Identification of Genomic Regions That Interact With a Viable Allele of the Drosophila Protein Tyrosine Phosphatase Corkscrew

Lucy Firth,* Jebecka Manchester,† James A. Lorenzen,† Martin Baron*,[1] and Lizabeth A. Perkins†,[1]

**Department of Biological Sciences, University of Manchester, Manchester M13 9PT, England and †Pediatric Surgical Research Laboratories, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114*

Manuscript received March 2, 2000
Accepted for publication June 21, 2000

ABSTRACT

Signaling by receptor tyrosine kinases (RTKs) is critical for a multitude of developmental decisions and processes. Among the molecules known to transduce the RTK-generated signal is the nonreceptor protein tyrosine phosphatase Corkscrew (Csw). Previously, Csw has been demonstrated to function throughout the Drosophila life cycle and, among the RTKs tested, Csw is essential in the Torso, Sevenless, EGF, and Breathless/FGF RTK pathways. While the biochemical function of Csw remains to be unambiguously elucidated, current evidence suggests that Csw plays more than one role during transduction of the RTK signal and, further, the molecular mechanism of Csw function differs depending upon the RTK in question. The isolation and characterization of a new, spontaneously arising, viable allele of *csw*, *csw^{lf}*, has allowed us to undertake a genetic approach to identify loci required for Csw function. The rough eye and wing vein gap phenotypes exhibited by adult flies homo- or hemizygous for *csw^{lf}* has provided a sensitized background from which we have screened a collection of second and third chromosome deficiencies to identify 33 intervals that enhance and 21 intervals that suppress these phenotypes. We have identified intervals encoding known positive mediators of RTK signaling, *e.g.*, *drk*, *dos*, *Egfr*, *E(Egfr)B56*, *pnt*, *Ras1*, *rolled/MAPK*, *sina*, *spen*, *Src64B*, *Star*, *Su(Raf)3C*, and *vein*, as well as known negative mediators of RTK signaling, *e.g.*, *aos*, *ed*, *net*, *Src42A*, *sty*, and *su(ve)*. Of particular interest are the 5 lethal enhancing intervals and 14 suppressing intervals for which no candidate genes have been identified.

RECEPTOR tyrosine kinases (RTKs) control a number of diverse cellular processes including growth, differentiation, migration, and viability (reviewed in Van Der Geer *et al.* 1994). The primary function of a cell surface receptor tyrosine kinase (RTK) is the conversion of extracellular information into a biological signal that is often transduced into the nucleus where it modulates the activity of transcription factors. Identification of the molecules involved in the transduction signal initiated from activated RTKs has led to the realization that all RTKs share common signaling components. This conserved signaling cassette permits the transmission of instructive data in a wide variety of developmental contexts leading to a range of different responses (Perrimon and Perkins 1997).

A combination of genetic and biochemical data has led to the following model of RTK signal transduction. Upon activation by a ligand, the RTK becomes autophosphorylated on specific tyrosyl residues. The adapter protein GRB-2 (Downstream of receptor kinase, Drk) then binds the activated receptor via its SH2 domain, leaving two SH3 domains to associate with Son of Sevenless (Sos), a ubiquitously expressed Ras guanine nucleotide exchange factor (Oliver *et al.* 1993; Simon *et al.* 1993). The formation of the Drk:Sos complex results in the relocalization of Sos to the cell plasma membrane where it promotes the exchange of GDP to GTP on Ras, thereby inducing a conformational change that activates Ras. The GTPase activating protein (Gap1) stimulates the hydrolysis activity of Ras, causing it to hydrolyze GTP for GDP and switch off the signal (Gaul *et al.* 1992). The cycling between these two states is essential for signal relay to proceed in a regulated fashion. Activation of Ras1 serves as a molecular switch, which leads to the activation of a kinase cascade that includes Raf, a serine/threonine specific protein kinase, MAPKK (MEK) a dual specific tyrosine/threonine kinase, and the serine/threonine kinase MAPK (ERK). Once activated, MAPK homodimerizes and is imported into the nucleus (Fukuda *et al.* 1997; Khokhlatchev *et al.* 1998) where it phosphorylates target nuclear proteins that initiate transcription, the ultimate goal of the signaling pathway.

A further component of RTK signaling pathways in Drosophila is Corkscrew (Csw), a cytoplasmic, nonreceptor protein tyrosine phosphatase (PTP; Perkins *et al.* 1992), which is the functional homologue of the vertebrate protein SHP-2 (Perkins *et al.* 1996). Csw was first identified as a downstream positive mediator of the signal from the Torso (Tor) RTK as loss-of-function *csw*

Corresponding author: Lizabeth A. Perkins, Pediatric Surgical Research Labs, Warren 10, Massachusetts General Hospital, 32 Fruit St., Boston, MA 02114. E-mail: perkins@helix.mgh.harvard.edu

[1]These authors contributed equally to this work.

mutations were found to suppress the gain-of-function phenotypes of *tor* (Perkins *et al.* 1992). The phenotypes of *csw* mutations are similar to mutations in other RTKs, suggesting that the Tor RTK is not alone in using Csw to positively transduce its signal. Loss-of-function phenotypes generated by a dominant negative mutation in the Drosophila epidermal growth factor receptor (EGFR) were enhanced by a decrease in the activity of *csw*, thus placing Csw within the EGFR signaling pathway (Perkins *et al.* 1996). Csw activity has also been shown to be involved in the transduction of the signal from the Drosophila fibroblast growth factor (FGF) receptor, Breathless (Btl; Perkins *et al.* 1996) as well as the Sevenless (Sev) RTK (Allard *et al.* 1996).

While a role for Csw in the RTK signaling cassette has been clearly demonstrated, the biochemical function of Csw remains to be unambiguously elucidated. Current evidence suggests that Csw plays more than one role during transduction of the RTK signal and, further, the molecular mechanism of Csw function differs depending upon the RTK in question (Perkins *et al.* 1992, 1996; Allard *et al.* 1996, 1998; Cleghon *et al.* 1996, 1998; Herbst *et al.* 1996). In the Tor signaling pathway, upon activation, most likely by the ligand Trunk (Casanova *et al.* 1995; Furriols *et al.* 1998), the RTK is phosphorylated at two major sites, tyrosine 630, Y^{630}, and tyrosine 918, Y^{918}. Mutational analysis has revealed that Csw interacts with phosphorylated Y^{630} through one of its SH2 domains. Upon this interaction Csw becomes tyrosine phosphorylated at Y^{666}, the residue through which it interacts with the SH2 domain of the adapter protein Drk. Significantly, Drk does not interact with Tor, supporting the current model that Tor transmits its positive signal through Csw to Drk. Y^{918} of Tor, when phosphorylated, binds RasGap. Following Tor activation and recruitment of Csw to pY^{630}, Csw is able to dephosphorylate pY^{918} and this presumably lowers the local concentration of RasGap and sustains the positive Tor signal by increasing the overall level of Ras activity (Cleghon *et al.* 1996, 1998).

Csw, therefore, plays at least two functions in the Tor pathway, as an adaptor protein for Drk and as a phosphatase to dephosphorylate the negative regulatory RasGap binding site. However, while the Csw:Tor interaction is dependent on RTK activation and tyrosine phosphorylation of the RTK, interaction between Csw and the Sev RTK is constitutive; that is, the Csw:Sev interaction is not activation dependent and does not require tyrosine phosphorylation (Allard *et al.* 1996). These differing results support the idea that the molecular mechanism of Csw function differs depending upon the RTK under consideration.

For most RTKs the precise molecular mechanisms of function of Drosophila Csw and its homologues, nematode PTP-2 and vertebrate SHP-2, remain unclear; however, genetic experiments have shown that in nearly all cases these SH2-containing phosphatases serve positive functions during signal transduction (reviewed by Van Vactor *et al.* 1998 and Hertog 1999). While molecular data are lacking for PTP-2, both Csw and SHP-2 are known to interact, through their SH2 domains, with various RTKs and/or members of a family of membrane-targeting, scaffolding proteins such as DOS in Drosophila and Gab1, IRS-1, and FRS-2 in vertebrates (reviewed by Van Vactor *et al.* 1998). Additionally, substrates for these SH2-containing phosphatases remain rather elusive. In Drosophila, two Csw substrates, Tor (Cleghon *et al.* 1998) and Dos (Herbst *et al.* 1996), have been reported; however, it is likely many more substrates remain to be identified.

In this article we report the isolation and characterization of a new, spontaneously arising, viable allele of *csw*, which we designate csw^{lf}. Adult homozygous csw^{lf} flies exhibit rough eyes and a wing vein gap phenotype. Both of these phenotypes are consistent with the requirement of Csw in the Sev and EGFR pathways during eye development (Allard *et al.* 1996) and the EGFR pathway during formation of wing veins (Perkins *et al.* 1996). The phenotypes exhibited by the csw^{lf} mutation are not limited to imaginal development. Homozygous csw^{lf} females lay ventralized eggs and embryos generated from females bearing csw^{lf} germline clones exhibit a number of mutant phenotypes. Together, these results suggest that the csw^{lf} mutant lesion is sufficient to compromise Csw function in a number of developmental processes that require RTK signaling. Finally, and reasoning that not all transducers and regulators of RTK signaling have been identified, we have identified, in a deficiency screen, autosomal regions that enhance or suppress the csw^{lf} mutant background. To date, among the genomic regions that we have determined encode at least one gene capable of modifying the csw^{lf} phenotype are several with no known components of RTK signaling.

MATERIALS AND METHODS

Drosophila strains: All flies were raised on standard Drosophila media at 25°. Chromosomes and mutations that are not described in the text, or below, can be found in FlyBase (FlyBase 1999).

To date, csw^{lf} is the 10th *csw* allele to be characterized. Unlike the other 9 alleles where *csw* mutant animals (csw^{-}/Y) derived from heterozygous females ($csw^{-}/+$) die during early pupal stages, csw^{lf} is not required for viability. However, all 10 alleles exhibit similar fully penetrant maternal effect phenotypes on embryonic development; that is, all embryos derived from females that lack maternal activity for any of the *csw* alleles fail to hatch; we refer to these as *csw* mutant embryos.

Deficiency screen: Males carrying deficiencies on the second and third chromosomes were crossed to $csw^{lf}/FM7$. All progeny from this cross were genotyped and counted. Male progeny carrying both the csw^{lf} mutation and the deficiency were scored for enhancement and suppression using three criteria: wing vein gaps, rough eye phenotype, and viability. The wing vein gaps of these flies were compared to that of males mutant for csw^{lf} alone, and likewise for the rough eye phenotype. Viability was scored by comparing the number of males bear-

ing csw^{lf} and the deficiency to the number of males with csw^{lf} and the balancer for that particular deficiency. In cases where $csw^{lf}/Y; Df/+$ males were not obtained, we controlled for the possibility that the deficiency deleted a haplo-insufficient locus by determining whether the sibling $FM7/Y; Df/+$ males were present in expected numbers.

Phenotypic analysis of adult structures: The eyes of live flies were examined in an Electroscan microscope under a wet vent chamber. Fixation and sectioning of the adult eyes were performed essentially as described in Tomlinson and Ready (1987). Wings were mounted in Gary's Magic Mountant (Ashburner 1989). All photomicrographs were acquired on a Zeiss Axioskop microscope using Improvision Openlab data capture software. Images were assembled using Adobe PhotoShop (Adobe Systems Inc., San Jose, CA) and Microsoft PowerPoint (Microsoft Corp.).

Production of *csw* germline mosaics: csw^{lf} germline clones were generated using the "dominant female sterile or FLP-DF5 technique" as previously described (Chou and Perrimon 1996). Both null (csw^{-}/Y) and paternally rescued ($csw^{-}/+$) animals, derived from females lacking maternal *csw* activity during oogenesis, die during embryogenesis. To distinguish between these two classes of embryos, mosaic females possessing *csw* germline clones were crossed with males carrying *FM7, ftz-lacZ/Y,* a balancer chromosome that contains a *lacZ* gene under the control of the *fushi-tarazu* (*ftz*) promoter. The genotypes of embryos were determined by following the expression pattern of the *lacZ* gene, which was detected by its β-galactosidase activity. Embryos without the *lacZ* marker are referred to as "null *csw* mutant embryos" since they lack both maternal and zygotic copies of the *csw* wild-type gene. Their siblings, which express the *lacZ* gene, are referred to as the "paternally rescued *csw* mutant embryos" since they lack only the maternal gene.

***In situ* hybridization and immunohistochemistry:** *In situ* hybridization on whole-mount embryos using digoxigenin-labeled probes was performed according to Tautz and Pfeifle (1989). Single-stranded sense and antisense digoxygenin-containing DNA probes were prepared by the PCR labeling technique (N. Patel, personal communication) using appropriate primers. Probes were prepared from plasmids containing the *tailless* (*tll*; Pignoni *et al.* 1990, 1992) and *huckebein* (*hkb*; Weigel *et al.* 1990; Bronner and Jäckle 1991; Bronner *et al.* 1994) cDNAs. For visualization, embryos were dehydrated through an ethanol series and mounted in Euparol (Carolina Biological Supply).

For immunohistochemistry, embryos were fixed with 4% formaldehyde and immunostained according to Michelson (1994) except that the blocking step was omitted. The primary antibodies were to evenskipped (eve; 1:50; provided by N. Patel), Kruppel (Kr; 1: 300; provided by D. Kosman and J. Reinitz), pericardial cell antigen (PC; 1:3; provided by T. Volk), and 2A12 (1:10; provided by M. Krasnow and the Developmental Studies Hybridoma Bank, Iowa City, IA). Biotin-conjugated secondary antibodies (BMB) were detected with the Vectastain Elite ABC kit (Vector Labs, Burlingame, CA) in combination with TSA-Indirect (New England Nuclear, Boston).

All embryos were analyzed and photographed with a Nikon FXA equipped with Nomarski optics. Larval cuticles were prepared in Hoyer's mountant as described by Van Der Meer (1977). Cuticles were examined using dark-field or phase contrast illumination.

RESULTS

Isolation of the viable allele csw^{lf}: Previously all known alleles of the Drosophila gene *csw* have been found to be lethal at the early pupal stage. We have recently isolated a recessive, viable spontaneous mutation that exhibits reproducible rough eye and wing vein gap phenotypes (Figure 1, compare A and E with B and F). The mutation displays a degree of temperature sensitivity with stronger phenotypes displayed at higher temperatures (data not shown). Linkage analysis and deficiency/duplication mapping determined that the mutation is located to the distal tip of the X chromosome in the vicinity of the previously described *corkscrew* (*csw*) locus. When placed *in trans* to any of the other *csw* alleles the resulting csw^{-}/csw^{lf} females were largely nonviable. However, when occasional females did emerge they exhibited eye and wing phenotypes more severe than those observed in csw^{lf}/csw^{lf} females (data not shown). The phenotypes of the new mutation were completely rescued by expression of the $csw^{wild\ type}$ minigene under the control of its endogenous promoter (J. A. Lorenzen, M. Melnick and L. A. Perkins, unpublished results). This rescue, together with the complementation analysis, allowed us to conclude that the new mutation represents a reduced activity, viable allele of *csw,* which we have designated csw^{lf}.

Sections of the adult ommatidium reveal that the rough eye phenotype results from a loss of photoreceptors, commonly R7; however, there is also occasional loss of outer photoreceptors as well (Figure 1, I and J). These results further support a role for Csw in both the EGF (DER; TOP) and SEV RTK pathways (Allard *et al.* 1996; Perkins *et al.* 1996).

Ovary and embryonic phenotypes of csw^{lf}: Under favorable culture conditions 10 to 15% of females from a balanced stock are homozygous for csw^{lf}. These homozygous csw^{lf} females are essentially sterile and only rarely produce fertilized eggs. The eggs from homozygous csw^{lf} mothers have shells that are partially ventralized (Figure 2). This defect, previously demonstrated to be the result of Csw function in the Torpedo EGF RTK pathway during oogenesis (Perkins *et al.* 1996), is likely responsible for the high percentage of unfertilized eggs produced by the homozygous females.

Fertilized eggs are rarely obtained from homozygous csw^{lf} mothers, so we chose to analyze csw^{lf} mutant phenotypes during embryogenesis by producing csw^{lf} germline clones using the FLP-DFS technique (Chou and Perrimon 1996). All of these csw^{lf} mutant embryos derived from females bearing csw^{lf} germline clones mated to csw^{lf}/Y males failed to hatch. While the cuticles of these embryos are largely like wild type (Figure 3A), mild defects, similar to those observed in stronger alleles of *csw* (partial fusion of denticle bands along the midline, mild twisting, incomplete germ band shortening), were frequently observed (Figure 3B).

Since the observed cuticular defects, alone, could not account for the lethality exhibited by csw^{lf} mutant embryos, specific tissues, whose specification and/or differentiation are dependent upon RTK signaling, were ex-

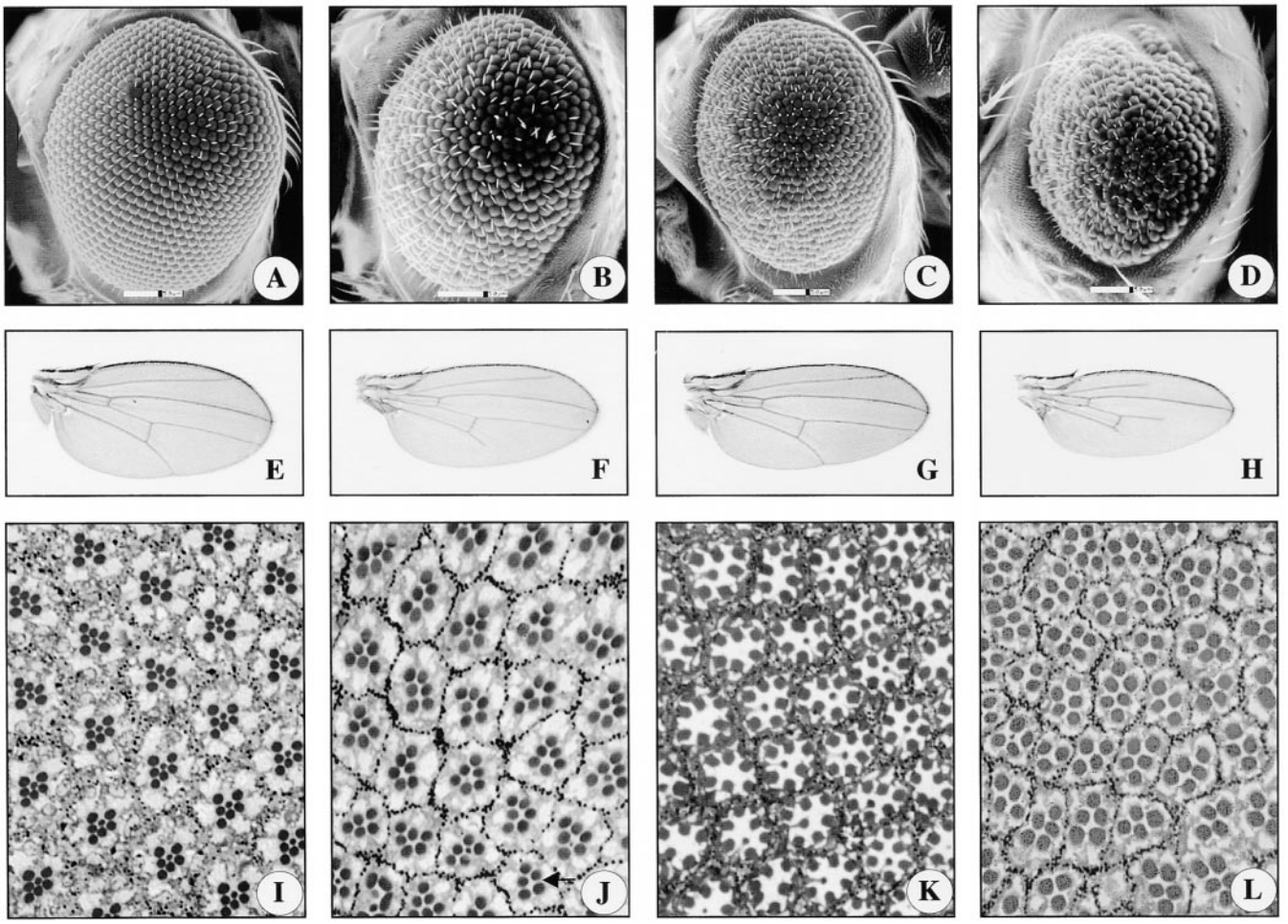

FIGURE 1.—The adult phenotypes of flies homo- or hemizygous for *csw*^{lf} create a sensitized background from which suppressors and enhancers of the mutant phenotypes can be identified. Scanning electron micrographs, thin transverse sections of adult eyes, and adult wings are shown as follows: wild-type flies (A, E, and I) and homo- or hemizygous *csw*^{lf} flies (B, F, and J) exhibit a rough eye phenotype (B) manifested by loss of photoreceptor R7 (J) and occasional loss of additional outer photoreceptors (see arrow J). Further, *csw*^{lf} mutant flies also exhibit wing vein gaps, most frequently manifested in the distal regions of L5 and L2 (F) and occasional gaps in L4 (not shown). A *csw*^{lf} mutant background was the starting point to identify genomic regions containing genes that either suppress (C, G, and K) or enhance (D, H, and L) the *csw*^{lf} adult phenotypes.

amined for defects resulting from the *csw*^{lf} mutation. Consistent with the mild cuticular phenotypes observed, residual *csw*^{lf} activity is sufficient for normal function of the Tor RTK pathway, the first RTK pathway known to be active during embryogenesis. In this pathway, which initiates patterning of the acron (anteriormost head) and telson (posteriormost tail), we used the expression patterns of *tll* and *hkb* as indicators of a loss of anterior and posterior patterning information (WEIGEL *et al.* 1990; GHIGLIONE *et al.* 1999). We observed no significant differences in the expression patterns of either *tll* or *hkb* in *csw*^{lf} mutant embryos (Figure 3, C and D).

At later stages of embryogenesis, tissues whose specification and/or differentiation requires signaling by the EGFR or either of the Drosophila FGF RTKs [Breathless (Btl) and Heartless (Htl)] were assayed in *csw*^{lf} mutant embryos. For specification and proper positioning of the segmentally reiterated precursor cells that give rise to both the DA1/#1 larval muscles and a subset of pericardial cells, input from both the EGF and Htl pathways is essential (CARMENA *et al.* 1998). That is, both the DA1 and pericardial cells are deleted from embryos mutant for either the EGF or Htl receptors. Similarly, in *csw*^{lf} mutant embryos, at germ band elongation these precursors, visualized with antibodies to the pair rule protein Even-skipped (Eve), are variably missing from one to four hemisegments per embryo (Figure 3, E and F). Since loss of these Eve positive precursor cells could be due to Csw function in either EGFR and/or Htl signaling, we utilized two additional molecular markers to determine if the *csw*^{lf} lesion is sufficient to compromise the signal from each of these RTKs. Specifically, the gap gene *Kruppel* (*Kr*) is expressed in the larval muscle precursor VA2/#27 in response to EGFR signaling alone (BUFF *et al.* 1998). As is the case for the Eve positive precursor, the VA2/#27 muscle precursor is variably

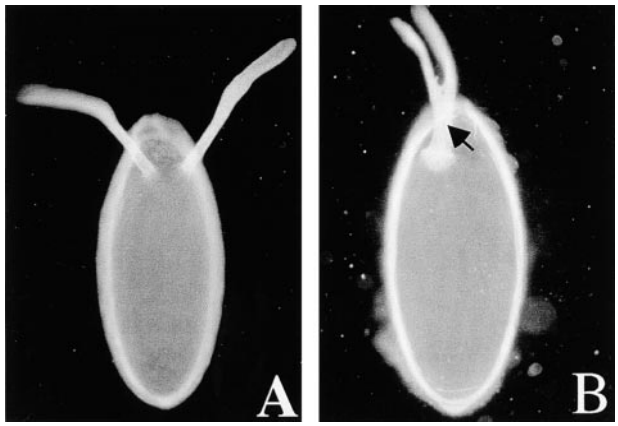

FIGURE 2.—The eggshells from females homozygous for *csw^{lf}* are partially ventralized. In wild type (A) paired dorsal appendages extend from the anterodorsal surface of the egg shell (or chorion). The dorsal appendages of eggshells homozygous for *csw^{lf}* are partially fused (arrow in B); this ventralized phenotype is due to loss of dorsal eggshell fates and a concomitant expansion of ventral cell fates. This fusion, which partially or completely deletes the site of sperm entry into the oocyte, is the likely cause of the almost total sterility of females homozygous for *csw^{lf}*. Both eggs are dorsal views and anterior is at the top.

deleted from random segments (Figure 3, G and H). Similarly, antibodies to a pericardial cell antigen (YARNITZKY and VOLK 1995), whose expression requires signaling by the Htl RTK (MICHELSON *et al.* 1998), reveals that in *csw^{lf}* mutant embryos these heart precursor cells are disrupted and/or deleted (Figure 3, I and J).

Finally, to assay the effect of the *csw^{lf}* lesion on signaling by the Btl RTK, we utilized a molecular marker that highlights the tracheal lumen (SAMAKOVLIS *et al.* 1996). Approximately 40 to 50% of the *csw^{lf}* mutant embryos examined exhibited only a very mild tracheal phenotype, most frequently manifested as gaps in the dorsal tracheal trunk (Figure 3K). However, the remaining mutant embryos exhibited a severe tracheal phenotype where not only was the dorsal tracheal trunk disrupted, but also all the major dorsoventral tracheal branches are misrouted (Figure 3L).

Taken together, our results suggest that the *csw^{lf}* lesion is sufficient to compromise four RTK signaling pathways that are utilized throughout development. EGFR signaling is disrupted during oogenesis, embryogenesis, and imaginal stages. SEV signaling is disrupted during specification of the R7 photoreceptor, and both FGF RTKs, Btl and Htl, are disrupted during embryogenesis. Surprisingly, specification of the larval head and tail by the Torso RTK is unaffected by *csw^{lf}*.

Genetics interactions of *csw^{lf}* with altered forms of Ras and Raf: Previously, KARIM *et al.* (1996) used genetic interactions with engineered Ras and Raf proteins to position additional components of Ras signaling within the pathway. *sev-Ras^{V12}* is a gain-of-function mutation that transforms nonneuronal cone cells into supernumerary R7 cells (KARIM *et al.* 1996). The differentiation of these extra photoreceptors disrupts the normal eye morphology, causing it to look rough. Expression of the dominant negative Ras1 allele, *Ras^{N17}* (SIGAL *et al.* 1986), expressed as a *sev-Ras1^{N17}* transgene, also results in a rough eye; however, this phenotype is due to missing R7 photoreceptors and occasionally outer photoreceptor cells (KARIM *et al.* 1996). Finally, immediately downstream to Ras in RTK signaling is Raf. The transgene *sev-Raf^{Torso}* is an activated form of Raf that uses the Torso membrane spanning region to target the chimeric protein to the cell surface (DICKSON *et al.* 1992). Presence of this transgene results in the constitutive activation of signaling downstream of Raf and in the eye results in the formation of ectopic photoreceptors.

We investigated the interactions of the above transgenes in males hemizygous for *csw^{lf}* (Figure 4). Further supporting a role for Csw during Ras signaling, *csw^{lf}* partially suppressed the rough eye phenotypes of the activated forms of Ras and Raf, *sev-Ras^{V12}* and *sev-Raf^{Torso}*, while it enhanced the rough eye phenotype of dominant negative Ras, *sev-Ras^{N17}* (Figure 4, compare A with G, B with H, and C with I). We characterized the interactions at the cellular level by examining transverse sections through ommatidia. Consistent with the interactions observed by SEM, above, in combination with activated forms of both Ras and Raf, *csw^{lf}* partially suppressed the transformation of the nonneuronal cone cells into R7 photoreceptors (Figure 4, compare D with J and F with L). Conversely, the photoreceptor loss resulting from *sev-Ras^{N17}* was enhanced in combination with *csw^{lf}* (Figure 4, compare E with K).

Taken together these data suggest that in the developing eye the *csw^{lf}* lesion is sufficient to alter the activities of both activated and dominant negative forms of Ras and an activated form of Raf. These phenotypes, developing within the context of signaling by both the EGFR and Sev pathways, firmly support a role for Csw as a modifier of the strengths of the inductive signals elicited by both the EGF and SEV RTKs.

Genetic interactions of *csw^{lf}* with downstream transcription factors in the eye: We have tested for genetic interactions between *csw^{lf}* and two downstream effectors of Ras signaling, *yan* and *pointed*, in the developing eye. Within the context of R7 formation during signaling by the Sev RTK, Yan and Pnt antagonize each other; that is, while *pnt* promotes the formation of the R7 photoreceptor, *yan* opposes or negatively regulates R7 formation (LAI and RUBIN 1992). Flies heterozygous for a gain-of-function mutation in *yan*, *yan$^{XS\text{-}2382}$*, have a very mild rough eye due to the absence of only a few of the R7 photoreceptors (KARIM *et al.* 1996). Flies doubly mutant for *csw* and *yan* (genotype: *csw^{lf} /Y; yan$^{XS\text{-}2382}$/+*) are poorly viable; however, flies that do eclose possess extremely small, rough eyes (data not shown). An amorphic *pnt* mutation, *pnt$^{\Delta 88}$*, and a recessive lethal mutation, *pnt^{07825}*, both dominantly enhanced the rough eye

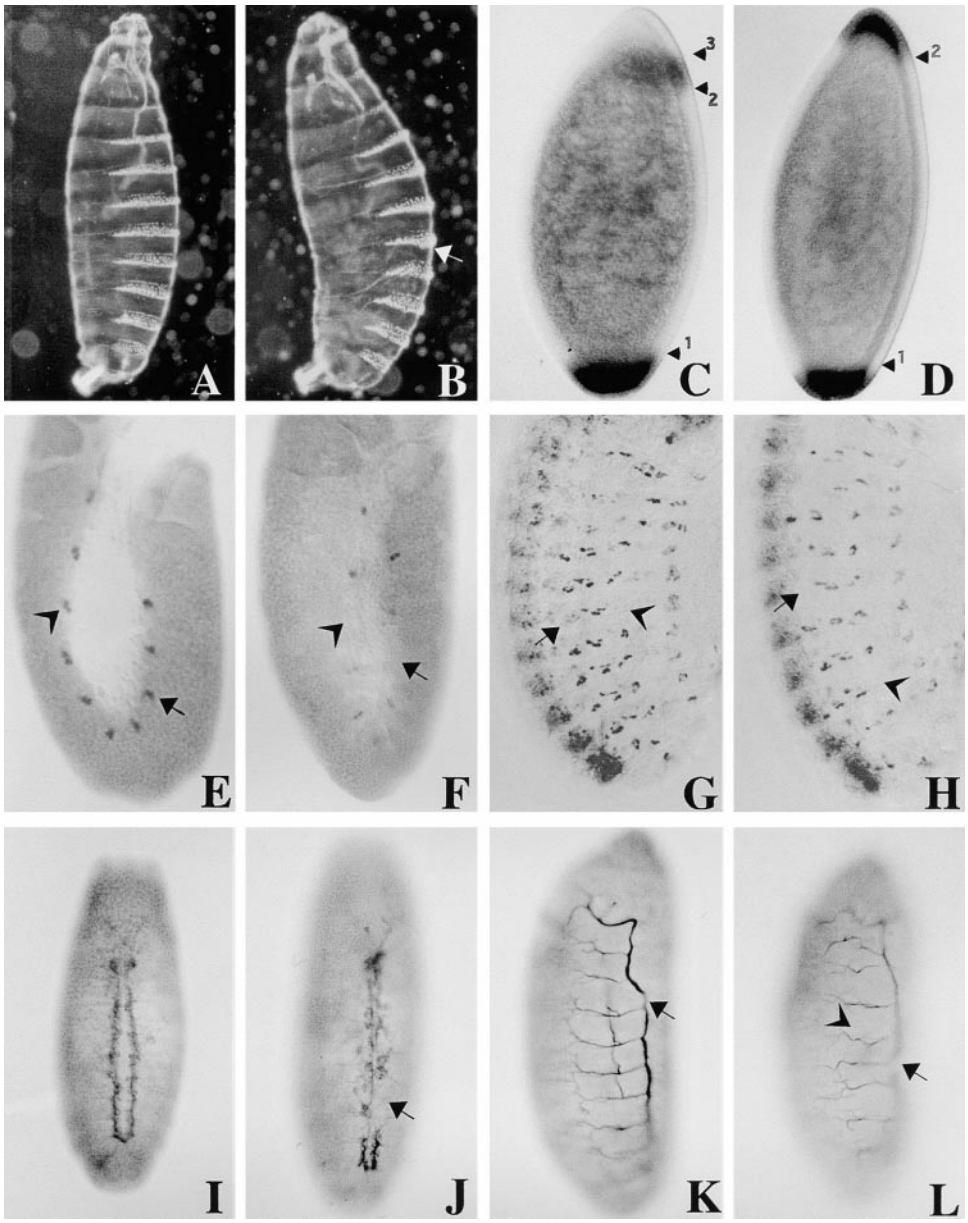

FIGURE 3.—Embryos homozygous for *csw*^{lf} exhibit phenotypes reminiscent of mutations in genes that function during RTK signaling. Homo- and hemizygous embryos derived from females bearing *csw*^{lf} germline clones fail to hatch; however, the cuticles from these embryos resemble wild type (A) or exhibit mild curvature and occasional loss or mispositioned/extra denticles (arrow in B). Molecular probes were used to examine embryonic tissues whose development is dependent on signaling by RTKs. The *csw*^{lf} lesion does not appear to affect signaling by the Torso RTK (C and D); however, tissues requiring the EGFR (E–H) and both the Btl and Htl FGFRs (I–L) are affected. Using the RNA expression patterns of *tll* (C) and *hkb* (D) to assay the effects of *csw*^{lf} on Torso signaling, relative to the wild-type expression patterns, no discernible differences were observed [percentage egg length (EL) of *tll* in *csw*^{lf} mutant embryos: 1, 13.3% (SD = 1.1; n = 23; wild type = 12.8% EL); 2, 78.0% EL (SD = 2.2; n = 23; wild type = 78.0% EL); 3, 88.6% EL (SD = 2.2; n = 23; wild type = 86.3% EL)] [percentage EL of *hkb* in *csw*^{lf} mutant embryos: 1, 9.1% EL (SD = 0.43; n = 13; wild type = 9.4% EL); 2, 90.0% EL (SD = 1.0; n = 13; wild type = 89.3% EL). Using the protein expression patterns of Eve (E and F) and Kr (G and H) to assay the effects of *csw*^{lf} on EGFR signaling in the developing mesoderm, defects were observed with both probes. At germ band elongation, in response to the EGFR pathway Eve is expressed, segmentally, in the precursors of both the DA1 larval muscle and pericardial cells (E). However, in *csw*^{lf} mutant embryos (F), Eve expression is variably lost from these cells. As the germ band shortens, Kr, also in response to the EGFR, is expressed in the precursors of the abdominal VA2/#27 larval muscle. As is the case for the Eve positive precursor, the VA2/#27 muscle precursor is variably deleted from random segments (arrows in G and H). Kr is also a marker for other developing muscles that are variably deleted *csw*^{lf} mutant embryos (arrowheads in G and H); however, RTK input has not been definitively established for these muscle precursors. Using the protein expression pattern of a pericardial cell antigen (YARNITZKY and VOLK 1995; I and J) to assay the effects of *csw*^{lf} on Htl signaling in the developing embryonic heart, relative to wild type (I) significant disruption and deletions of heart precursor cells were observed in *csw*^{lf} mutant embryos. Finally, the protein expression pattern of the tracheal lumen antigen 2A12 (K and L) was used to assay the effects of *csw*^{lf} on Btl signaling in the developing trachea. *csw*^{lf} mutant embryos exhibited either relatively little tracheal disruption (*e.g.*, interruption of the dorsal trunk, arrow in K) or significant disruption where the dorsal trunk was interrupted one or more times along the anteroposterior axis (arrowhead in L) and the dorsoventral tracheal branch patterns were misrouted (compare with K).

phenotype of males carrying *csw*^{lf}. An enhancement of the wing vein gap phenotype of *csw*^{lf} males was observed with *pnt*^{07825}, but not *pnt*$^{\Delta 88}$.

Together these results suggest that the *csw*^{lf} lesion is sufficient to alter the signal received by the transcription factors whose activities are essential for proper formation of the R7 photoreceptor.

Screening for modifiers of *csw*^{lf} adult phenotypes: We have utilized the *csw*^{lf} mutation as a starting point to conduct a sensitized genetic screen (see MATERIALS AND METHODS) to identify chromosomal regions that are able to dominantly enhance or suppress the *csw*^{lf} phenotype when their dosage is reduced by one-half. Enhancing deficiencies (Table 1) were defined as those defi-

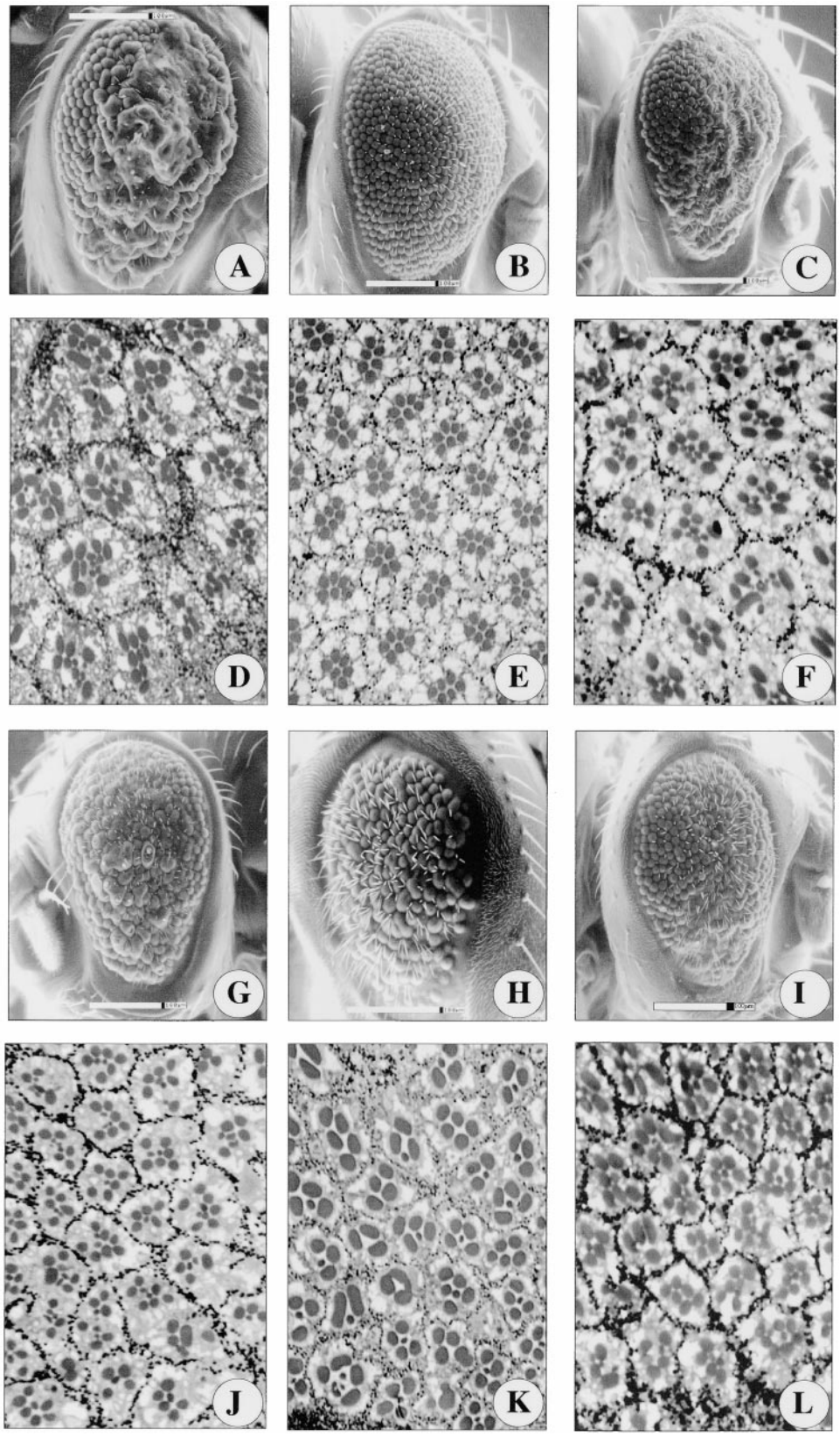

FIGURE 4.—In the developing eye, *csw*^{lf} interacts genetically with transgenes encoding modified forms of both Ras and Raf. Scanning electron micrographs and thin transverse sections through the adult eye are shown as follows: (A and D) *T2B/+ (TM3, Sb, P*[*sev-Ras1*^{V12}]/+); (B and E) P(*sev-Ras1*^{N17})/+; (C and F) P(*sev-Raf*^{torso})/+; (G and J) *csw*^{lf}*/Y; T2B/+*; (H and K) *csw*^{lf}*/Y;* P(*sev-Ras1*^{N17})/+; (I and L) *csw*^{lf}*/Y;* P(*sev-Raf*^{Torso})/+. The supernumerary R7 cells (D) and the rough eye (A) produced by the activated *Ras1* allele (*sev-Ras1*^{V12}) is suppressed by *csw*^{lf} (G and J). The dominant negative *Ras1* allele, *P*(*sev-Ras1*^{N17}), produces a mild rough eye (B) resulting from the loss of R7 cells (E). *In trans* with hemizygous *csw*^{lf} the signaling efficiency is reduced. Both the loss of photoreceptor cells and the external eye roughness are enhanced. An activated *Raf* allele (*sev-Raf*^{Torso}) also produces a rough eye (C) due to the production of ectopic R7 cells (F); this, too, is suppressed by hemizygous *csw*^{lf}.

ciencies that result in absence, *i.e.*, lethality, of *csw*^{lf}*/Y; Df/+* males or those deficiencies in which the *csw*^{lf} mutant phenotype was more severe but not lethal (examples shown in Figure 1, D, H, and L). Suppressing deficiencies (Table 2) were defined as those deficiencies that in conjunction with *csw*^{lf} (genotype: *csw*^{lf}*/Y; Df/+*) result in marked improvement of the *csw*^{lf} adult phenotypes (examples shown in Figure 1, C, G, and K). The

TABLE 1

Summary of the enhancers of *csw*^{lf}

Deficiency (BL stock nos.)	Location	Class of interaction	Candidate modifiers	Reference
		Second chromosome		
Df(2L)net-PMF (3638)[a]	21A; 21B7-8	Eye only	Spen (21A1-B6)	1
Df(2L)net18 (3633)[b]	21A4; 21B3-4	S		
Df(2L)al (3548)	21B8-C1; 21C8-D1	N		
Df(2L)ast2 (3084)	21D1-2; 22B2-3	Lethal	Star (21E2)	2
Df(2L)S3 (3446)[b]	21D2-3; 21F2-22A1	Lethal	Star (21E2)	2
Df(2L)dp-79b (3133)	22A2-3; 22D5-E1	N		
Df(2L)sc19-8 (693)	24C2-8; 25C8-9	N		
Df(2L)sc19-5 (627)	25A4-5; 25D5-7	Eye only	—	
Df(2L)cl-h3 (781)	25D2-4; 26B2-5	N		
Df(2L)E110 (490)	25F3-26; 26D2-11	N		
Df(2L)Dwee (3571)	27A; 28A	Lethal	—	
Df(2L)spd[j2] (2414)	27C1; 28A	N		
Df(2L)Trf-C6R31 (140)	28D; 28E	N		
Df(2L)TE29 (179)	28E4-7; 29B2-C1	Eye and vein gaps	—	
Df(2L)N22-14 (2892)	29C1-2; 30C8-9	N		
Df(2L)J2 (3366)	31B; 32A	N		
Df(2L)J39 (1469)	31C-D; 32D-E	Lethal	—	
Df(2L)Prl (3079)	32F1-3; 33F1-2	N		
Df(2L)H20 (3180)	36A8-9; 36E1-2	S		
Df(2L)TW137 (420)	36C2-4; 37B9-C1	Lethal	—	
Df(2L)M36F (3186)[b]	36D1-E1;36F1-37A1	Lethal	—	
Df(2L)TW50 (3189)	36E4-F1; 38A6-7	S		
Df(2R)M41A4 (739)	41A	S		
Df(2R)bw (749)	41A-B; 42A2-3	Lethal	Rolled/MAPK (h41)	3
Df(2R)nap1 (1006)	41D2-E1; 42B1-3	N		
Df(2R)cn9 (3368)	42E; 44C	N		
Df(2R)H3C1 (198)	43F;44D3-8	Vein gaps only	—	
Df(2R)cn83c (3136)[b]	43C5-D1; 44B6-C1	Vein gaps only	—	
Df(2R)44CE (3643)[b]	44C4-5; 44E2-4	N		
Df(2R)vg135 (1642)	49A3; 49D-E	N		
Df(2R)vg-C (754)	49A4-13; 49E7-F1	Vein gaps only	E(Egfr)B56 (49D1-4)	4
Df(2R)vg-D (434)[b]	49C1-2; 49E2-6	Vein gaps only	E(Egfr)B56 (49D1-4)	4
Df(2R)vg-B (752)[b]	49D3-4; 49F15-50A3	N		
Df(2R)CX1 (442)	49C1-4; 50C23-D02	Eye and vein gaps	Drk (50A12-14)	5
Df(2R)trix (1896)	51A1-2; 51B6	S		
Df(2R)Pcl7B (3064)	54E8-F1; 55B9-C1	N		
Df(2R)Pcl11B (3120)	54F6-55A1;55C1-3	Vein gaps only	—	
Df(2R)PC4 (1547)	55A; 55F	N		
Df(2R)Pl13 (1916)[b]	57B13-14; 57D8-9	N		
Df(2R)Pu-D17 (2606)	57B4; 58B	Lethal	Egfr (57F1)	6
Df(2R)PK1 (3469)[b]	57C5; 57F5-6	Lethal	Egfr (57F1)	6
Df(2R)X58-7 (283)	58A1-2; 58E4-10	N		
Df(2R)59AB (590)	59A1-3; 59B1-2	N		
Df(2R)BR6 (1682)	59D5-10; 60B3-8	Eye and vein gaps	—	
Df(2R)Px[4] (1473)	60B; 60D1-2	S		

(*continued*)

TABLE 1

(Continued)

Deficiency (BL stock nos.)	Location	Class of interaction	Candidate modifiers	Reference
		Third chromosome		
Df(3L)R-G7 (2400)	62B8-9; 62F2-5	S		
Df(3L)M21 (3650)	62F; 63D	Eye and vein gaps	Dos (62F)	7
Df(3L)HR232 (3648)	63C1; 6303	S		
Df(3L)HR119 (3649)	63C6; 63E	S		
Df(3L)GN50 (3687)	63E1-2; 64B17	Eye and vein gaps	Src64B (64B12-17)	8
Df(3L)GN24 (3686)	63F4-7; 64C13-15	Eye and vein gaps	Src64B (64B12-17)	8
Df(3L)10H[b]	64B10-12; 64C5-9	Eye and vein gaps	Src64B (64B12-17)	8
Df(3L)ZN47 (3096)	64C; 65C	Eye and vein gaps	Vn (64F1-2)	9
Df(3L)h-i22 (3024)	66D10-11; 66E1-2	Eye and vein gaps	—	
Df(3L)1xd6 (89)	67E1-2; 68C1-2	N		
Df(3L)vin2 (2547)	67F2-3;68D6	N		
Df(3L)vin5 (2611)	68A2-3; 69A1-3	Eye and vein gaps	—	
Df(3L)vin7 (2612)	68C8-11; 69B4-5	Eye and vein gaps	—	
Df(3L)BK10 (2992)	71C; 71F	S		
Df(3L)brm11 (3640)	71F1-4; 72D1-10	Eye and vein gaps	—	
Df(3L)th102 (3641)[b]	71F3-5; 72D12	Eye and vein gaps	—	
Df(3L)st-f13 (2993)	71C1-D1; 73A3-4	Lethal	—	
Df(3L)st-e4 (1317)[b]	72D5-10; 73A5-8	S		
Df(3L)st-e4 (1317)[b]	72D5-10; 73A5-8	S		
Df(3L)st7P (2997)[b]	73A1; 73A7	S		
Df(3L)st-b11 (197)[b]	72D10-11; 73D1-2	Eye and vein gaps	—	
Df(3L)81K19 (2998)	73A3; 74F	Eye and vein gaps	Sina (73D3)	10
Df(3L)rdgC (2052)	77A1; 77D1	N		
Df(3L)ri79C (3127)	77B-C; 77F-78A	Vein gaps only	—	
Df(3L)Pc-MK (3068)	78A3; 79E1-2	Lethal	—	
Df(3L)Pc-101 (4876)[b]	78C3-4; 78C8-9	Eye and vein gaps	—	
Df(3L)Pc2q (4430)[b]	78C5-6; 78E3-79A1	Eye and vein gaps	—	
Df(3R)ME15 (1518)	81F3-6; 82F5-7	Eye and vein gaps	—	
Df(3R)p712 (1968)	84D4-6; 85B6	N		
Df(3R)pXT103 (1962)	84F14; 85C-D	Eye and vein gaps		
Df(3R)p819 (1964)	85A3; 85B6	Eye and vein gaps	—	
Df(3R)by10 (1931)	85D8-12; 85E7-F1	Lethal	Ras1 (85D18-19)	11
Df(3R)by62 (1893)[b]	85D11-14; 85F16	Lethal	Ras1 (85D18-19)	11
Df(3R)M-Kx1 (3128)	86C1; 87B1-5	N		
Df(3R)T-32 (3003)	86E2-4; 87C6-7	Lethal	Svp (87B4)	12
Df(3R)ry615 (3007)	87B11-13; 87E8-11	N		
Df(3R)ChaM7 (3011)	91A; 91F5	N		
Df(3R)DlBX12 (3012)	91F1-2; 92D3-6	Eye and vein gaps	—	
Df(3R)e-N19 (2425)	93B; 94	Vein gaps only	Pnt (94F1-2)	13
Df(3R)e-R1 (3340)	93B3-5; 93D2-4	N		
Df(3R)crbS87-4 (2362)	95E8-F1; 95F15	Eye and vein gaps	—	
Df(3R)crbS87-5 (2363)	95F7; 96A17-18	N		
Df(3R)Tl-P (1910)	97A; 98A1-2	Eye and vein gaps	Su(Raf)3C (98A-F)	1

1, Dickson *et al.* (1996); 2, Kolodkin *et al.* (1994); 3, Biggs and Zipursky (1992); 4, Price *et al.* (1997); 5, Simon *et al.* (1993); 6, Clifford and Schüpbach (1989); Baker and Rubin (1989); 7, Herbst *et al.* (1996); Raabe *et al.* (1996); 8, Cooper *et al.* (1996); 9, Simcox *et al.* (1996); 10, Carthew and Rubin (1990); 11, Simon *et al.* (1991); 12, Begemann *et al.* (1995); and 13, Brunner *et al.* (1994). N, no interaction; S, suppressor.

[a] *Df(2L)net-PMF* (3638) suppressed the wing vein gaps of *csw*^{lf} and enhanced the rough eye.

[b] Additional Bloomington deficiencies (*i.e.*, not from the kit).

TABLE 2

Summary of the suppressors of *csw^{lf}*

Deficiency (BL stock nos.)	Location	Class of interaction	Candidate modifiers	Reference
		Second chromosome		
Df(2L)net-PMF (3638)[a]	21A; 21B7-8	Vein gaps only	Net (21B3)	1
Df(2L)net-18 (3633)	21A4; 21B3-4	Vein gaps only	Net (21B3)	1
Df(2L)al (3548)	21B8-C1; 21C8-D1	N		
Df(2L)C144 (90)	23A1-2;23C3-5	Eye only	—	
In(2LR)DTD16 (3573)	23C; 23E3-6	Vein gaps only	—	
Df(2L)ed1 (712)	24A3-4; 24D3-4	Eye only	Ed (24D4)	2
Df(2L)ed-dp (702)[b]	24C3-5; 25A2-3	Eye and vein gaps	Ed (24D4)	2
Df(2L)sc19-8 (693)	24C2-8; 25C8-9	N		
DF(2L)cact (2583)	35F-36 A; 36D	N		
Df(2L)H20 (3180)	36A8-9; 36E1-2	Eye only	—	
Df(2L)TW137 (420)	36C2-4; 37b9-c1	Lethal	—	
Df(2L)TW50 (3189)	36E4-F1; 38A6-7	Eye only	—	
Df(2L)M36F (343)[b]	36E6-F1; 36F7-9	Eye only	—	
Df(2L)TW3 (3781)[b]	36F7-9; 37B2-7	N		
Df(2L)TW158 (3784)[b]	37B2-8; 37E2-F1	Eye only	—	
Df(2L)pr-A16 (567)[b]	37B2-12; 38D2-5	Eye only	—	
Df(2L)TW130 (1961)[b]	37B9-C1; 37D1-2	Eye only	—	
Df(2L)VA16 (3172)[b]	37B9; 38A1	Eye only	—	
Df(2L)VA17 (3785)[b]	37C1; 37F5	Eye only	—	
Df(2L)E55 (3076)[b]	37D2-F1; 37F5-38A1	N		
Df(2L)TW161 (167)	38A6-B1; 40A4-B1	N		
Df(2R)M41A4 (739)	41A	Eye only	—	
Df(2R)bw (749)	41A-B; 42A2-3	E		
Df(2R)nap1 (1006)	41D2-E1; 42B1-3	N		
Df(2R)nap9 (1007)	42A1-2; 42E6-F1	Eye only	Src42A (42A1-2)	3
Df(2R)pk78s (1930)	42C1-7; 43F5-8	Eye only	—	
Df (2R)cn88b (3134)[b]	42C; 42E	Eye only	—	
Df (2R)Drl[rv3] (3920)[b]	42E1-4; 43C3	Eye only	—	
Df (2R)pk78k (1594)[b]	42E3; 43C3	Eye only	—	
Df(2R)cn9 (3368)	42E; 44C	N		
Df(2R)CX1 (442)	49C1-4; 50C23-D02	E		
Df(2R)trix (1896)	51A1-2; 51B6	Vein gaps only	—	
Df(2R)knSA3 (1150)[b]	51B5-11; 51F5-13	Vein gaps only	—	
Df(2R)Jp1 (3518)	51C3; 52F5-9	N		
Df(2R)PC4 (1547)	55A; 55F	N		
Df(2R)P34 (757)	55E2-4; 56C1-11	Vein gaps only	—	
Df(2R)017 (543)	56F5; 56F15	N		
Df(2R)BR6 (1682)	59d5-10; 60B3-8	E		
Df(2R)Px[4] (1473)	60B; 60D1-2	Eye and vein gaps	Bs (60C1; D1)	4
Df(2R)Px2 (2604)	60C5-6; 60D9-10	Eye and vein gaps	Bs (60C1; D1)	4

(*continued*)

eye and wing phenotypes of outcrossed *csw^{lf}* males (genotype: *csw^{lf}/Y; +/+*) were used as controls. From this screen we have identified regions on both the second and third chromosomes that encompass loci that modify the expressivity of *csw^{lf}*. Frequently, the intervals containing loci that modify the *csw^{lf}* phenotypes could be refined by deficiencies that do not interact with *csw^{lf}*. These are included in Tables 1 and 2 as deficiencies showing no (N) interaction with *csw^{lf}*.

Enhancers of *csw^{lf}*: Our deficiency screen identified 14 genomic regions on the second chromosome and 19 genomic regions on the third chromosome that dom-

TABLE 2

(Continued)

Deficiency (BL stock nos.)	Location	Class of interaction	Candidate modifiers	Reference
		Third chromosome		
Df(3L)emc5 (439)	61C3-4; 62A8	Eye and vein gaps	Su(ve) (~61F8)	5
Df(3L)R-G5 (2399)	62A10-B1; 62C4-D1	N		
Df(3L)R-G7 (2400)	62B8-9; 62F2-5	Vein gaps only	—	
Df(3L)HR232 (3648)	63C1; 63D3	Eye and vein gaps	Sty (63D1-2)	6
Df(3L)HR119 (3649)	63C6; 63E	Eye and vein gaps	Sty (63D1-2)	6
Df(3L)GN50 (3687)	63E1-2; 64B17	N		
Df(3L)fzGF3b (3124)	70C1-2; 70D4-5	Vein gaps only	—	
Df(3L)fzM21 (3126)	70D2-3; 70E4-5	Vein gaps only	—	
Df(3L)BK10 (2992)	71C; 71F	Eye and vein gaps	—	
Df (3L)brm11 (3640)	71F1-4; 72D1-10E	E		
Df(3L)st-f13 (2993)	72C1-D1; 73A3-4	E		
Df(3L)st-e4 (1317)[b]	72D5-10; 73A5-8	Eye and vein gaps	Aos (73A3)	7
Df(3L)st7P (2997)[b]	73A1; 73A7	Eye and vein gaps	Aos (73A3)	7
Df(3L)81K19 (2998)	73A3; 74F	E		
Df(3L)W10 (2608)	75B3-6; 75C	N		
Df(3L)W4 (2607)	75B10; 75C1-2	Vein gaps only	Term (75C1)	8
Df(3R)red1 (3341)	88B1; 88D3-4	Eye and vein gaps	—	
Df(3R)sbd45 (3678)	89B4; 89B10	N		
Df(3R)P115 (1467)[b]	89B7-8; 89E7-8	Vein gaps only	—	
Df(3R)sbd26 (1705)	89B9-10; 89C7-D1	Vein gaps only	—	
Df(3R)C4 (3071)	89E3-4; 90A1-7	N		
Df(3R)awd-KRB (3369)	100C-D	Eye and vein gaps	—	

1, Garcia-Bellido and de Celis (1992); 2, Hsu *et al.* (1998); 3, Lu and Li (1999); 4, Fristrom *et al.* (1994); 5, FlyBase (1999); 6, Casci *et al.* (1999); Kramer *et al.* (1999); 7, Freeman *et al.* (1992); Sawamoto *et al.* (1994); 8, Baldarelli *et al.* (1988). N, no interaction; E, enhancer.

[a] *Df(2L)net-PMF* (3638) suppressed the wing vein gaps of csw^{lf} and enhanced the rough eye.

[b] Additional Bloomington deficiencies (*i.e.*, not from the kit).

inantly enhanced the csw^{lf} eye and/or wing vein phenotypes (Table 1). Consistent with a role for Csw in RTK signaling, as well as validating the screen, a significant number of the genomic regions identified encompass loci that function positively during RTK signaling or genetically interact with known components of RTK signaling. Briefly, deficiencies were identified that encode the *Egfr* receptor, the RTK transducers *drk*, *dos*, *Ras1*, *rolled/MAPK*, *Src64B*, the Egfr ligand *vein*, the Egfr ligand processing protein *Star*, and the transcription factors *pnt*, *seven-in-absentia* (*sina*), and *seven-up* (*svp*). We also identified enhancer interactions with deficiencies encoding candidate loci that have been previously identified as modifiers of RTK phenotypes and whose molecular identities are unknown, *e.g.*, *E(Egfr)B56* and *Su(Raf)3C*, or known molecules whose connection to RTK signaling is undescribed, *e.g.*, the RNP motif containing protein *split-ends* (*spen*).

When possible, we tested for genetic interactions between csw^{lf} and mutations in the candidate loci. As outlined above, we observed strong genetic interactions with csw^{lf} and known positive components of RTK signaling, Ras, Raf, and Pnt. In addition, and supporting their designated "candidate genes" status, loss-of-function mutations in several loci, dos^{R31}, drk^{TZ160}, $Egfr^{1F26}$, $ras^{\Delta C40b}$, and $Star^{1}$, were observed to strongly enhance the csw^{lf} mutant phenotypes, while the gain-of-function mutation $rolled^{SEM}$ strongly suppressed the csw^{lf} phenotypes. We were satisfied to learn that, in combination with csw^{lf}, the deficiency that contains the *E(Egfr)B56* candidate, like the mutation, enhanced only the vein gap phenotype (Price *et al.* 1997). The adult viable, hypomorphic allele of *Src64B*, $Src64B^{\Delta 17}$, also enhanced the wing phenotype of csw^{lf} but, like *E(Egfr)B56*, no obvious enhancement of the csw^{lf} rough eye phenotype was observed in combination with $Src64B^{\Delta 17}$.

Of particular interest are the enhancing deficiencies for which no candidate genes have been identified. Five genomic regions, three on the second chromosome and two on third chromosome, are lethal in combination

with csw^{lf} (Table 1). It is possible, however, that the lethal interactions with *Df(3L)st-f13* (72C1-D1; 73A3-4) and *Df(3L)Pc-MK* (78A3; 79E1-2) result from the combined effects of nonlethal enhancer loci that also map to these regions.

Of the remaining nonlethal enhancing loci, most are enhancers of both the wing vein and eye csw^{lf} phenotypes, five are enhancers of the vein gap phenotype only, and two are enhancers of the eye phenotype only. One of the latter, covered by the deficiency *Df(2L)net-PMF*, is an enhancer of the rough eye phenotype while also completely suppressing the wing vein phenotype, suggesting that two interacting loci are in this region. Interestingly, a candidate gene, *net*, maps to this region and displays ectopic veins and may thus be responsible for the suppression of the wing vein gap phenotype (see below).

Finally, there are several genomic intervals for which we expected interactions with csw^{lf}; however, no interactions were observed. Three notable examples include the genomic intervals encoding the exchange factor Sos (34D4), the kinase Ksr (83A5), and the transcription factor Phyllopod (51A2). There are several reasons why interactions may not have been detected in these genetic intervals, the most likely being that the dosage of an interacting gene removed by the deficiency is not critical and, thus, removal of one copy would be insufficient to modify the csw^{lf} phenotypes. Another likely possibility is that the regions deleted remove not only these positive components of RTK signaling but also nearby negative components. This latter hypothesis is thought to be the case for *ksr* and *sos* since, as expected, mutant alleles for two of these expected interacting loci, ksr^{S638} and sos^{34G}, exhibit strong dominant enhancing interactions with csw^{lf}.

Suppressors of csw^{lf}: Our deficiency screen identified 13 genomic regions on the second chromosome and 10 genomic regions on the third chromosome that dominantly suppressed the csw^{lf} eye and/or wing vein phenotypes (Table 2). Among the suppressing loci 8 suppress both the wing vein and eye csw^{lf} phenotypes, 8 are suppressors of the vein gap phenotype only, and 7 are suppressors of the eye phenotype only.

Also validating the screen, several of the genomic regions identified encompass loci that function to negatively regulate RTK signaling; that is, deficiencies were identified that encode the known negative regulators of RTK signaling *argos* (*aos*) and *sprouty* (*sty*). We also identified suppressing interactions with deficiencies encoding candidate loci that have been previously identified as modifiers of RTK phenotypes and whose molecular identities are both known, *e.g.*, the serum response factor gene *blistered* (*bs*) and the tyrosine kinase gene *Src42A*, and unknown, *e.g.*, *echinoid* (*ed*), *net*, and *suppressor of veinlet* [*su(ve)*], as well as a gene whose expression pattern suggests involvement in RTK signaling, *e.g.*, the zinc finger encoding gene *terminus* (*term*).

When possible, we tested for genetic interactions between csw^{lf} and mutations in the candidate loci. Supporting their designated candidate genes status, loss-of-function mutations in several loci, aos^{delta7}, net^{1}, $Src42A^{k10108}$, and $sty^{\Delta 5}$, were found to suppress csw^{lf} mutant phenotypes. Fourteen additional suppressor loci, 8 on the second chromosome and 6 on the third chromosome, contain no obvious candidate genes (Table 2).

Interestingly, two deficiencies that delete the candidate locus *bs* (*Df(2R)Px4* and *Df(2R)Px2*) and are strong suppressors of both the eye and vein gap phenotypes of csw^{lf}, on their own, exhibit a dominant blistered wing and ectopic vein phenotype, which is, in turn, suppressed by interaction with csw^{lf} (Figure 5). The dominant phenotypes of the deficiencies are due to the loss of the *bs* gene; however, a role for *bs* in eye development has not previously been reported (Fristrom *et al.* 1994; Montagne *et al.* 1996; Roch *et al.* 1998). We tested several *bs* alleles for their dominant effects on csw^{lf} mutant phenotypes. Briefly, two recessive lethal *bs* alleles, bs^{K07909} and bs^{03267}, which display dominant blistered wing and ectopic vein phenotypes alone, are strong suppressors of the csw^{lf} eye and wing vein phenotypes (Figure 5). Of the three recessive viable alleles tested, bs^{1} exhibited a strong suppression with csw^{lf} while bs^{2} and bs^{3} showed only partial suppression of the vein gap phenotype and weak or no suppression of the eye phenotype, respectively. We observed a reciprocal suppression of the dominant *bs* phenotypes by csw^{lf} similar to the interactions obtained with both deficiencies of *bs*.

Finally, there are several genomic intervals for which we expected interactions with csw^{lf}; however, no interactions were observed. Two notable examples include the genomic intervals encoding the GTPase-activating protein Gap1 (67D2-3) and the Ets-domain transcription factor Yan (22D1-2). As expected, however, the loss-of-function allele $Gap1^{B2}$ exhibits a strong suppressing interaction with csw^{lf}, and the gain-of-function allele $yan^{XS-2382}$ (see above) exhibits a strong enhancing interaction with csw^{lf}.

DISCUSSION

csw^{lf} is a hypomorphic allele that affects several RTK pathways: We have isolated and characterized a novel allele of the Drosophila *csw* gene, csw^{lf}, that bypasses the pupal lethality associated with all other known *csw* alleles and, when homozygous, results in rough eye and wing vein loss adult phenotypes (Figure 1). Complementation analysis with different alleles of *csw*, as well as deficiencies that remove the *csw* gene, always result in lethality or weakly viable adults with eye and wing phenotypes much more severe than homozygous csw^{lf} flies. Further, the phenotypes of homozygous csw^{lf} adults can be rescued by addition of one copy of a *csw* minigene. Together, these results suggest that the csw^{lf} allele is a hypomorphic or residual activity mutation in the *csw*

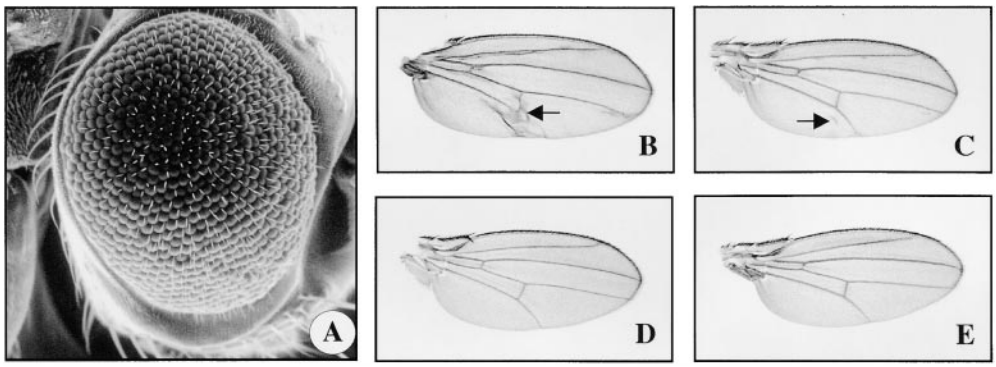

FIGURE 5.—The ectopic wing vein phenotype of *bs*, a suppressor of the csw^{lf} adult phenotypes, is in turn, suppressed by csw^{lf}. Scanning electron micrograph from csw^{lf}/Y; *Df(2R)Px2*/+ (compare A to Figure 1B). Wings from adult flies: (B) a deficiency containing the *bs* gene *Df(2R)Px2*/+; (C) a strong allele of *bs*, bs^{K07909}/+; (D) csw^{lf}/Y; *Df(2R)Px2*/+; and (E) csw^{lf}/Y; bs^{K07909}/+. The deficiency *Df(2R)Px2* dominantly suppresses the csw^{lf} rough eye (A) and wing vein gaps (D). In turn, the ectopic wing veins of *Df(2R)Px2* (see arrow in B) are suppressed by hemizygous csw^{lf} (D). The mutual suppression was confirmed to be due to the *bs* gene, using the bs^{K07909} allele, which also exhibits an ectopic wing vein phenotype (see arrow in C). Again the wing vein gaps of csw^{lf}/Y and the ectopic veins of bs^{K07909} were suppressed (E).

gene. This viable csw^{lf} allele has provided a highly useful tool for both genetic and phenotypic analysis of *csw* function, as well as the identification of genomic regions and loci that genetically interact with *csw*.

Csw function had previously been shown to be essential for many developmental processes throughout the life cycle, including both the Sev and Egfr signaling pathways during imaginal development (PERKINS *et al.* 1992, 1996; ALLARD *et al.* 1996). In the course of analyzing the csw^{lf} adult phenotypes we observed a preferential loss of the R7 photoreceptor, along with the occasional loss of outer photoreceptors, again supporting a role for Csw in Sev and Egfr signaling in the eye. Likewise, the ability of csw^{lf} to suppress wing vein differentiation, a developmental process requiring the Egfr (reviewed by DE CELIS 1998), further supports a role for Csw in this process. However, we also wanted to determine whether the csw^{lf} mutation affected only RTK pathways during imaginal development or whether RTK signaling at other stages could also be disrupted by the mutation. Although homozygous adult male and female csw^{lf} flies can be obtained, a homozygous stock cannot be maintained due to almost complete sterility of the homozygous females; however, adult males appear to be fully fertile. Further examination of homozygous csw^{lf} females revealed that the eggs they lay are ventralized; *i.e.*, the dorsal appendages are fused (Figure 2). This is a phenotype exhibited by mutations in the Egf RTK pathway in the determination of the dorsal eggshell during oogenesis (reviewed by VAN BUSKIRK and SCHÜPBACH 1999).

To investigate the effect of the csw^{lf} lesion during embryonic RTK signaling, we mated females bearing csw^{lf} germline clones to hemizygous csw^{lf} males and examined the resulting csw^{lf}/csw^{lf} female and csw^{lf}/Y male progeny. That csw^{lf} is the weakest of the series of *csw* alleles is supported by the analysis of the expression patterns of *tll* and *hkb*, transcription factors whose expression directs the formation of larval terminal structures and is dependent on the activity of the Torso RTK (reviewed by PERRIMON *et al.* 1995). All of the previously identified *csw* alleles reduced, to varying degrees, the posterior expression of both *tll* and *hkb* (PERKINS *et al.* 1992, 1996; GHIGLIONE *et al.* 1999); however, the csw^{lf} mutation did not affect the expression of either *tll* or *hkb* (Figure 3).

While the reduction in Csw function by the csw^{lf} lesion does not show any obvious effects on Torso signaling, this mutation mildly affects signaling by other essential embryonic RTKs (Figure 3). As is the case for signaling by the Egfr during oogenesis and imaginal development, the csw^{lf} mutation reduces the Egfr pathway signal, as evidenced by a partial fusion of denticle bands along the midline, mild twisting, and incomplete germ band shortening, all phenotypes exhibited by mutations affecting Egfr signaling (reviewed by PERRIMON and PERKINS 1997; SCHWEITZER and SHILO 1997). Other RTK pathways whose activities are presumed to be reduced by the csw^{lf} mutation include both of the Drosophila FGF receptors, Btl required for larval tracheal development (reviewed by METZGER and KRASNOW 1999) and Htl required for larval muscle and heart development (BEIMAN *et al.* 1996; GISSELBRECHT *et al.* 1996). In the case of Btl signaling, homozygous csw^{lf} embryos exhibit a disconnected system of tracheal branches that are often misrouted and, in the case of Htl signaling, specific mesodermally derived larval muscles and heart precursor cells appear to be missing (Figure 3).

Collectively, our phenotypic analysis of the csw^{lf} lesion suggests that, with the exception of the RTK Torso, this mutation affects an aspect of Csw function that is utilized within each of the RTK signaling pathways examined. This point is important in the context of our deficiency screen where this lesion was used to identify genomic regions that either enhance or suppress the csw^{lf} adult mutant phenotypes. In this regard, we can reasonably assume that among the interacting genomic intervals that modify both the Sev and Egfr pathways required for proper eye and wing formation are candidate loci

that function to modify additional RTK pathways, *e.g.*, the Btl and Htl signaling pathways.

Role of Csw in the RTK signaling cassette: We tested for interactions between components of the pathway and csw^{lf}. Uniformly, we observed that loss-of-function or dominant negative mutations in positive transducers of the RTK signal enhanced the csw^{lf} phenotypes, while gain-of-function or activated forms of positive transducers suppressed the csw^{lf} phenotypes. Similarly, loss-of-function mutations in negative transducers of the RTK signal suppressed the csw^{lf} phenotypes and a gain-of-function mutation in a negative transducer, yan, enhanced the csw^{lf} phenotypes. These results firmly support previous work demonstrating a role for Csw as a positive mediator of RTK pathway signaling, and our results are consistent with previous reports that have suggested that Csw appears to function either upstream or downstream of Ras1 and/or D-Raf depending upon the RTK under investigation (Lu *et al.* 1993; Allard *et al.* 1996). We found that the csw^{lf} mutation was capable of suppressing the phenotypes resulting from the expression in the eye of activated forms of both Ras1 and Raf. In a large-scale screen for suppressors of the activated Ras phenotype, Karim *et al.* (1996) isolated multiple alleles of several genes known to act downstream of Ras, but did not detect mutations in genes upstream of Ras, *e.g.*, *sos*, *drk*, or *Egfr*. It is unlikely therefore that the activated Ras phenotype is sensitive to downregulation of the endogenous RTK pathway upstream of Ras, which leads us to conclude that Csw has an additional role(s) downstream of Ras and Raf.

Use of csw^{lf} in a sensitive genetic screen: csw^{lf} is a viable mutation with easily scored adult phenotypes, thus making it an ideal sensitized genetic background with which to perform an F_1 screen for modifier loci. The efficacy of the screen is indicated by the detection of several loci containing known components of the Ras pathway, *e.g.*, Aos, Dos, Drk, Egfr, Pnt, Ras1, Rl/MAPK, Star, and Sty. The screen detected both modifiers of the adult phenotypes and also lethal interactions. This was to be expected, as our phenotypic analysis shows that csw^{lf} reduces the efficiency of various RTK signals throughout development, not just in the eyes and wing veins. Some of the modifier loci included the map positions of mutations previously detected in genetic screens for modifiers of the Ras pathway; *e.g.*, E(Egfr)B56 and Spen/E(raf)3A were detected in screens for modifiers of Egfr and Raf phenotypes (Dickson *et al.* 1996; Price *et al.* 1997).

Interestingly, we also observed enhancing and suppressing genetic interactions with genomic intervals containing the nonreceptor tyrosine kinases Src64B and Src42A, respectively. With regard to Src64B, we tested a small deletion that encompasses the Src64B gene, *Df(3L)10H* as well as the adult viable hypomorphic mutation $Src64B^{\Delta 17}$, both of which enhance the wing phenotype of csw^{lf}; however, unlike the deficiency *Df(3L)10H*, we observed no obvious enhancement of the csw^{lf} rough eye phenotype in combination with $Src64B^{\Delta 17}$. While in Drosophila the role of Src64B in RTK signaling has not been broadly explored, ectopic expression studies have suggested that Src64B plays a positive role during photoreceptor differentiation (Cooper *et al.* 1996). Here we extend these observations to include a putative role for this gene in Egfr-mediated specification of wing veins. With regard to Src42A, there are conflicting reports with respect to Ras pathway regulation. Takahashi *et al.* (1996) has reported that this tyrosine kinase maps to 41A (*Src41A*) and plays a positive role in Ras signaling. More recently, however, Lu and Li (1999) have mapped the same gene to 42A and demonstrated a negative role for this kinase in Egfr signaling. When we tested for a genetic interaction between csw^{lf} and a *P*-element insertion allelic to *Src42A*, we observed a suppression of the csw^{lf} phenotypes, consistent with a negative regulatory role for this gene.

Other enhancer loci did not appear to have any obvious candidate interacting genes, including four of the lethal interactions on the second chromosome and two lethal interactions on the third chromosome. For some of these loci we were able to confirm the interaction with overlapping deficiencies (see Table 1), demonstrating that the modifiers most likely reside in the deficiency location and not elsewhere on the chromosome. Similarly, a number of the nonlethal modifier loci were also located in overlapping deficiencies.

Among the suppressor loci we identified are *net*, a mutation with a vein promoting phenotype; *ed*, which has previously been shown to be a suppressor of reduced Egfr signaling; and *sty*, a known negative regulator of multiple RTK pathways throughout development. In addition, two strong suppressors were contained within overlapping deficiencies that removed the *bs* gene. The latter deficiencies result in dominant wing blistering and ectopic vein phenotypes, which in turn are suppressed by csw^{lf}, suggesting that the interacting allele is indeed *bs*, a finding we have confirmed by testing interactions with a number of *bs* alleles. Bs has previously been shown to act autonomously in the intervein cells of the pupal wing in order to limit the width of the wing veins and is a Drosophila homologue of the mammalian serum response factor, a MADS-box containing transcriptional regulator (Montagne *et al.* 1996). Mutual suppression between the csw^{lf} and *bs* alleles may indicate that the balance of vein differentiation observed reflects antagonistic activity between Bs and the Egfr pathway. This is supported by the observation that in the pupal wing the activities of *bs* and *veinlet* mutually repress the expression of the other (Roch *et al.* 1998). Our finding that strong alleles of *bs* suppress the eye phenotypes of csw^{lf} is interesting as this is the first report of a role for Bs in the developing eye.

Perspective: The fortuitous isolation of a novel, viable allele of *csw*, a known positive transducer of multiple

RTK initiated signals, has provided a powerful tool to identify autosomal genomic intervals that genetically enhance or suppress the adult *csw* mutant phenotypes. It is anticipated that several of the autosomal intervals identified will yield hitherto unknown transducers and/or regulators of RTK signaling, each of which may provide clues toward the identification of new or novel ways to regulate the activities of these universally conserved, essential signaling pathways.

For antibodies we thank M. Krasnow, T. Volk, Dave Kosman, and John Reinitz. For fly stocks we thank the Bloomington Stock Center, Y. Hiromi, N. Perrimon, J. Price, G. Rubin, F. Takahashi, and C.-t Wu. We thank C. Gilpin for assistance with electron microscopy and W. Li for sharing data prior to publication. L.F. is supported by the Biotechnology and Biological Sciences Research Council. M.B. is supported by the Medical Research Council and the Wellcome Trust. J.A.L. is supported by Postdoctoral Fellowships from the National Institutes of Health, F32GM17901, and the American Cancer Society, Massachusetts Division. L.A.P is supported by a National Science Foundation grant IBN-9904606 and by the Department of Surgery at the Massachusetts General Hospital.

LITERATURE CITED

Allard, J. D., H. C. Chang, R. Herbst, H. McNeill and M. A. Simon, 1996 The SH2-containing tyrosine phosphatase Corkscrew is required during signaling by Sevenless, Ras1 and Raf. Development **122:** 1137–1146.

Allard, J. D., R. Herbst, P. M. Carroll and M. Simon, 1998 Mutational analysis of the SRC homology 2 domain protein-tyrosine phosphatase Corkscrew. J. Biol. Chem. **273:** 13129–13135.

Ashburner, M., 1989 *Drosophila: A Laboratory Handbook and Manual*, Vols. 1 and 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Baker, N. E., and G. M. Rubin, 1989 Effect on eye development of dominant mutations in Drosophila homologue of the EGF receptor. Nature **340:** 150–153.

Baldarelli, R. M., P. A. Mahoney, F. Salas, E. Gustavson, P. D. Boyer *et al.*, 1988 Transcripts of the Drosophila blastoderm-specific locus, terminus, are concentrated posteriorly and encode a potential DNA-binding finger. Dev. Biol. **125:** 85–95.

Begemann, G., A. M. Michon, L. van der Voorn, R. Wepf and M. Mlodzik, 1995 The Drosophila orphan nuclear receptor Seven-up requires the Ras pathway for its function in photoreceptor determination. Development **121:** 225–235.

Beiman, M., B. Z. Shilo and T. Volk, 1996 Heartless, a Drosophila FGF receptor homolog, is essential for cell migration and establishment of several mesodermal lineages. Genes Dev. **10:** 2993–3002.

Biggs, W. H., and S. L. Zipursky, 1992 Primary structure, expression, and signal-dependent tyrosine phosphorylation of a Drosophila homolog of extracellular signal-regulated kinase. Proc. Natl. Acad. Sci. USA **89:** 6295–6299.

Bronner, G., and H. Jäckle, 1991 Control and function of terminal gap gene activity in the posterior pole region of the Drosophila embryo. Mech. Dev. **35:** 205–211.

Bronner, G., Q. Chu-LaGraff, C. Q. Doe, B. Cohen, D. Weigel *et al.*, 1994 Sp 1/egr-like zinc-finger protein required for endoderm specification and germ layer formation in Drosophila. Nature **369:** 664–668.

Brunner, D., K. Ducker, N. Oellers, E. Hafen, H. Scholz *et al.*, 1994 The ETS domain protein Pointed-P2 is a target of MAP kinase sevenless signal transduction pathway. Nature **370:** 386–389.

Buff, E., A. Carmena, S. Gisselbrecht, F. Jimenez and A. M. Michelson, 1998 Signalling by the Drosophila epidermal growth factor receptor is required for the specification and diversification of embryonic muscle progenitors. Development **125:** 2075–2086.

Carmena, A., S. Gisselbrecht, J. Harrison, F. Jimenez and A. M. Michelson, 1998 Combinatorial signaling codes for the progressive determination of cell fates in the Drosophila embryonic mesoderm. Genes Dev. **12:** 3910–3922.

Carthew, R. W., and G. M. Rubin, 1990 seven in absentia, a gene required for specification of R7 cell fate in the Drosophila eye. Cell **63:** 561–577.

Casanova, J., M. Furriols, L. A. McCormick and G. Struhl, 1995 Similarities between trunk and spatzle, putative extracellular ligands specifying body pattern in Drosophila. Genes Dev. **9:** 2539–2544.

Casci, T., J. Vinos and M. Freeman, 1999 Sprouty, an intracellular inhibitor of Ras signaling. Cell **96:** 655–665.

Chou, T.-Z., and N. Perrimon, 1996 The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. Genetics **144:** 1673–1679.

Cleghon, V., U. Gayko, T. D. Copeland, L. A. Perkins, N. Perrimon *et al.*, 1996 Specific phosphotyrosine residues on the Drosophila Torso RTK mediate both positive and negative signaling events that are compensatory. Genes Dev. **10:** 566–577.

Cleghon, V., P. Feldman, C. Ghiglione, T. D. Copeland, N. Perrimon *et al.*, 1998 Opposing actions of CSW and RasGAP modulate the strength of Torso RTK signaling in the Drosophila terminal pathway. Mol. Cell **2:** 719–727.

Clifford, R. J., and T. Schüpbach, 1989 Coordinately and differentially mutable activities of torpedo, the Drosophila melanogaster homolog of the vertebrate EGF receptor gene. Genetics **123:** 771–787.

Cooper, J. A., M. A. Simon and S. J. Kussick, 1996 Signaling by ectopically expressed Drosophila Src64 requires the protein tyrosine phosphatase Corkscrew and the adapter Downstream of Receptor Kinases. Cell Growth Differ. **7:** 1435–1441.

de Celis, J. F., 1998 Positioning and differentiation of veins in the Drosophila wing. Int. J. Dev. Biol. **42:** 335–343.

Dickson, B., F. Sprenger, D. Morrison and E. Hafen, 1992 Raf functions downstream of Ras1 in the sevenless signal transduction pathway. Nature **360:** 600–603.

Dickson, B. J., A. van der Straten, M. Dominguez and E. Hafen, 1996 Mutations modulating Raf signaling in Drosophila eye development. Genetics **142:** 163–171.

FlyBase, 1999 The FlyBase database of the Drosophila Genome Projects and community literature. Available from http://flybase.bio.indiana.edu/. Nucleic Acids Res. **27:** 85–88.

Freeman, M., C. Klambt, C. S. Goodman and G. M. Rubin, 1992 The argos gene encodes a diffusible factor that regulates cell fate decisions in the Drosophila eye. Cell **69:** 963–975.

Fristrom, D., P. Gotwals, S. Eaton, T. B. Kornberg, M. Sturtevant *et al.*, 1994 *blistered*: a gene required for vein/intervein formation in wings of Drosophila. Development **120:** 2661–2671.

Fukuda, M., Y. Gotoh and E. Nishida, 1997 Interaction of MAP kinase with MAP kinase kinase: its possible role in the control of nucleocytoplasmic transport of MAP kinase. EMBO J. **16:** 1901–1908.

Furriols, M., A. Casali and J. Casanova, 1998 Dissecting the mechanism of Torso receptor activation. Mech. Dev. **70:** 111–118.

Garcia-Bellido, A., and J. de Celis, 1992 Developmental genetics of veination pattern. Annu. Rev. Genet. **26:** 277–304.

Gaul, U., G. Mardon and G. M. Rubin, 1992 A putative Ras GTPase activating protein acts as a negative regulator of signaling by the sevenless receptor tyrosine kinase. Cell **68:** 1007–1019.

Ghiglione, C., N. Perrimon and L. A. Perkins, 1999 Quantitative variations in the level of MAPK activity control patterning of the embryonic termini in Drosophila. Dev. Biol. **205:** 181–193.

Gisselbrecht, S., J. B. Skeath, C. Q. Doe and A. M. Michelson, 1996 heartless encodes a fibroblast growth factor receptor (DFR1/DFGF-R2) involved in the directional migration of early mesodermal cells in the Drosophila embryo. Genes Dev. **10:** 3003–3017.

Herbst, R., P. M. Carrol, J. D. Allard, J. Schilling, T. Raabe *et al.*, 1996 Daughter of Sevenless is a substrate of the phosphotyrosine phosphatase Corkscrew and functions during Sevenless signaling. Cell **85:** 899–909.

Hertog, J. D., 1999 Protein-tyrosine phosphatases in development. Mech. Dev. **85:** 3–14.

Hsu, J. C., C.-t. Wu, N. Perrimon and J. M. Bai, 1998 Echinoid, a negative regulator of the Drosophila EGF receptor signaling pathway. A. Dros. Res. Conf. **39:** 193A.

Karim, F. D., H. C. Chang, M. Therrien, D. A. Wasserman, T. Laverty *et al.*, 1996 A screen for genes that function downstream of Ras1 during Drosophila eye development. Genetics **143:** 315–329.

Khokhlatchev, A. V., B. Canagarajah, J. Wilsbacher, M. Robinson, M. Atkinson *et al.*, 1998 Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell **93:** 605–615.

Kolodkin, A. L., A. T. Pickup, D. M. Lin, C. S. Goodman and V. Banjeree, 1994 Characterization of Star and its interactions with Sevenless and Egf receptor during photoreceptor development in Drosophila. Development **120:** 1731–1745.

Kramer, S., M. Okabe, N. Hacohen, M. A. Krasnow and Y. Hiromi, 1999 Sprouty: a common antagonist of FGF and EGF signaling pathways in Drosophila. Development **126:** 2515–2525.

Lai, Z.-C., and G. M. Rubin, 1992 Negative control of photoreceptor development in Drosophila by the product of the *yan* gene, an ETS domain protein. Cell **70:** 609–620.

Lu, X., and Y. Li, 1999 Drosophila Src42A is a negative regulator of RTK signaling. Dev. Biol. **208:** 233–243.

Lu, X., T. B. Chou, N. G. Williams, T. Roberts and N. Perrimon, 1993 Control of cell fate determination by p21ras/Ras1, an essential component of torso signaling in Drosophila. Genes Dev. **7:** 621–632.

Metzger, R. J., and M. A. Krasnow, 1999 Genetic control of branching morphogenesis. Science **284:** 1635–1639.

Michelson, A. M., 1994 Muscle pattern diversification in *Drosophila* is determined by the autonomous function of homeotic genes in the embryonic mesoderm. Development **120:** 755–768.

Michelson, A. M., S. Gisselbrecht, E. Buff and J. B. Skeath, 1998 Heartbroken is a specific downstream mediator of FGF receptor signalling in Drosophila. Development **125:** 4379–4389.

Montagne, J., J. Groppe, K. Guillemin, M. A. Krasnow, W. J. Gehring *et al.*, 1996 The Drosophila serum response factor gene is required for the formation of intervein tissue of the wing and is allelic to *blistered*. Development **122:** 2589–2597.

Oliver, J. P., T. Raabe, M. Henkemyer, B. Dickson, G. Mbamula *et al.*, 1993 Biochemical and genetic analysis of Drk SH2/SH3 adapter protein implicated in coupling the sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, Sos. Cell **73:** 179–191.

Perkins, L. A., I. Larsen and N. Perrimon, 1992 *corkscrew* encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase *torso*. Cell **70:** 225–236.

Perkins, L. A., M. R. Johnson, M. B. Melnick and N. Perrimon, 1996 The nonreceptor protein tyrosine phosphatase corkscrew functions in multiple receptor tyrosine kinase pathways in Drosophila. Dev. Biol. **180:** 63–81.

Perrimon, N., and L. A. Perkins, 1997 There must be 50 ways to rule the signal: the case of the Drosophila EGF receptor. Cell **89:** 13–16.

Perrimon, N., X. Lu, X. S. Hou, J. C. Hsu, M. B. Melnick *et al.*, 1995 Dissection of the torso signal transduction pathway in Drosophila. Mol. Reprod. Dev. **42:** 515–522.

Pignoni, F., R. M. Baldarelli, E. Steingrimsson, R. J. Dias, A. Patapoutian *et al.*, 1990 The Drosophila gene *tailess* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. Cell **62:** 151–163.

Pignoni, F., E. Steingrimsson and J. A. Lengyel, 1992 *bicoid* and the terminal system activate *tailess* expression in the early Drosophila embryo. Development **115:** 239–251.

Price, J. V., E. D. Savenye, D. Lum and A. Breitkreutz, 1997 Dominant enhancers of the Egfr in *Drosophila melanogaster*: genetic links between the Notch and Egfr signaling pathways. Genetics **147:** 1139–1153.

Raabe, T., J. Riesgo-Escovar, X. Liu, B. S. Bausenwein, P. Deak *et al.*, 1996 DOS, a novel pleckstrin homology domain-containing protein required for signal transduction between Sevenless and Ras1 in Drosophila. Cell **85:** 911–920.

Roch, F., A. Baonza, E. Martín-Blanco and A. García-Bellido, 1998 Genetic interactions and cell behaviour in blistered mutants during proliferation and differentiation of the Drosophila wing. Development **125:** 1823–1832.

Samakovlis, C., N. Hacohen, G. Manning, D. C. Sutherland, K. Guillemin *et al.*, 1996 Development of the Drosophila tracheal system occurs by a series of morphologically distinct but genetically coupled branching events. Development **122:** 1395–1407.

Sawamoto, K., H. Okano, Y. Kobayakawa, S. Hayashi, K. Mikoshiba *et al.*, 1994 The function of argos in regulating cell fate decisions during Drosophila eye and wing vein development. Dev. Biol. **164:** 267–276.

Schweitzer, R., and B.-Z. Shilo, 1997 A thousand and one roles for the Drosophila EGF receptor. Trends Genet. **13:** 191–196.

Sigal, I. S., J. B. Gibbs, J. S. D'Alonzo, G. L. Temeles and B. S. Wolanski, 1986 Mutant ras-encoded proteins with altered nucleotide binding exert dominant biological effects. Proc. Natl. Acad. Sci. USA **83:** 952–956.

Simcox, A. A., G. Grumbling, B. Schnepp, C. Bennington-Mathias, E. Hersperger *et al.*, 1996 Molecular, phenotypic, and expression analysis of vein, a gene required for growth of the Drosophila wing disc. Dev. Biol. **177:** 475–489.

Simon, M. A., D. D. L. Bowtell, G. S. Dodson, T. R. Laverty and G. M. Rubin, 1991 Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. Cell **67:** 701–716.

Simon, M. A., G. S. Dodson and G. M. Rubin, 1993 An SH3-SH2-SH3 protein is required for p21Ras1 activation and binds to sevenless and SOS proteins in vitro. Cell **73:** 169–177.

Takahashi, F., S. Endo, T. Kojima and K. Saigo, 1996 Regulation of cell-cell contacts in developing Drosophila eyes by Dsrc41, a new, close relative of vertebrate c-Src. Genes Dev. **10:** 1645–1656.

Tautz, D., and C. Pfeifle, 1989 A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. Chromosoma **98:** 81–85.

Tomlinson, A., and D. F. Ready, 1987 Cell fate in the Drosophila ommatidium. Dev. Biol. **123:** 264–275.

van Buskirk, C., and T. Schüpbach, 1999 Versatility in signalling: multiple responses to EGF receptor activation during Drosophila oogenesis. Trends Cell Biol. **9:** 1–4.

Van Der Geer, P., T. Hunter and R. A. Lindberg, 1994 Receptor protein-tyrosine kinases and their signal transduction pathways. Annu. Rev. Cell Biol. **10:** 251–337.

Van Der Meer, J., 1977 Optical clean and permanent whole mount preparation for phase-contrast microscopy of cuticular structures of insect larvae. Dros. Inf. Serv. **52:** 160.

Van Vactor, D., A. M. O'Reilly and B. G. Neel, 1998 Genetic analysis of protein tyrosine phosphatases. Curr. Opin. Genet. Dev. **8:** 112–126.

Weigel, D., G. Jurgens, M. Klingler and H. Jäckle, 1990 Two gap genes mediate maternal terminal pattern information in Drosophila. Science **248:** 495–498.

Yarnitzky, T., and T. Volk, 1995 Laminin is required for heart, somatic muscles and gut development in the Drosophila embryo. Dev. Biol. **169:** 609–618.

Communicating editor: T. Schüpbach