Differential Activity of *Ras1* during Patterning of the Drosophila Dorsoventral Axis

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ABSTRACT

In Drosophila, the *Ras1* gene is required downstream of receptor tyrosine kinases for correct eye development, embryonic patterning, wing vein formation, and border cell migration. Here we characterize a *P*-element allele of *Ras1*, $Ras1^{5703}$, that affects viability, eye morphogenesis, and early and late stages of oogenesis. Flies transheterozygous for $Ras1^{5703}$ and existing EMS-induced *Ras1* alleles are viable and exhibit a range of eye and eggshell defects. Differences in the severity of these phenotypes in different tissues suggest that there are allele-specific effects of *Ras1* in development. Analysis of rescue constructs demonstrates that these differential phenotypes are due to loss of function in *Ras1* alone and not due to effects on neighboring genes. Females mutant at the *Ras1* locus lay eggs with reduced or missing dorsal eggshell structures. We observe dominant interactions between *Ras1* mutants and other dorsoventral pathway mutants, including $Egfr^{top}$ and *gurken*. *Ras1* is also epistatic to *K10*. Unlike $Egfr^{top}$ and *gurken* mutants, however, *Ras1* females are moderately fertile, laying eggs with ventralized eggshells that can hatch normal larvae. These results suggest that *Ras1* may have a different requirement in the patterning of the eggshell axis than in the patterning of the embryonic axis during oogenesis.

THE Ras protein is a member of a highly conserved family of GTPase proteins that function in signal transduction pathways in a wide variety of organisms and developmental processes (Boguski and McCormick 1993). Originally identified as an oncogene, *Ras* has since been found to be important for cell proliferation and differentiation as a conserved component downstream of receptor tyrosine kinases (RTKs) (Moodie and Wolfman 1994). In recent years, the combined evidence from genetic and biochemical investigation has led to a model for RTK activation of Ras that leads to subsequent activation of the mitogen-activated protein (MAP) kinase cascade (reviewed by McCormick 1994). The current model postulates that ligand binding to a RTK results in autophosphorylation of tyrosine residues. These phospho-tyrosine residues then serve as binding sites for a class of SH2-SH3 domain-containing adapter proteins that include Drk (Downstream of receptor kinase), the Drosophila homologue of the Grb2/Sem5 proteins (Olivier *et al.* 1993; Simon *et al.* 1993). Drk is thought to bind activated RTKs through its SH2 domain while interacting with a guanine nucleotide exchange factor, Son-of-sevenless, via its SH3 domains. These interactions serve to translocate the Sos protein to the plasma membrane where it can stimulate the exchange of GDP for GTP on Ras1. The binding of GTP by Ras1 activates Ras1 and leads to the binding and localization of Raf at the plasma membrane. Raf becomes activated and initiates a kinase cascade beginning with the phosphorylation of MEK (MAP kinase-kinase encoded by *Dsor1*). MEK in turn phosphorylates MAP kinase (encoded by the *rolled* gene), which then translocates to the nucleus and phosphorylates the appropriate transcription factors; these directly induce a gene response (reviewed in Marshall 1994).

In Drosophila, much of the original understanding of Ras signaling grew out of work on the Sevenless (Sev) RTK, which functions in eye development (reviewed by Wassarman *et al.* 1995), and the Torso (Tor) RTK, required for terminal differentiation of the embryo (reviewed by Duffy and Perrimon 1994). Additional investigation has demonstrated the importance of Ras signaling downstream of the Drosophila homologue of the epidermal growth factor receptor (Egfr). Unlike the highly restricted functions of Sev and Tor, however, Egfr is utilized repeatedly in Drosophila development; *Egfr* mutations affect the eye (*Ellipse*, $Egfr^{Elp}$) (Baker and Rubin 1992), the wing, the embryonic ventral midline (*faint little ball*, $Egfr^{flb}$) (Price *et al.* 1989; Schejter and Shilo 1989; Clifford and Schüpbach 1992), and the specification of primary axes of the eggshell and embryo (*torpedo*, $Egfr^{top}$) (reviewed in Munn and Steward 1995; Ray and Schüpbach 1996). Recent evidence suggests that the Ras signaling pathway may operate to some extent in all of these processes. Mosaic analyses demonstrate that Drk, Sos, Ras1, Raf, and Rolled are required downstream of Egfr in the epidermis (Diaz-Benjumea and Hafen 1994), and in oogenesis, Raf and MEK have been shown to be involved in dorsoventral axis specification downstream of Egfr in the follicle cells

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(BRAND and PERRIMON 1994; HSU and PERRIMON 1994). These studies raise important questions about Egfr and Ras signaling. Does Egfr signal exclusively through the Ras pathway in Drosophila development? Is this pathway conserved from tissue to tissue?

To answer these questions, we examine the role of Ras1 and Egfr signaling in the establishment of the dorsoventral axis during Drosophila oogenesis. Previous studies have demonstrated that multiple signaling events contribute to the establishment of dorsoventral polarity. Early in oogenesis, Gurken (Grk), the presumptive ligand for Egfr, signals from the germline to specify the fate of the posterior follicle cells. A subsequent signal back to the oocyte leads to the reorganization of the cytoskeleton within the germ cells, a key process that is responsible for the ultimate creation of the anterior-posterior (A-P) and dorsoventral (D/V) axes of the embryo (GONZ'ALEZ-REYES *et al.* 1995; ROTH *et al.* 1995).

In later stages of oogenesis, the Grk-Egfr signaling pathway is required to specify the identity of the dorsal follicle cells. These cells go on to secrete the dorsal structures of the eggshell, including the dorsal respiratory filaments, or dorsal appendages. In addition to the visible outer structures of the eggshell, these somatic follicle cells are thought to deposit into the inner vitelline membrane a gradient of molecules that is later used to correctly establish the dorsoventral axis of the embryo. A candidate protein for such a prepattern molecule is Nudel, a transmembrane protein whose expression pattern is regulated by Egfr signaling (HONG and HASHIMOTO 1995). The establishment of this prepattern produces an extraembryonic activity in the ventral portion of the perivitelline space that leads to the localized processing of Spätzle protein, the presumptive ligand for the Toll receptor (MORISATO and ANDERSON 1994; SCHNEIDER *et al.* 1994). Locally processed Spätzle activates Toll, eventually resulting in the nuclear import of Dorsal, the ventral morphogen (reviewed by CHASAN and ANDERSON 1993). The linkage between eggshell and embryonic axes is evident in analysis of loss-of-function mutations in *grk* and *Egfr;* defects in these genes lead to expansion of ventral tissues in both the eggshell and the embryo.

In a search for other components involved in dorsoventral patterning during oogenesis, we have isolated and characterized the first viable allele of *Ras1*, $Ras1^{5703}$. *Ras1* mutant flies lay eggs that completely lack dorsal eggshell structures, but these eggs hatch normal larvae at high frequencies.

MATERIALS AND METHODS

Fly stocks and culture media: Flies were maintained on standard medium at 25° unless otherwise indicated. Embryos were collected on apple juice agar plates at 25°. The $Ras1^{5703}$ line was generated in a *P[lacZ, ry*$^+$*]* mutagenesis screen conducted in the laboratory of ALLAN SPRADLING (KARPEN and SPRADLING 1992). $Ras1^{5703}$, also known as *85D5703* or *fs(3)5703*, contains a single *P[lacZ, ry*$^+$*]* element at 85D10. The excision alleles $Ras1^{ix12a}$, $Ras1^{ix13b}$, $Ras1^{\Delta C40b}$, and $Ras1^{\Delta G17b}$ were generated by mobilizing the *P* element in the strain $Ras1^{5703}$. $Ras1^{E62K}$, $Ras1^{D38N}$ (SIMON *et al.* 1991), $Ras1^{su(tor)404}$ and $Ras1^{su(tor)341}$ (DOYLE and BISHOP 1993) are lethal, EMS-induced alleles of *Ras1*. The HSP-*Rlb1* rescue line was generated by EZER *et al.* (1994). top^{18a} ($Egfr^{Df}$) (PRICE *et al.* 1989), grk^{HK36} (SCHÜPBACH 1987) and *fs(1)K10* (WIESCHAUS *et al.* 1978) were provided by TRUDI SCHÜPBACH. *Df(3R)by10* (85D8-11; 85E10-13) was obtained from the Bloomington Stock Center. Canton-S was used as a wild-type control.

Microscopy of egg chambers, embryos, and eyes: Ovaries were fixed according to COOLEY *et al.* (1992). To visualize cell nuclei, ovaries were stained in phosphate-buffered saline (PBS) plus 1 ng/ml DAPI (4,6-diamidino-2-phenylindole) for 10 min. Individual egg chambers were dissected and mounted in glycerol:PBS (1:1) and examined using differential interference contrast optics on a Nikon Microphot FXA. Cuticles were prepared for microscopy essentially as described by WIESCHAUS and NÜSSLEIN-VOLHARD (1986). For scanning electron microscopy (SEM) of fly eyes, flies were stored at −70° and then shadowed according to standard methods. SEM images were generated by LIZ CALDWELL at the Fred Hutchinson Cancer Research Center.

Excision screen: Isochromosomal strains were generated in which either *sbd* or *cv-c* and *sbd* were recombined onto the $Ras1^{5703}$ chromosome as markers. These strains, designated $Ras1^{5703x}$ and $Ras1^{5703c}$, respectively, were then used separately but identically to generate excision lines. For example, $Ras1^{5703}$ *P[lacZ, ry*$^+$*]*(85D10) ry^{506} *cv-c sbd/* TM3, ry^{RK} *Sb* males were crossed to ry^{506} *Sb P[ry*$^+$*, Δ2-3]*(99B)/TM6 (ROBERTSON *et al.* 1988) females *en mass*. In the subsequent generation, males were selected containing ry^{506} *Sb P[ry*$^+$*, Δ2-3]*(99B)/$Ras1^{5703}$ *P[lacZ, ry*$^+$*]*(85D10) ry^{506} *cv-c sbd*, and two such males were mated to five ry^{506}/TM3, ry^{RK} *Sb* virgins per vial for a total of 100 crosses. In the next generation, excision of the *P* element was identified by loss of the ry$^+$ eye color marker, yielding flies of the genotype $Ras1^{ry^-}$ ry^{506} *cv-c sbd*/TM3, ry^{RK} *Sb*. At most, two ry$^-$ males were selected from each of the 100 crosses and mated individually to virgins of the starting strain, $Ras1^{5703}$ *P[lacZ, ry*$^+$*]*(85D10) ry^{506} *cv-c sbd*/TM3, ry^{RK} *Sb*. From this cross, transheterozygotes between the excision allele and the original *P* allele were tested for fertility, and balanced stocks were generated for each excision allele.

Molecular analysis of $Ras1^{5703}$ and excision lines: For Southern analysis, DNA was isolated and analyzed according to methods previously described (HOROWITZ and BERG 1995). Blots were hybridized with probes generated from a 12-kb *Xho*I-*Not*I genomic fragment con-

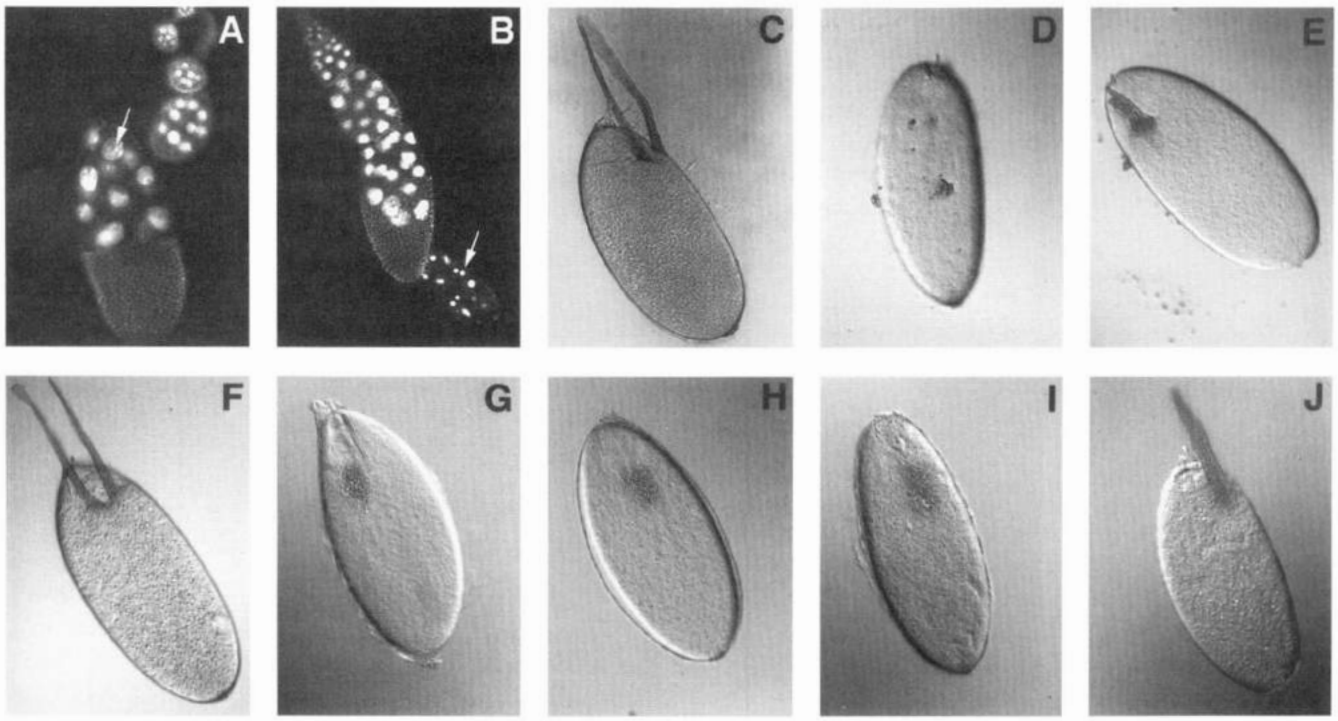


FIGURE 1.—*Ras1* is required early and late in oogenesis. (A and B) Early defects. At a low frequency (<5%), we observe early oogenesis defects in *Ras1* mutant females including degeneration of egg chambers. (A) Wild-type ovariole stained with DAPI to visualize cell nuclei. Arrow indicates a large, brightly staining nurse cell nucleus of a stage 10 egg chamber. Ovariole from a $Ras1^{5703}/Ras1^{5703}$ female (B). Arrow indicates punctate DAPI staining observed in degenerating egg chambers. (C–J) Late defects. *Ras1* mutant eggs lack dorsal appendage structures. Anterior is up, dorsal is facing out of the page. (C) Wild-type egg from a $Ras1^{5703}$/TM3 mother: note prominent dorsal appendage structures. (D) Severe Ras1 eggshell phenotype from $Ras1^{5703}/Ras1^{5703}$ female. Few eggs are laid and those produced have only a patch of chorion instead of the dorsal appendages. Overall egg shape appears normal and only one micropyle is ever observed. (E) Example of a moderate Ras1 phenotype. $Ras1^{ix13b}$ eggs have some dorsal appendage material, usually a short single appendage as pictured here. The phenotype can range from a smaller nub to a longer single dorsal appendage. Wild-type dorsal appendages are never observed. (F) Weak Ras1 phenotype. $Ras1^{ix12a}$ females lay eggs that range from wild type to those exhibiting a slight expansion of the chorion at the base of the dorsal appendages. (G–J) Moderate Ras1 eggshell phenotypes observed in heteroallelic combinations between $Ras1^{5703}$ and EMS-induced *Ras1* mutations: (G) $Ras1^{5703}/Ras1^{D38N}$, (H) $Ras1^{5703}/Ras1^{su(tor)341}$, (I) $Ras1^{5703}/Ras1^{su(tor)404}$, (J) $Ras1^{5703}/Ras1^{E62K}$. Every egg from females of this genotype has a single dorsal appendage.

taining *Ras1* (SIMON *et al.* 1991) and from a 3′ *P*-element clone (HOROWITZ and BERG 1995). For plasmid rescue, the genomic DNA surrounding the $Ras1^{5703}$ *P*[*lacZ*, ry^+](85D10) insertion was recovered much as described in ASHBURNER (1989) except that electrotransformation using a Bio-Rad Gene Pulser was employed. Sequencing of the genomic DNA bordering the *P*-element insertion was carried out by the dideoxy chain-termination method using reagents from US Biochemicals.

***In vitro* mutagenesis and *P* element-mediated germline transformation:** A 5.5-kb *Eco*RI fragment containing *Ras1*, *Rlb1* and *Rlc1* was subcloned from the 12-kb *Xho*I-*Not*I genomic fragment (SIMON *et al.* 1991) into pBlueScript KS (Stratagene). All three rescue elements derive from this 5.5-kb fragment. The positive control, pR5.5, was generated by cloning a 5.5-kb *Xho*I-*Bam*I fragment into the pCaSpeR4 vector (THUMMEL and PIRROTTA 1992). For the other constructs, *in vitro* mutagenesis of the *Ras1* and *Rlb1* genes was carried out using the Promega Altered Sites II kit. Briefly, a *Xho*I-*Bam*I fragment was subcloned into the pAlter1 vector, a primer containing the mutagenic change annealed, second strand synthesis completed, DNA transformed into *Escherichia coli* and then putative clones screened by sequencing the pertinent region. The pR5.5ΔRlb1 construct was created by deleting a nucleotide at position 9 in the open reading frame of *Rlb1* (ATG GAC AGA TTG AAA) resulting in a frameshift that should produce a three-amino acid peptide. The pR5.5ΔRas1 construct was created by deleting a nucleotide at position 12 (ATG ACG GAA TAC AAA), similarly resulting in a frameshift predicting a peptide of three amino acids. Mutant inserts were subcloned into pCaSpeR4 and sequenced across the mutation again before injection. *P* element-mediated transformation was carried out as described (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982).

RESULTS

***5703* contains a *P*[*lacZ*, ry^+] element in the 5′ region of *Ras1*:** In a *P*-element mutagenesis screen described

in KARPEN and SPRADLING (1992), we recovered a semilethal, female sterile mutant line designated *5703*. *In situ* hybridization mapped the insertion to 85D10 on the right arm of the third chromosome, the approximate cytological position of *Ras1* (NEUMAN-SILBERBERG *et al.* 1984), and eye phenotypes associated with the mutation were consistent with a known function of *Ras1* in the fly (SIMON *et al.* 1991). Subsequent Southern analysis suggested that the *5703* line contained a *P* element inserted in the 5′ regulatory region of the *Ras1* gene (data not shown). Using plasmid rescue, we isolated genomic DNA flanking the 5′ end of the *P* element (See MATERIALS AND METHODS), and sequence analysis demonstrated that the *P* element is located 28 bp upstream of a putative *Ras1* transcription start site as determined by cDNA analysis (EZER *et al.* 1994). These observations are consistent with the hypothesis that the *5703* element disrupts expression of the *Ras1* gene, and genetic tests (described below) further support this conclusion. We therefore renamed this line $Ras1^{5703}$.

Eggs from $Ras1^{5703}$ flies have defects in early oogenesis and lack dorsal appendage structures: Since existing mutations in the *Ras1* gene are lethal, $Ras1^{5703}$ provides the first opportunity to characterize the adult phenotypes of *Ras1* mutants directly. Females homozygous for the $Ras1^{5703}$ mutation exhibit both early and late stage oogenesis defects. A low percentage of egg chambers from any given mutant female degenerate, generally between stages 8 and 10. This degeneration is characterized by the loss of distinct cellular morphology within the egg chamber and punctate DAPI staining of nurse cell nuclei (arrow in Figure 1B). At a lower frequency (<1%), we observe egg chambers that contain too few nurse cells or fail to correctly position the developing oocyte within the egg chamber (data not shown). In addition, flies carrying *5703* in *trans* to a deficiency for the region have rudimentary ovaries. These results suggest an essential role for *Ras1* in early oogenesis.

All late-stage egg chambers from $Ras1^{5703}$ females lack dorsal appendage structures and possess only a patch of chorion centered on the dorsal midline (Figure 1D). Few eggs are laid. The reduction in dorsal appendage material is a characteristic of ventralizing mutations, *e.g.*, *grk* and *Egfr*, that reduce the number of cells adopting the dorsal follicle cell fate. Thus, the eggs produced by $Ras1^{5703}$ appear ventralized with respect to the dorsal appendage structures, but at the same time their overall shape is normal; these eggs are approximately the same length as normal eggs and possess a ventral surface that is longer than the dorsal surface.

Generation and characterization of excision alleles of the $Ras1^{5703}$ line: To expand the spectrum of phenotypes available for analysis, we mobilized the *P* element in $Ras1^{5703}$ to generate additional alleles. We crossed in a source of transposase, Δ2-3 (ROBERTSON *et al.* 1988), and screened flies for loss of the ry^+ marker contained in the *5703 P* element. Of 134 ry^- lines established in this manner, 66 (49%) are fully viable and fertile and represent true revertants of the phenotype, evidence that the mutation in the $Ras1^{5703}$ line is due to insertion of the *P* element. The remaining lines consist of 30 lethals (22%), 21 semilethals similar to the starting strain (16%), and 17 phenotypically weaker alleles (13%).

TABLE 1

Measure of viability of *Ras1* mutant alleles

Genotype	% Viability[a]	*n*
5703/5703	0.84	3815
Df[b]*/5703*	0.13	1598
ix13b/ix13b	48	1053
Δ*C17b*[c]*/5703*	0	98
Δ*C40b*[c]*/5703*	0	199
Δ*C17b/E62K*[d]	0	116
Δ*C40b/E62K*	0	91
D38N[d]*/5703*	55	464
E62K/5703	53	559
su(tor)341[d]*/5703*	19	429
su(tor)404[d]*/5703*	9.7	346

[a] Percentage viability is based on the number of observed/number of expected flies. The number of +/*Ras1* sibling flies was used to calculate the number of expected $Ras1^-/Ras1^-$ flies.

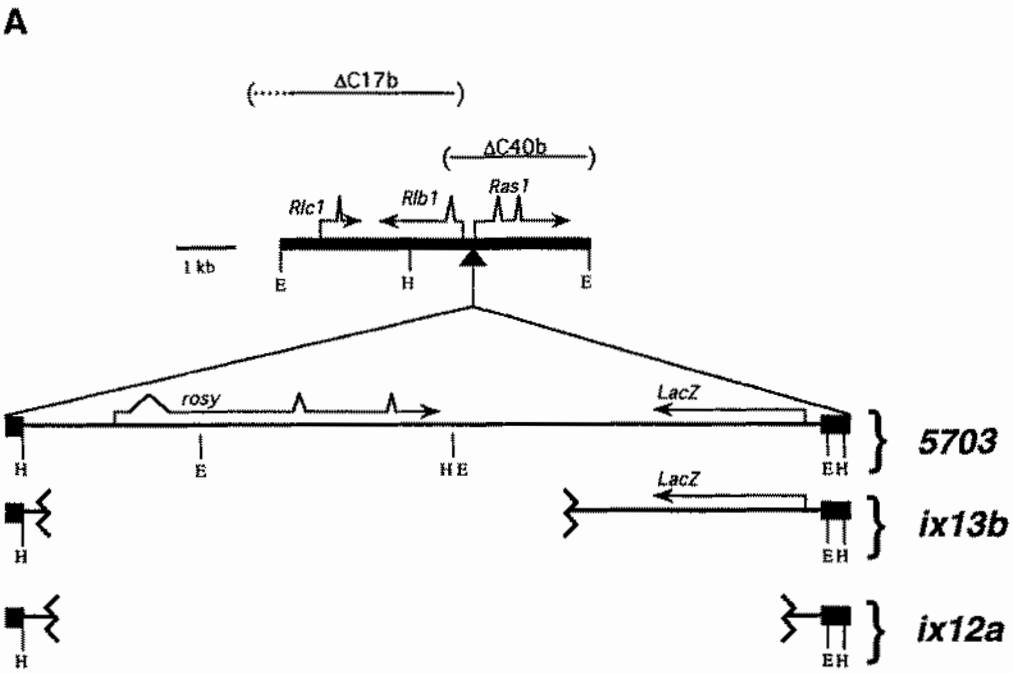
[b] *Df* = *Df(3R)by10* (85D8-11; 85E10-13).

[c] Lethal excision allele.

[d] Lethal EMS-induced *Ras1* allele.

Each excision line can be classified according to its viability and eggshell phenotype, resulting in a simple phenotypic series. Furthermore, the severity of the allele correlates with the molecular lesion. The strongest alleles are lethal and contain deletions of flanking DNA; examples include $Ras1^{\Delta C17b}$ and $Ras1^{\Delta C40b}$ (Table 1; Figure 2). Moderate alleles such as $Ras1^{ix13b}$ are viable, and homozygous females lay eggs that possess a single dorsal appendage or a short nub of chorion material along the dorsal midline (Figure 1E). $Ras1^{ix13b}$ results from an internal deletion of the *P* element, retaining 5.6 kb (Figure 2) of the original 15-kb transposon. The weakest excision lines are similar to the wild type, but some eggs from these females have slightly defective chorions as evidenced by unusually close spacing and slight enlargement of the bases of the dorsal appendages ($Ras1^{ix12a}$, Figure 1F). Genetic tests confirm that $Ras1^{ix12a}$ is a hypomorphic allele; more severe phenotypes become apparent when $Ras1^{ix12a}$ is placed in *trans* to a deficiency (Figure 5B). Consistent with the weaker phenotype, $Ras1^{ix12a}$ consists of a large internal deletion of the *P* element, retaining 2 kb of the transposon sequence (Figure 2).

Flies carrying *P* element/EMS-induced allelic combinations exhibit eggshell and eye defects: We characterized the phenotypes of flies carrying $Ras1^{5703}$ in *trans* to existing EMS-induced mutations in *Ras1* (SIMON *et al.* 1991; DOYLE and BISHOP 1993). All of these transhetero-



B

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                                          ***          <--Rlb1
     .....CAAATTATTGAATTTCAATCTGTCCATGCTGTTTTCGTTACAGTggtggttgaaataat 3100

agactcaggcttgagtccgtgctaagaaacggtgatgccagcccgttgaacttaaattaacaattattaaaagca 3175

gaaagcgtctgccagtttttacagaaagctgtattgatcaaatcatttattagtttaagcaaattatagagagtc 3250

gactgaagtgcatccacctctttagattagttttcatatagcaatcgctattgcaagtccatcgatggtgccggc 3325

                                                                  P element
cagaatgcagccatcgctatcgatagtgccatcggcttggtgccagctctagtatcttcacagcta▼cgtacagc 3399

                      Ras1-->
ctagatttcatcttcatttcACATACGGATTTATTGTTTTCCTGCGCTGTTAATTGCAATTACCGCACGGCAATT 3475
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FIGURE 2.—Molecular map of the *Ras1* region and *P* element alleles. (A) *Ras1*, *Rlb1*, and *Rlc1*, characterized by EZER *et al.* (1994), are contained on a 5.5-kb *Eco*RI fragment. The direction of transcription is indicated by arrows, and the position of the *5703 P* element is indicated below the solid line with an arrowhead. $Ras1^{ix13b}$ and $Ras1^{ix12a}$ are excision alleles generated from $Ras1^{5703}$; they contain internal deletions of the *P*-element sequence in which only 5.6 and 2 kb, respectively, of transposon sequence remain. ΔC40b and ΔC17b are lethal deletions of the *Ras1* region. The lines indicate the approximate extent of genomic DNA deleted. By Southern analysis, ΔC40b contains a deletion of the *Ras1* open reading frame (HOU *et al.* 1995). ΔC17b contains a deletion 5′ of *Ras1* that extends through *Rlb1*. The extent of the deletion is not known. Some *P*-element transposon sequence may remain. (H, *Hin*dIII and E, *Eco*RI) (B) Position of the *5703 P* element at the sequence level. The *P* element is 28 bp upstream of the putative transcription start site as determined by cDNA analysis (EZER *et al.* 1994) and 306 bp from *Rlb1*. Although the *Ras1* gene does not appear to have a TATA sequence, it does possess two transcriptional regulatory sequences: a DNA replication related element (DRE), tatcgata at 3343, and an initiation sequence, **tcattt,** near the predicated transcription start site. The DRE sequence has been found in a number of Drosophila genes and shown to be important for the transcriptional regulation of DNA polymerase α and proliferating cell nuclear antigen (HIROSE *et al.* 1993; MATSUKAGE *et al.* 1996). The initiator (Inr) or cap-site sequence matches with the Drosophila consensus Inr sequence, $TCA^{G}/_{T}T^{T}/_{G}$, found in both TATA-containing and TATA-less promoters (HULTMARK *et al.* 1986; PURNELL *et al.* 1994; ARKHIPOVA 1995). Arrows indicate direction of transcription, small letters indicate untranscribed regions, capitals indicate transcribed regions, dashed underlines indicate untranslated regions, solid underlines indicate translated sequence with three asterisks indicating initiation of *Rlb1* translation. The numbering and molecular characterization is based on EZER *et al.* (1994). The position of the *P* element is indicated by ▼.

zygous individuals exhibit oogenesis and eye phenotypes consistent with a role for *Ras1* in these processes. Three of the four heteroallelic combinations lay eggs with severely disrupted eggshells (Figure 1, G–I), exhibiting only a patch of chorion instead of dorsal appendages. The eggs from $Ras1^{E62K}/Ras1^{5703}$ females exhibit a weaker phenotype in which a single dorsal appendage is present on the midline (Figure 1J).

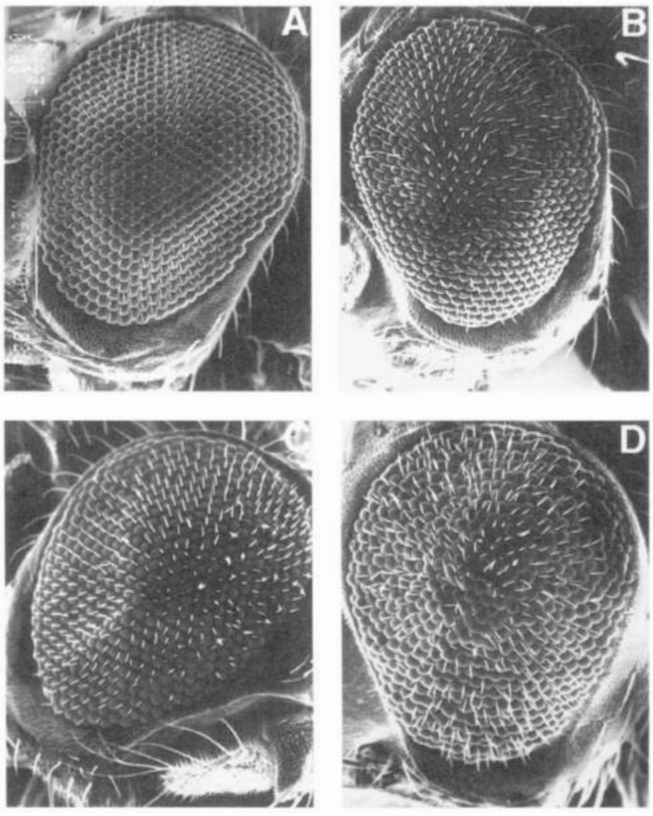

FIGURE 3.—Scanning electron micrographs of eyes. (A) Wild-type eye (*Ras1*^{5703} *sbd*/TM3, *Sb*). (B) Slightly rough eye from *Ras1*^{5703} *sbd*/*Ras1*^{5703} *sbd* fly. These eyes appear nearly normal under the dissecting microscope. (C) More extreme rough eye from *Ras1*^{5703}/*Ras1*^{D38N} fly. These flies exhibit a noticeable rough eye when viewed under the dissecting microscope. (D) Extreme rough eye exhibited by *Ras1*^{5703}/*Ras1*^{E62K} fly. Array is disrupted and there is an increased frequency of fused ommatidia.

The severity of the eye phenotypes in these animals, however, does not correlate with the eggshell phenotypes. The strictly ordered array of ommatidia present in wild type is slightly disrupted in *Ras1*^{5703} homozygotes and the heteroallelic lines *Ras1*^{D38N}/*Ras1*^{5703}, *Ras1*$^{su(tor)341}$/*Ras1*^{5703}, and *Ras1*$^{su(tor)404}$/*Ras1*^{5703}. In contrast, *Ras1*^{E62K}/*Ras1*^{5703} animals exhibit extremely rough eyes. We examined these differences more closely by employing SEM. Analysis of flies with slightly rough eyes reveals a small number of fused, missing, or misplaced ommatidia (Figure 3, B and C). *Ras1*^{E62K}/*Ras1*^{5703} flies exhibit an increased number of fused and misplaced ommatidia compared to the other allelic combinations (Figure 3D). Thus, the *Ras1*^{E62K}/*Ras1*^{5703} flies exhibit the roughest eyes, yet have the highest viability and weakest eggshell phenotype of the heteroallelic combinations characterized. These observations suggest that there are allele-specific effects of *Ras1* in development.

Since all four of the EMS-induced *Ras1* alleles are lethal, while the *Ras1*^{5703} allele is semilethal (viability ≤1%) (Table 1), we expected that heteroallelic combinations between the EMS alleles and the *P* allele would be semilethal. We were surprised to find, however, that flies carrying these combinations of alleles eclose at significantly higher than expected frequencies (9–55%) (Table 1). Work by EZER *et al.* (1994) has defined two other transcripts in the vicinity of *Ras1*, *Rlb1* and *Rlc1*. *Rlb1* and *Ras1* are divergently transcribed and are separated by a 334-bp region (Figure 2). Given the proximity and orientation of the *Rlb1* gene to the *5703* insertion site, the observed partial complementation could be explained by hypothesizing that *Ras1*^{5703} is a complex mutation affecting the levels of both *Ras1* and *Rlb1* mRNAs. To test the involvement of *Rlb1* in the Ras1^{5703} phenotype, we designed rescue elements that would restore either *Rlb1* function alone or *Ras1* function alone (Figure 4). First, we tested the 5.5-kb *Eco*RI fragment containing *Ras1*, *Rlb1*, and *Rlc1* (pR5.5) and showed that it completely rescues the *Ras1*^{5703} mutation, restoring viability (Figure 4) and proper ovarian development (data not shown). We then attempted to rescue the Ras1^{5703} phenotype with a construct containing *Rlc1* and *Rlb1*, but lacking *Ras1* function (pR5.5ΔRas1). To retain all the regulatory elements that might be necessary for *Rlb1* expression, we used the 5.5-kb *Eco*RI fragment and engineered a single nucleotide deletion early in the *Ras1* coding region (Figure 4 and MATERIALS AND METHODS). This change is predicted to cause premature translational termination and the production of a nonfunctional three amino acid protein. We examined four independent insertion lines of the *Rlb1*$^{+}$ *Ras1*$^{-}$ rescue construct and found no measurable change in the Ras1^{5703} phenotype. To verify this result, we used an *hsp70* promoter-*Rlb1* construct (EZER *et al.* 1994) as an alternative source of Rlb1 product. Test animals were heat shocked daily, but no amelioration was observed in the Ras1^{5703} phenotype (data not shown). The observation that *Rlb1* alone cannot rescue the semilethality or oogenesis phenotypes of *Ras1*^{5703} flies argues that these phenotypes are due entirely to a reduction in Ras1 activity. To strengthen the conclusion that *Ras1*^{5703} only affects the levels of *Ras1* mRNA, we designed and tested a construct that would only rescue *Ras1* function and not *Rlb1* (pR5.5ΔRlb1; Figure 4). Full rescue of all Ras1^{5703} phenotypes was achieved with two independent transformant lines. These results demonstrate that, at least for the viability, eye, and eggshell phenotypes we have examined, only *Ras1* is affected in the *Ras1*^{5703} line.

***Egfr*^{Df} dominantly enhances a Ras1 mutant phenotype:** To understand the role of *Ras1* in development more fully, we asked if mutations in the *Ras1* gene interact with *Egfr* mutations. We focused our efforts on analyzing the process that establishes dorsoventral polarity in egg and embryo. Since previous studies indicated that Ras signaling is sensitive to dosage effects (SIMON *et al.* 1991), we reasoned that if Ras1 were operating downstream of Egfr, removal of just one copy of *Egfr* or *grk* in a *Ras1* background would result in an enhanced

Rescue of *Ras1*^{5703}

Rescue Construct	Genotype and Number of Flies Recovered: P[Rescue]; 5703/5703	+/+; 5703/5703	P[Rescue]; +/5703	+/+; +/5703
pR5.5	44	0	85	102
	30	0	83	93
pR5.5ΔRas1	4	9	230	190
	0	1	188	198
pR5.5ΔRlb1	37	2	124	112
	35	0	84	75

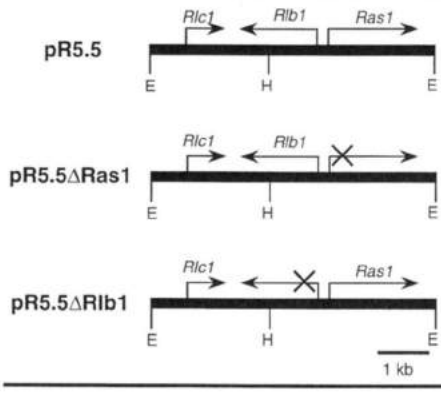

FIGURE 4.—Genomic constructs and rescue results demonstrating that only *Ras1* is affected in the 5703 line. Rescue constructs were made using a 5.5-kb genomic Eco*RI* fragment containing *Rlc1*, *Rlb1*, and *Ras1* inserted into the pCaSpeR4 vector. On the left are the three constructs: pR5.5 is the wild-type sequence, pR5.5ΔRas1 was mutagenized *in vitro* to create an early stop codon (marked with ×) in the *Ras1* gene, pR5.5ΔRlb1 was mutagenized to create an early stop codon in the *Rlb1* gene. The table on the right contains the results of *Ras1*^{5703} rescue using two independent transformant lines for each construct. The columns show the classes of progeny produced by *w*/Y; *P*[*Rescue Construct*, *w*$^{+}$]/+; *Ras1*^{5703} *ry*^{506} *sbd*/+ males mated to *w*; +/+; *Ras1*^{5703} *ry*^{506} *sbd*/*TM3*, *ry*^{RK} *Sb* females. The *w*$^{+}$ marker was used to follow the rescue construct, the sbd marker was used to follow the *Ras1*^{5703} chromosome. pR5.5 and pR5.5ΔRlb1 rescue the semi-lethality of *Ras1*^{5703} (Table 1) and all associated oogenesis phenotypes. pR5.5ΔRas1 has no apparent ability to ameliorate the *Ras1*^{5703} phenotype.

phenotype. First we identified a weak excision allele, *Ras1*^{ix12a}, that produces quantities of Ras1 barely adequate to signal during axis determination in oogenesis. Flies homozygous for the *Ras1*^{ix12a} allele lay nearly wild-type eggs (Figure 1F). When *Ras1*^{ix12a} is placed in *trans* to a deficiency for the region, however, females now lay eggs that have a single, fused dorsal appendage (Figure 5B). We then tested *Egfr*^{Df}/+; *Ras1*^{ix12a}/*Ras1*^{ix12a} and *grk*^{HK36}/+; *Ras1*^{ix12a}/*Ras1*^{ix12a} flies and found that such females produce eggs with forked and fused single dorsal appendages (Figure 5, C and D). These results are consistent with a role for the *Ras1* gene product downstream of *Egfr*.

Embryos from *Ras1* mutant females rarely exhibit dorsoventral defects: Flies carrying mutations in *Egfr* or *grk* lay eggs that lack dorsal appendages, and the embryos that develop within these eggs also lack dorsal structures (SCHÜPBACH 1987). Strong alleles produce strong effects on both embryonic and eggshell phenotypes, and weak alleles exhibit weak effects on both

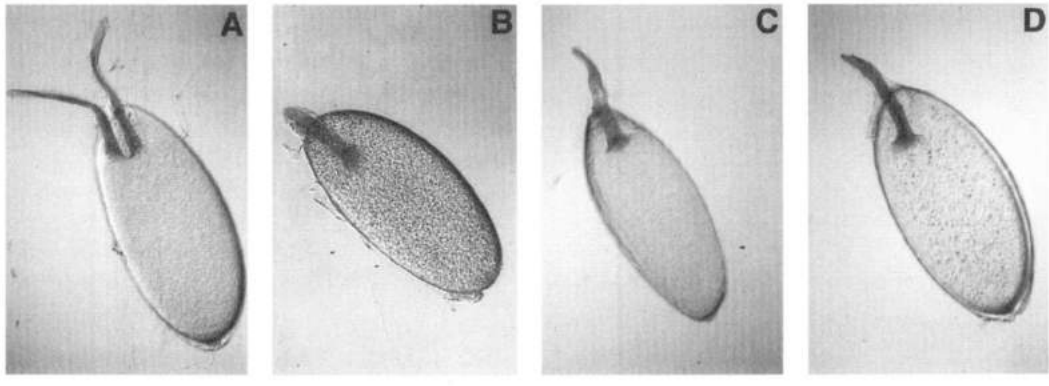

FIGURE 5.—Weak eggshell phenotype of *Ras1*^{ix12a}/*Ras1*^{ix12a} is dominantly enhanced by mutations in the genes of the dorsoventral pathway. (A) Wild-type egg from *Egfr*^{Df}/*CyO* female. The eggshell phenotypes produced by *Egfr*^{Df} (*Df*(2*R*)*top*^{18a}), *grk*, and *Ras1* mutations are completely recessive. (B) Single dorsal appendage phenotype of *Ras1*^{ix12a}/*Df*(3*R*)*by10*. Eggs from these females vary from almost wild type to that pictured. This variation is similar to that observed for (C) *grk*/+; *Ras1*^{ix12a}/*Ras1*^{ix12a} eggs and (D) *Egfr*^{Df}/+; *Ras1*^{ix12a}/*Ras1*^{ix12a} eggs.

TABLE 2

Maternal effect of *Ras1* alleles

Genotype[a]	% hatching	*n*
+/+ (control)	96	384
ix13b/ix13b	13	675
E62K/5703	84	510
D38N/5703	57	213
su(tor)341/5703	40	114
su(tor)404/5703	25	147

[a] Genotype of virgin females mated to Canton S males.

axes (ROTH and SCHÜPBACH 1994). Given this strong correlation between the eggshell and embryo axes, we asked what effect a mutation in the *Ras1* gene would have upon the embryo. Surprisingly, a high proportion of embryos from *Ras1* females develop normally and hatch (Table 2). As many as 57% of eggs laid by $Ras1^{D38N}/Ras1^{5703}$ females hatch normal larvae, and even higher frequencies are obtained with the allelic combination $Ras1^{E62K}/Ras1^{5703}$ (84%). Since little variation is observed between eggs, all hatching larvae must develop within eggs that lack dorsal appendage structures. The fact that *Ras1* mutants produce eggs with defective chorions but are moderately fertile contrasts sharply with *Egfr* and *grk* mutants that produce similar eggshell phenotypes but are usually sterile.

To more fully understand this difference, we examined the cuticle patterns of nonhatching eggs from $Ras1^{D38N}/Ras1^{5703}$ females. Of the nonhatching eggs that are fertilized, 54% contain embryos with normal cuticles (Figure 6B), while 27% exhibit normal ventral denticles and posterior structures but are missing elements of the head skeleton (Figure 6C). Of the remaining unhatching embryos, 19% have defects in the anterior and posterior termini, holes in the cuticle, or rarely, normal head and abdominal structures but disordered posterior segments. Similar phenotypes are observed when unhatched eggs from $Ras1^{ix13b}$ homozygous females are examined (data not shown). Although we do not detect overt dorsoventral defects in these unhatching embryos, two possible interpretations of these phenotypes can be made. It is possible that the majority of the head and tail phenotypes arise from a failure to correctly specify the terminal segments of the embryo via the Torso pathway, known to require *Ras1* (DOYLE and BISHOP 1993; LU *et al.* 1993). These head skeleton defects, however, might also be consistent with a weak ventralization of the embryo, and the other less frequent defects such as disordered posterior segments support this interpretation (ROTH and SCHÜPBACH 1994).

***Ras1* is required for the dorsalization of the embryo by the *K10* mutation:** Since the *Ras1* mutants do not exhibit the obvious embryonic dorsoventral defects observed with *grk/Egfr* mutants, we considered the possibility that Ras1 does not operate downstream of Egfr

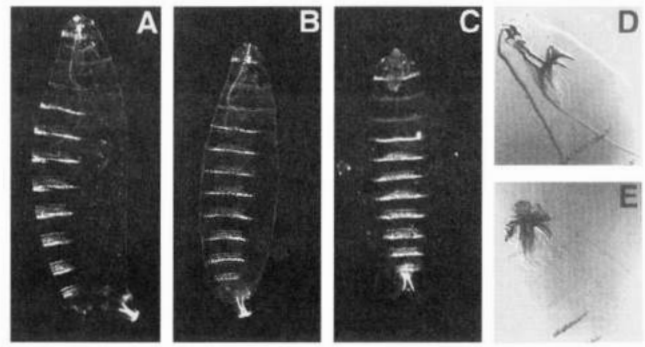

FIGURE 6.—Cuticle phenotype of nonhatching embryos produced by *Ras1* mutant mothers. To examine the strict maternal effect of $Ras1^-$ on the embryo, mutant females were collected as virgins and mated to wild-type males, then allowed to lay eggs. (A) Cuticle preparation of an embryo from a wild-type mother. (B) Cuticle preparation of an embryo from a $Ras1^{D38N}/Ras1^{5703}$ female. The embryo failed to hatch, but appears wild type. (C) Cuticle preparation of an abnormal embryo from a $Ras1^{D38N}/Ras1^{5703}$ female. The head skeleton failed to form properly, but there is no expansion of the ventral denticle belts. In addition, the posterior terminal region developed normally, as the filzkörper are clearly present. (D, E) Nomarski enlargements of the head structures present in embryos produced by wild-type (D) or $Ras1^{D38N}/Ras1^{5703}$ (E) females.

to specify the dorsoventral pattern in the embryo, but acts only to specify the pattern of the eggshell. To test this hypothesis, we constructed double mutants of *K10* and *Ras1*. Mutations in *K10* result in dorsalized eggshells and dorsalized embryos (WIESCHAUS *et al.* 1978). The K10 product operates in the germline to restrict the localization of *grk* mRNA to a dorsal-anterior location in the oocyte, in a cap just over the oocyte nucleus. In *K10* mutants, *grk* mRNA is present uniformly along the entire anterior cortex of the oocyte, resulting in inappropriate Egfr signaling that leads to dorsalized eggshells and embryos (NEUMAN-SILBERBERG and SCHÜPBACH 1993). Double mutants of *K10; grk* or *K10; top* produce eggs with ventralized eggshells and embryos because the K10 mutant phenotype requires active Grk and Egfr for dorsal patterning to occur. If *Ras1* is not required for embryonic dorsoventral patterning, *K10; Ras1* double mutants should exhibit ventralized eggshells that contain dorsalized embryos. If, however, *Ras1* is required for embryonic D/V patterning, *K10; Ras1* mutants should produce eggs resembling *Ras1* mutants alone.

As shown in Figure 7, the *K10* homozygous females produce strongly dorsalized eggs (Figure 7A) that develop into dorsalized embryos if fertilized (Figure 7D). As expected, $K10/K10; Ras1^{ix13b}/Ras1^{D38N}$ females produce eggs with eggshells (Figure 7C) that resemble those produced by $Ras1^{ix13b}/Ras1^{D38N}$ flies (Figure 7B). In addition, a role for *Ras1* in embryonic dorsoventral patterning is demonstrated by these studies. $K10/K10; Ras1^{ix13b}/Ras1^{D38N}$ double mutant females produce embryos that range from strongly dorsalized (18%, *n* =

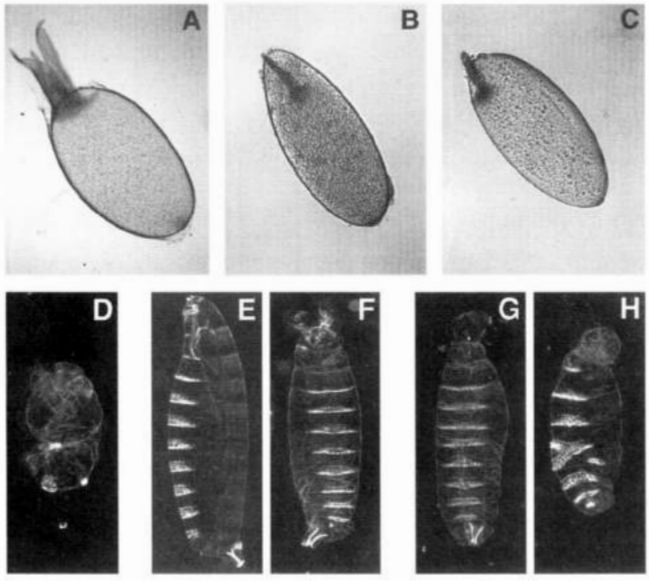

FIGURE 7.—*Ras1* is necessary for the manifestation of the K10 phenotype in the eggshell and the embryo. *K10/K10; Ras1/TM3* females lay strongly dorsalized eggs (A) and these contain dorsalized embryos (D). *K10/FM7; Ras^{ix13b}/$Ras1^{D38N}$* females lay eggs that exhibit a moderate Ras1 mutant phenotype of a single dorsal appendage (B). Many of these eggs hatch normal larvae (36%), and of those that do not hatch, some contain embryos that exhibit normal cuticular structures (E) or that fail to fully develop a head skeleton (F). *K10/K10; $Ras1^{ix13b}$/$Ras1^{D38N}$* females lay eggs (C) that are indistinguishable from *Ras1* mutant eggs. In addition, embryos that develop within these eggshells resemble those from *$Ras1^{-}$* females (G). These phenotypes range from strongly dorsalized to weakly ventralized (H). We interpret these phenotypes to mean that *Ras1* is required to produce the dorsalization caused by the *K10* mutation.

85), to weakly dorsalized (19%) to almost normal with slight head defects (30%) (Figure 7G). The remaining 27% of embryos have twisted and/or fused denticle bands similar to those found in weakly ventralized mutants. We observe these defects most frequently in posterior segments (*e.g.*, Figure 7H). In addition, a small number of eggs (0.3%) laid by *K10/K10; $Ras1^{ix13b}$/$Ras1^{D38N}$* females develop into larvae (Table 3). We never observe hatching larvae, wild-type cuticles, or even weakly dorsalized cuticles in eggs from *K10* mutants alone. These results indicate that *Ras1* is required to determine the dorsoventral fate of the embryo in addition to that of the eggshell.

DISCUSSION

In Drosophila, genetic studies of *Ras* genes have played an important role in furthering our understanding of Ras-mediated signal transduction (SIMON *et al.* 1991; DOYLE and BISHOP 1993; KARIM *et al.* 1996). Three *Ras* genes, *Ras1*, *Ras2*, and *Ras3*, were originally identified in Drosophila through homology to the human *Ras* genes (NEUMAN-SILBERBERG *et al.* 1984). *Ras3* has since been renamed *Rap1;* it is more homologous to

TABLE 3

Maternal effect of *K10;Ras1*

Genotypes[a]	% hatching	*n*
K10/FM7;ix13b/TM3	57	169
K10/K10;ix13b/TM3	0	731
K10/FM7;ix13b/D38N	36	329
K10/K10;ix13b/D38N	0.3	955

[a] Genotype of virgin females mated to Canton S males.

the human k-rev1 proteins, putative antagonists of Ras function (HARIHARAN *et al.* 1991). When *Ras2* is activated and expressed ectopically in flies, bristle defects can result (BISHOP and CORCES 1988), but transgenic studies suggest that *Ras2* cannot substitute for *Ras1* in eye development (FORTINI *et al.* 1992). In addition, no loss of function mutations in *Ras2* have been identified, although extensive mutagenesis has been undertaken (HARRISON *et al.* 1995). *Ras1* thus appears to be the closest Drosophila homologue of the human transforming *Ras* genes, *K-ras, N-ras,* and *H-ras.*

In this work, we present the characterization of the first viable allele of *Ras1* in Drosophila, *$Ras1^{5703}$*. Our genetic and molecular studies demonstrate that the *P*[*lacZ, ry^{+}*] element in insertion line *5703* disrupts *Ras1* function. Sequence analysis localizes the *P* element to a position just 5′ to the putative *Ras1* transcription start site, and complementation tests are consistent with *$Ras1^{5703}$* being a hypomorphic allele. The position of the *P* element outside the coding region indicates that only the quantity and not the quality of Ras1 protein has been altered, but it is not clear how the presence of the *P* element reduces the transcription of the *Ras1* gene. Although *Ras1* lacks a TATA box sequence, it does contain the transcriptional regulatory element, TATCGATA, an 8-bp palindrome called a DNA replication-related element (DRE) because of its presence in the promoters of the proliferating cell nuclear antigen and DNA polymerase α genes (HIROSE *et al.* 1993). The *P* element in the *5703* line resides between the DRE sequence and the possible transcription start site of *Ras1,* and thus it might impair the efficiency of transcription that depends on the DRE. Alternatively, *Ras1* transcription might occur independently of upstream sequences and instead occur through the initiator sequence and a possible downstream promoter element (DPE) found in some Drosophila TATA-less promoters (BURKE and KADONAGA 1996). We cannot locate a DPE by sequence comparison downstream of the start site in *Ras1,* but this is not surprising as previous comparisons of Drosophila promoter regions suggest that downstream sequences vary in position and sequence more so than the highly conserved initiator and TATA elements (ARKHIPOVA 1995; BURKE and KADONAGA 1996).

In addition, it is not clear if the *$Ras1^{5703}$* mutation lowers Ras1 levels in a uniform manner throughout

development. *Ras1* mRNA is loaded into the oocyte and found uniformly throughout the early embryo, but *Ras1* embryonic transcription is spatially localized in later stages of development, especially in the central nervous system (SEGAL and SHILO 1986; EZER *et al.* 1994), suggesting that there may be tissue-specific regulation of expression. If the *P* element disrupts a tissue or temporal enhancer element, specific developmental processes dependent on *Ras1* function may he adversely affected while others remain unaffected.

Analysis of the genomic organization of the *Ras1* locus revealed that *Ras1* is very close to *Rlb1* and *Rlc1* (EZER *et al.* 1994). We therefore tested the possibility that the *5703 P* element was affecting these other transcription units as well as the *Ras1* gene. Using rescue constructs designed to restore only *Rlb1* function or *Ras1* function, we demonstrated that the 5703 phenotype is due solely to the loss of *Ras1*. These results are consistent with the analysis by EZER *et al.* (1994) who showed that *Rlb1* is not transcribed in the same tissues as *Ras1*, even though these genes are separated by only 334 bp. The deletion lines generated in this study may be helpful in genetically characterizing *Rlb1* and *Rlc1* and assist in obtaining a hetter understanding of the regulation in this region.

The large number of adult flies obtained when the semilethal allele $Ras1^{5703}$ is placed in *trans* to the lethal EMS-induced mutations (*E62K, D38N, 341,* and *404*) remains to be explained. One possibility is that *Ras1* can participate in conventional intra-allelic complementation. This phenomenon, however, would be expected to have some allele specificity, and we observe substantially higher than expected viability with all four of the EMS-induced mutations. Given this result and the fact that the *P* element is disrupting a 5′ regulatory sequence, we favor a pairing-dependent mechanism such as transvection (LEWIS 1954). Pairing-dependent complementation was originally identified for alleles of *bithorax* and subsequently found at other loci, including *yellow, decapentaplegic,* and *eyes absent* (LEISERSON *et al.* 1994; reviewed by PIRROTTA 1990). We are currently testing the role of transvection in mediating the exceptional viability observed in our heteroallelic lines.

Different requirements for *Ras1* exist in different developmental pathways: Genetic analyses of *Ras1* mutations indicate that specific developmental pathways respond differently to the amount of Ras1 protein. For example, the $Ras1^{5703}$ allele and the derived excision lines exhibit more severe defects in viability and oogenesis than in eye morphology. Moderate alleles exhibit single dorsal appendage eggshell phenotypes and 50% of the expected viability, but have no detectable eye phenotype. In a more severe case, $Ras1^{5703}/Df$, only 0.13% of the expected hemizygotes eclose and such females exhibit rudimentary ovaries. The eye morphology, however, is only slightly more disrupted than in $Ras1^{5703}$ flies (data not shown) and not nearly as disrupted as in other heteroallelic combinations (see below). It may be that the levels of Ras1 required for correct eye development are less than that required for oogenesis and *Ras1*-mediated viability functions. Alternatively, these differential phenotypes might be explained by the character of the *P*-element mutation; $Ras1^{5703}$ may not reduce eye-specific transcription to the degree that it reduces transcription in the follicle cells.

In addition to the differences in the amount of Ras1 required for different developmental pathways, the heteroallelic combinations of lethal EMS-induced alleles and $Ras1^{5703}$ suggest that developmental pathways respond differently to specific EMS mutations in *Ras1*. We have shown that three alleles in *trans* to $Ras1^{5703}$ exhibit very similar moderate eye and eggshell phenotypes, but show a difference in viability indicating a varying requirement for Ras1. The fourth combination examined, $Ras1^{E62K}$ in *trans* to $Ras1^{5703}$, produces extremely rough eyes, significantly more disrupted than other heteroallelic combinations. In contrast, $Ras1^{E62K}/Ras1^{5703}$ flies produce eggs with both the weakest eggshell phenotype and the highest hatching frequency. This difference in severity of eye and egg phenotypes suggests that the mutational change in the $Ras1^{E62K}$ allele disrupts a required function in the eye that has little effect in the egg, indicating a possible difference in the signaling pathway in these tissues. Such allele-specific effects have been observed for a dominant mutation of *Egfr*, $Egfr^{Ellipse}$. The $Egfr^{Ellipse}$ mutation affects viability, eye development, and wing morphogenesis but does not cause obvious eggshell defects. This result may be a consequence of *Egfr* interacting with a different ligand in different tissues. Likewise, the allele-specific effects observed for *Ras1* could be due to differences in components and/or the mechanism of *Ras1* signal transduction in different tissues.

The *Ras* signaling pathway may also function differently when a gradient of signaling activity is required. In the eye, the *sevenless-Ras* pathway is responsible for determining the identity of the R7 cell in the developing ommatidium. The developmental decision to assume R7 identity is essentially binary, and there is no continuum or gradient within an ommatidium, or across the eye (WASSARMAN *et al.* 1995). Follicle cell patterning during oogenesis, however, occurs at the level of a field of cells that presumably experiences a gradient of signaling activity. Over- or underactivation of the Egfr signaling pathway through genetic manipulation results in characteristic phenotypes that can be classified into a range from more dorsal to more ventral in identity (NEUMAN-SILBERBERG and SCHÜPBACH 1994). Clearly, the Ras signaling pathway must be able to generate a range of outcomes from the same quality of signal, and these outcomes must be the result of the level of activation. Identifying the mechanism for this process would greatly increase our understanding of signal transduction and development.

Model of Ras1 function in dorsoventral axis specification: Our analyses support the hypothesis that *Ras1* operates downstream of Egfr to specify the dorsoventral axis during oogenesis. Ras-mediated signal transduction in development has been demonstrated previously in the eye (SIMON *et al.* 1991), in the termini of the embryo (DOYLE and BISHOP 1993; LU *et al.* 1993), in the wing (STURTEVANT *et al.* 1993), and in the control of border cell migration during oogenesis (LEE *et al.* 1996). Our results are consistent with findings that other Ras signaling members including *Raf, MEK,* and *Gap1* function in dorsoventral specification during oogenesis (CHOU *et al.* 1993; BRAND and PERRIMON 1994; HSU and PERRIMON 1994).

Flies homozygous for moderate *Ras1* alleles produce eggs with fused dorsal appendages of nearly normal length, similar to weak *grk* or *top* mutants. This length decreases in stronger *Ras1* mutants until only a nub or patch of chorion material remains, similar to the strongest Egfr and grk phenotypes. Additionally, weak *Ras1* mutations interact synergistically with *grk* and *Egfr* mutations: *grk*/+ or *Egfr*/+ in a $Ras1^{ix12a}/Ras1^{ix12a}$ background results in an increase in the number of eggs with mutant eggshells.

We have also shown that *Ras1* is required to produce the dorsalized embryonic phenotype caused by the *K10* mutation, indicating a role for *Ras1* in establishing the prepattern of the dorsoventral axis of the embryo. Similar results were obtained for double mutants of *K10* and *MEK* (HSU and PERRIMON 1994). Furthermore, studies involving a hyperactivated *Ras1* signaling pathway also suggest a role for the Ras cassette in establishing embryonic polarity. A gain-of-function mutation in *Raf* or a loss-of-function mutation in GTPase activating protein, *Gap1,* leads to dorsalization of the eggshell axis and the embryonic axis (CHOU *et al.* 1993; BRAND and PERRIMON 1994). These data argue that Ras1-mediated signal transduction functions in prepatterning the embryonic dorsoventral axis and support a model in which Ras1 signaling alone transduces the Grk/Egfr signal during dorsoventral patterning (Figure 8A).

Thus, the majority of the data support a linear model, depicted in Figure 8A, in which Grk activates Egfr and this leads to the activation of the Ras signaling cascade, ultimately determining the axes of both the eggshell and the embryo. Unfortunately, this model does not adequately explain why loss-of-function mutations in *grk* or *Egfr* result in consistently ventralized eggshells and embryos, while mutations in *Ras1, Raf,* or *MEK* that exhibit a similar severity of eggshell phenotype have only a slight effect on the embryo. Even the weakest alleles of *grk* and *Egfr* show a high degree of correlation between the eggshell axis and the embryo axis (SCHÜPBACH 1987). We do not observe such a strong correlation between eggshell and embryo phenotypes in the *Ras1* mutants, but instead observe high hatching rates for embryos developing within defective eggshells. Similarly, hypomorphic alleles of *Raf* or *MEK* reduce dorsal appendage structures but do not result in a concomitant ventralization of the embryo (BRAND and PERRIMON 1994; HSU and PERRIMON 1994). Mutations in the neurogenic gene *brainiac* also result in ventralized eggshells but have no effect on embryonic dorsoventral patterning (GOODE *et al.* 1992), suggesting that this dissociation between dorsal follicle cell fate and embryonic polarity is not limited to Ras signaling components.

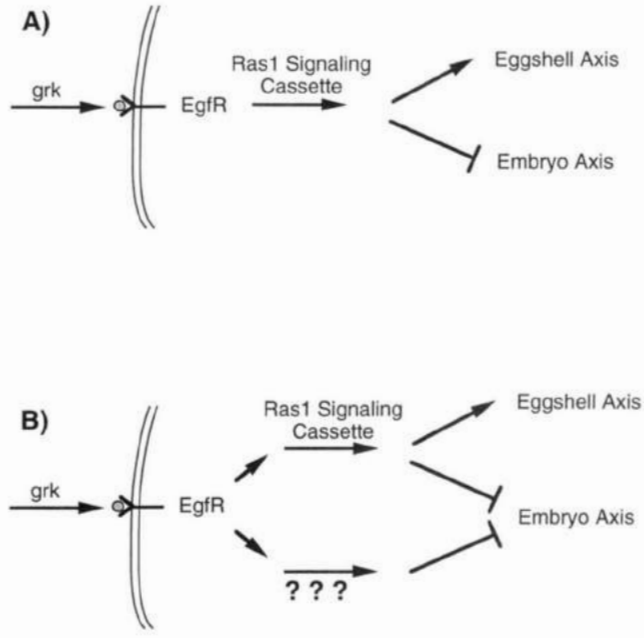

FIGURE 8.—Alternative models of Ras1 function in dorsoventral patterning during oogenesis. See text for details.

There are a number of explanations for this apparent qualitative difference between *grk/Egfr* mutations and *Ras1* mutations. One possible explanation focuses on the nature of the alleles characterized. All *grk* and $Egfr^{top}$ alleles were generated during screens for female sterile or lethal mutations; thus, only strong phenotypes are observed. A screen in which dorsal eggshell structures are assayed might generate weaker *grk* and $Egfr^{top}$ alleles that more closely resemble the phenotype of *Ras1* alleles. In support of this hypothesis, CLIFFORD and SCHÜPBACH (1989) noted that some *Egfr* mutant females lay rare eggs with a single dorsal appendage, and these eggs develop into hatching embryos, suggesting that there is not always a strict correlation between the eggshell and the embryonic axes. It should also be recognized that viable *Ras1* alleles still retain considerable *Ras1* function, and it could be that the null phenotype of *Ras1* in the follicle cells would closely resemble the null phenotype of *grk* or *Egfr* in the follicle cells. Given these genetic caveats, one can argue that the linear model in Figure 8A adequately explains the data, and it predicts that null alleles examined at similar times and places in the follicle cells should yield similar phenotypes, *i.e.,* grk = Egfr = Ras1.

In addition to these genetic arguments, two modifi-

cations of the above pathway could help explain the lack of correlation between eggshell defects and embryonic defects in *Ras1* mutants. For example, the linear pathway shown in Figure 8A could act to determine the fate of the dorsal follicle cells, affecting eggshell and embryo equally. If, however, a feedback loop existed that required Ras1 activity for continued input into the eggshell part of the pathway, differential phenotypes could be observed. Similarly, if the Ras1 signaling cassette acted downstream of a different RTK to mediate dorsal follicle cell migrations following the *grk/Egfr* determinative events, eggshell synthesis would be more strongly affected than embryonic patterning. Evidence of a role for Drosophila *Ras1* in cell migration has been demonstrated for the border cells, which require *Ras1* to both initiate and maintain cell movements (LEE *et al.* 1996).

A second model is shown in Figure 8B. In this model, embryonic dorsoventral patterning requires both *Ras* activity and some unidentified Ras-independent pathway. These pathways would be parallel, partially redundant, and dependent on activation through *Egfr*. This model would explain how activation of the Ras pathway could cause embryonic defects while accounting for the differential sensitivity of the eggshell and embryo axes observed with hypomorphic mutations. Evidence for a Ras-independent pathway has been presented for the Torso pathway (HOU *et al.* 1995), and there is some evidence that a parallel pathway could exist in eye development (THERRIEN *et al.* 1995).

To more fully understand these issues, it will be necessary to identify and characterize components acting downstream of the signaling cascade. Egfr signaling in the follicle cells is unique in that multiple developmental processes, eggshell patterning and embryonic patterning, depend on the receptor. The genetics of *Ras1* appear to separate these dependent processes, and it will be exciting to elucidate the biochemical basis for this differential activity. Downstream components such as transcription factors that respond to RTK signaling in the eye and the termini of the embryo are being identified (DICKSON 1995), and there is evidence that one such transcription factor, Pointed (p2), is activated by Egfr signaling in the follicle cells (MORIMOTO *et al.* 1996). It is evident that a clearer understanding of the mechanism leading to dorsoventral patterning will facilitate understanding similar signaling events that operate throughout development.

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