significant lethality. At 40 mM, the lethal effect of FTI-254, compared with the DMSO control, was statistically insignificant. Furthermore, injected animals examined for

Table 1. Inhibition of GGPTase I *in vitro*

Peptidomimetic	IC_{50}, μM
FTI-254	19
FTI-265	100
FTI-280	7.5
FTI-249*	4.4
FTI-279*	0.1

*FTI-249 and FTI-279 are free carboxylate non-prodrug analogs of FTI-254 and FTI-280, respectively.

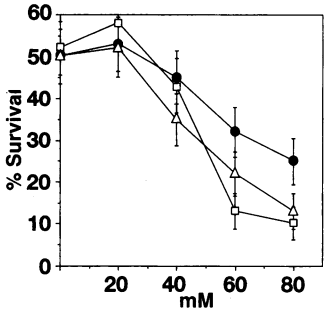


FIG. 4. Lethality of flies after injection with peptidomimetic FTI-254. $Ras1^{val12}$ flies were injected with DMSO (●) or FTI-254 (△). Ras^{Myr} flies were also injected with FTI-254 (□). The working stock solution of FTI-254 was 100 mM FTI-254/10 mM DTT in DMSO. This solution was diluted with 1 mM DTT, and 3 nl was injected for each value. Flies that received 0 mM FTI-254 or DMSO were injected with 1 mM DTT alone. A total of 60 larvae were injected for each value, and the percentage of flies for each group that eclosed into adults was calculated. The error bars represent the standard deviation calculated for a binomial distribution. The numerical values listed on the *x* axis refer to mM peptidomimetic and % DMSO.

developmental defects in the adult bristle pattern, legs, and wings displayed very few abnormalities, even at the highest doses of FTI-254 administered. The developmental abnormalities associated with DMSO injections alone included missing scutellar bristles and, in some cases, the development of extra bristles at these positions. Several additional defects were observed in flies injected with peptidomimetics that were not evident in the control population. Of 370 flies injected with FTI-254, FTI-265, or FTI-280, 1.3% had an incomplete outgrowth of one wing. Approximately one-half of these wing defects were associated with incomplete notum development. *Ras1* is involved in many different aspects of pupal growth and development, yet a low level of toxicity is associated with FTI-254 injections at concentrations that are sufficient to suppress the $Ras1^{val12}$ phenotype. This suggests that $Ras1^{val12}$ activity has a greater sensitivity to inhibition by FTI-254 than wild-type Ras1 activity or other isoprenylated proteins.

Concluding Remarks. Three lines of evidence suggest that normal Ras1 is not affected by peptidomimetic treatment to the same degree as $Ras1^{val12}$: FTI-254 suppresses supernumerary R7 cell development induced by $Ras1^{val12}$ but does not significantly suppress wild-type R7 cell development, the development of ectopic R7 cells transformed by Sev^{S11} is not inhibited even though wild-type Ras1 is required for cell transformation by Sev^{S11}, and pupal development is largely unaffected despite the involvement of Ras1 signaling in a number of developmental pathways. The greater sensitivity of oncogenic Ras activity to CAAX peptidomimetics has earlier precedents. Fibroblast or Rat1 cells grow in the presence of certain FPTase inhibitors at concentrations which inhibit growth of these cells when transformed by oncogenic *Ras* (15–17). While these effects may be attributed in part to a dominant-negative effect of oncogenic *Ras* on c-*Ras* signaling (7), we did not observe a dominant-negative interaction between $Ras1^{val12}$ and wild-type *Ras1* in the R7 cell. If $Ras1^{val12}$ is in a constitutively GTP-bound conformation prior to post-translational modification, the substrate affinity of $Ras1^{val12}$ for GGPTase I may be lower than normal Ras1 and consequently more sensitive to competitive inhibition. Studies on yeast mutants lacking FPTase support such a differential substrate affinity for Ras. The phenotype of an oncogenic form of the yeast *Ras2* gene is suppressed in an FPTase-deficient cell, whereas the wild-type *Ras* gene is not suppressed because of a low level of geranylgeranylation by GGPTase I (37). An alternative explanation for the greater sensitivity of $Ras1^{val12}$ is that high levels of $Ras1^{val12}$ may be required for ectopic R7

transformation. The *sev* promoter used to drive $Ras1^{val12}$ expression is likely producing significantly higher levels of $Ras1^{val12}$ than Ras1 driven by its own promoter.

Our results demonstrate that peptidomimetics designed to inhibit FPTase have few effects on overall growth and development at concentrations that are sufficient to inhibit $Ras1^{val12}$ modification by GGPTase I. Although *Drosophila* proteins such as lamin are farnesylated (27), high doses of FTI-254 do not appear to significantly disrupt their activities, as evidenced by overall morphology and survival. This biological specificity suggests that there may be a fairly broad range of doses that can be tolerated during peptidomimetic treatment.

We have used *Drosophila* as a model system for characterizing the effectiveness of anti-Ras compounds *in vivo*. Despite the great physiological differences between flies and humans, our results suggest that *Drosophila* may be valuable to assay compounds that antagonize oncogenes in the Ras pathway. The rapid, inexpensive, and genetically defined aspects of this approach may make *Drosophila* a useful test animal for certain pharmacological studies.

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