Development 122, 1137-1146 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV8311

The SH2-containing tyrosine phosphatase corkscrew is required during signaling by sevenless, Ras1 and Raf

John D. Allard[1], Henry C. Chang[2], Ronald Herbst[1], Helen McNeill[1] and Michael A. Simon[1,*]

[1]Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA
[2]Howard Hughes Medical Institute and the Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

*Author for correspondence (e-mail: msimon@leland.stanford.edu)

SUMMARY

The *sevenless* gene encodes a receptor tyrosine kinase which is required for the development of the R7 photoreceptor cell in each ommatidium of the *Drosophila* eye. We have previously used a sensitized genetic screen to identify mutations, designated *Enhancers of sevenless* (*E(sev)*), which affect genes that encode components of the sevenless signaling pathway. Here, we report that one of these mutations, *E(sev)1A*^{e0P} is a dominantly inhibiting allele of *corkscrew*, which encodes an SH2 domain-containing protein tyrosine phosphatase (Perkins et al., 1992). We show that *corkscrew* function is essential for sevenless signaling and that expression of a membrane-targeted form of corkscrew can drive R7 photoreceptor development in the absence of *sevenless* function. Furthermore, we have used the dominantly inhibiting *corkscrew* allele to examine the role of corkscrew during signaling by activated forms of Ras1 and Raf. Our analysis indicates that *corkscrew* function is still required during signaling by activated Ras1 and Raf proteins. These results define a function for corkscrew that is either downstream of Ras1 activation or in a parallel pathway that acts with activated Ras1/Raf to specify R7 photoreceptor development.

Key words: *corkscrew*, *sevenless*, *Drosophila*, ommatidium, cell signaling

INTRODUCTION

During the development of multicellular organisms, groups of undifferentiated cells become organized into complex tissues. Coordination of the differentiation decisions of adjacent cells within a tissue is essential in order to ensure that every cell is surrounded by appropriate neighbors. One mechanism for achieving this coordination is for each cell to link its differentiation decision to the reception of instructive signals from nearby cells. An important class of receptors for such signals is the family of receptor tyrosine kinases (RTKs) (reviewed by van der Geer et al., 1994). These transmembrane molecules serve to transduce signals, in the form of extracellular ligands, into changes in intracellular regulatory pathways that can ultimately regulate cell fate decisions. One example of how a RTK can be used to mediate a developmentally instructive cell-cell interaction is the role of the *sevenless* RTK (sev) during R7 photoreceptor development in *Drosophila* (reviewed by Zipursky and Rubin, 1994; Wolff and Ready, 1995). The R7 photoreceptor is the last of eight photoreceptors to be recruited into each of the approximately 800 ommatidial clusters that constitute each compound eye. The decision of the R7 precursor cell to differentiate as a photoreceptor is absolutely dependent upon sev activation after binding to its ligand, the *bride of sevenless* protein (boss), on the surface of the adjacent R8 cell. In the absence of either sev or boss function, the R7 cell precursor fails to become a photoreceptor and instead differentiates as a non-neuronal cone cell.

Genetic screens for mutations which affect the ability of sev to specify photoreceptor development have identified many of the genes that encode components of the sev-mediated signaling pathway (Simon et al., 1991; Rogge et al., 1991; Olivier et al., 1993). Subsequent analysis of the role of the products of these genes has indicated that the early steps in sev signaling are similar to those used by RTKs in other eukaryotes (reviewed by Zipursky and Rubin, 1994; Schlessinger, 1994). A key early event is the transition of the *Ras1* protein from its inactive GDP-bound form to its active GTP-bound form. This transition is achieved by the action of the products of *Son of sevenless* and *downstream of receptor kinases* genes. *Son of sevenless* (*Sos*) encodes a guanine nucleotide exchange factor which can catalyze the activation of Ras1. *downstream of receptor kinases* (*drk*), the *Drosophila* homolog of the mammalian *Grb2* gene, encodes a protein that consists of an amino-terminal SH3 domain which binds to proline-rich sequences within Sos, a central SH2 domain which binds to specific phosphorylated tyrosine residues present in activated forms of RTKs such as sev, and a carboxy-terminal SH3 domain whose role is not fully understood (Raabe et al., 1995). Binding of boss by sev thus leads to sev autophosphorylation and the subsequent association of drk:Sos with the phosphorylated cytoplasmic domain of sev. The formation of this

signaling complex is proposed to then lead to the accumulation of Ras1:GTP by increasing the activity and concentration of Sos on the inner face of the plasma membrane where Ras1 resides. Once Ras1 is activated, a protein kinase cascade is initiated that is similar to those found in other eukaryotic cells and includes the sequential activation of raf, MEK, and MAPK. Among the important consequences of this kinase cascade in the R7 cell is the regulation of two ets domain transcription factors, yan and pointed. pointed is a positive regulator of photoreceptor development whose activity is stimulated by Ras1 activation, while yan is a negative regulator whose activity is inhibited by Ras1 activation (O'Neill et al., 1994; Brunner et al., 1994a; Rebay and Rubin, 1995).

An unresolved question about signal transduction within the R7 cell is whether all the effects of sev activation are mediated through the activation of Ras1. Previous studies have indicated that the expression of a constitutively activated Ras1, Raf or MAPK can bypass the requirement for sev activation in the R7 precursor cell (Fortini et al., 1992; Dickson et al., 1992; Brunner et al., 1994b). These results indicate that strong activation of the Ras1 pathway can overcome the need for any other pathways to be directly regulated by sev. However, it remains possible that sev normally controls other signaling pathways that are required when Ras1 is activated only to normal physiological levels. If such parallel pathways exist, mutations that affect components of these pathways might have been isolated in the same genetic screen that yielded *Ras1*, *Sos* and *drk* mutations (Simon et al., 1991). We have investigated this possibility by characterizing the *E(sev)1A^{e0P}* mutation. Here, we report that *E(sev)1A^{e0P}* is a dominantly inhibiting allele of a previously identified gene, *corkscrew* (*csw*), which encodes an SH2 domain-containing phosphotyrosine phosphatase (PTP; Perkins et al., 1992). We demonstrate that csw function is essential for R7 photoreceptor development and that the expression of a membrane-bound form of csw can bypass the requirement for sev activity during R7 differentiation. In addition, we have used these dominant alleles of *csw* to perform epistasis experiments that strongly suggest that csw acts either downstream of Ras1 or in a parallel pathway that acts in conjuction with the Ras1 pathway.

MATERIALS AND METHODS

Genetics

Fly culture and crosses were performed using standard procedures. The *csw^{C114}* and *csw$^{13\text{-}87}$* alleles are each genetic nulls (Perkins et al., 1992). Marked clones of cells homozygous for the *csw$^{13\text{-}87}$* and *csw^{C114}* mutations were induced by X-ray irradiation of *w, csw*/+ 1st instar larvae as previously described (Simon et al., 1991). To test whether the P[*sevhs-csw*] element could rescue the lethality of either the *csw$^{13\text{-}87}$* or *csw$^{E(sev)1A}$* mutations, either *csw$^{13\text{-}87}$*, *w^{1118}/FM7c* or *y, csw$^{E(sev)1A}$, w^{1118}, sev^{d2}, f, car/FM7c* females were crossed to *w^{1118}*/Y; P[*sevhs-csw*] males. Embryos were heat-shocked daily for 1 hour at 37°C until eclosion and the progeny were scored for the appearance of *csw$^{13\text{-}87}$* or *csw$^{E(sev)1A}$* hemizygous males. The ability of the P[*sevhs-csw^{G547E}*] element to rescue the lethality of *csw$^{13\text{-}87}$* was tested in a similar manner.

Histology

Fixation and sectioning (0.5-2 μm) of adult eyes was performed as described by Tomlinson and Ready (1987). Scanning electron microscopy was performed as described by Kimmel et al. (1990). Antibody staining of eye imaginal discs was essentially as described previously (Gaul et al., 1992). The primary antibodies were either a rat monoclonal against the elav protein (provided by G. Rubin) or mAb BP104 (Hortsch et al., 1990). The primary antibody was detected with Cy5-conjugated anti-rat antibodies (Jackson Immunoresearch) that were diluted 1/200. The discs were then washed and mounted in Citifluor (Ted Pella) and observed with a Biorad MRC-1000 confocal microscope. Staining of cultured cells expressing csw was as described previously except that Cy5-conjugated anti-rat antibodies were used (Simon et al., 1989). The anti-csw antibodies were generated by injecting rats with a glutathione-S-transferase/csw fusion protein that contained the final 160 amino acids of csw (Guan and Dixon, 1991).

Sequencing

The majority of the *csw* coding region of the *E(sev)1A^{e0P}* allele was cloned from *E(sev)1A^{e0P}/FM7c* flies as an 18 kb *Bgl*II fragment into the λFix2 vector (Stratagene). The area containing the coding sequences was subcloned into pBluescript. A nested deletion series for sequencing was constructed using the Erase-a-Base Kit (Promega). Sequencing was performed using Sequenase v.2.0 (US Biochemicals).

Construction of *csw* mutations

Mutations in *csw* were generated by oligonucleotide mutagenesis using the *dut ung* method (Kunkel, 1985) and reagents and protocols in the Mutagene kit (Bio-Rad). To construct the template for making the *csw^{G547E}* mutation, the 2.7 kb *Dra*I fragment containing the entire *csw* cDNA was isolated from plasmid Y1229 (Perkins et al., 1992) and ligated to *Kpn*I linkers. The *csw* cDNA fragment was digested with *Kpn*I and cloned into the *Kpn*I site of pBluescript KS$^+$, creating plasmid pJA-csw. The mutagenic primer was 5′-GGCGGGCACTTCGTGATCCGG-3′. The resulting mutagenized plasmid was designated pJA-csw^{G547E}. The csw^{src90} construct was made by using the polymerase chain reaction to amplify a DNA fragment containing a 5′ *Xba*I site, the coding region from the first 90 amino acids of Src1 (Simon et al., 1985), followed by the codons for amino acids 2-7 of csw. The oligonucleotides used were 5′-GCTCTAGAATGTCATCGCGAAGATGGTT-3′ and CGCACCA-CACCAACCGGTTCATCGCGAAGATGGTTC-3′. This fragment was then mixed with plasmid Y1119 and a second PCR reaction was performed using the same 5′ oligonucleotide and the oligonucleotide 5′-TCCTGTTGCAGCGATTCG-3′ from within the *csw* coding sequences. The amplification product was then cut with *Xba*I and *Eco*RI to yield a *src-csw* junction fragment. This fragment was then cloned into *Xba*I/*Eco*RI cut pBluescript into which the *csw* cDNA from Y1119 had been cloned in the orientation such that the polylinker *Xba*I site was placed at the 5′ end of the cDNA. This created pcsw^{src90}. A version of pcsw^{src90} which is mutated from glycine to alanine at codon 2 of the *Src1* sequences was created by site-specific mutagenesis using the oligonucleotide 5′-CATTTGTTGGCCATTCTAGAGG-3′. This plasmid was called pcsw^{src90G2A}.

P element transformation

To generate P[*sevhs-csw*] and P[*sevhs-csw^{G547E}*], *Kpn*I *csw* cDNA fragments were isolated from the pJA-csw and pJA-csw^{G547E} plasmids and ligated into the *Kpn*I site of *w$^+$* transformation vector pKB267. pKB267 was kindly supplied by K. Basler and E. Hafen and contains 2 tandem copies of the *sevenless* enhancer region and a single copy of the hsp70 promoter (Basler et al., 1989). To generate P[*sevhs-csw^{src90}*] and P[*sevhs-csw^{src90G2A}*], pcsw^{src90} and pcsw^{src90G2A} were first modified to place *Kpn*I sites at each end of the coding region and then cloned as *Kpn*I fragments into pKB267. Injection and transformation was as previously described (Rubin and Spradling, 1982).

Tissue culture

In order to examine the subcellular localization of csw and csw^{src90}, *Kpn*I fragments containing the entire coding sequence were cloned into the expression vector pAT-Hygro. This vector has a single *Kpn*I site which is downstream of a constitutively expressed Actin5C promoter and upstream of polyadenylation sequences from a *Drosophila* tubulin gene. In addition, this plasmid contains a copia LTR-driven gene that encodes resistance to the antibiotic hygromycin B. Full details of pAT-Hygro are available upon request. Transfection and growth of *Drosophila* SL2 cells was as described previously (Simon et al., 1989).

RESULTS

The *E(sev)1A^{e0P}* mutation affects csw function

The *E(sev)1A^{e0P}* mutation was identified in a genetic screen for mutations that attenuate signaling by sev (Simon et al., 1991). The strategy utilized a temperature-sensitive sev to provide barely sufficient activity to support R7 photoreceptor development. Dominant mutations, called *Enhancers of sevenless*, were then isolated that made this reduced level of sev activity inadequate and consequently yielded an R7-minus phenotype. Under such limiting conditions for sev function, heterozygous loss-of-function mutations in *Ras*, *Sos*, and *drk* can yield a dominant R7-minus phenotype by causing a two-fold reduction in the activity of their protein products (Simon et al., 1991, 1993). Meiotic mapping of the *E(sev)1A^{e0P}* mutation indicated a position approximately 1 map unit from the telomere of the X chromosome. During these mapping experiments we noticed that the chromosome carrying the *E(sev)1A^{e0P}* mutation also carried a recessive lethal mutation that failed to separate from the *E(sev)1A^{e0P}* mutation in over 100 meioses. Since the other characterized *E(sev)* loci encode products which are essential for viability, we assumed that the recessive lethal phenotype was caused by the same lesion as the *E(sev)* mutation. This region of the X chromosome was known to contain two essential genes, *Raf1* and *corkscrew*, that encode proteins which act in RTK-initiated signaling pathways (Ambrosio et al., 1989; Dickson et al., 1992; Perkins et al., 1992). We tested whether the *E(sev)1A^{e0P}* mutation affects either *Raf1* or *corkscrew* function by assaying its ability to complement the recessive lethality of *Raf1* and *corkscrew* alleles. The *E(sev)1A^{e0P}* chromosome fully complemented *Raf1* mutations, but was unable to complement either loss-of-function *corkscrew* alleles (*csw$^{13\text{-}87}$* and *csw^{C114}*) or a deletion (Df(1)JA52) that removes *corkscrew*. Less than one percent of the expected progeny carrying both *E(sev)1A^{e0P}* and a *corkscrew* mutation were observed and these surviving progeny were extremely short-lived and had grossly abnormal eyes.

The complementation and mapping results suggested that the recessive lethality associated with the chromosome carrying the *E(sev)1A^{e0P}* mutation was due to a mutation affecting *corkscrew*. This conclusion was supported further by experiments with flies carrying a P element which expresses a *corkscrew* cDNA under the control of a hybrid promoter consisting of the transcriptional enhancer elements of *sevenless* and a heat-shock promoter. This construct, called P[*sevhs-csw*], drives expression in a subset of cells in the developing eye including the R7 cell precursor as well as heat-shock inducible expression in all cells (Basler et al., 1991, 1989). We found that the expression of csw induced by daily 1 hour heat shocks allowed P[*sevhs-csw*] males hemizygous for the *E(sev)1A^{e0P}* mutation to survive to adulthood at high frequency (data not shown). Furthermore, these individuals did not show the eye defects noted above for females heterozygous for *corkscrew* alleles and the *E(sev)1A^{e0P}* mutation.

Table 1. Effects of *corkscrew* on *sevenless* function

Genotype	Fraction of R7$^+$ ommatidia
at 21.5°C	
sev^{d2}/*sev^{d2}*; TM3, P[*sev^{B4}*]/+	1.0
E(sev)1A^{e0P}, *sev^{d2}*/*sev^{d2}*; TM3, P[*sev^{B4}*]/+	0.06
E(sev)1A^{e0P}, *sev^{d2}*/*sev^{d2}*; P[*sevhs-csw*]/+; TM3, P[*sev^{B4}*]/+	0.75
at 23.3°C	
sev^{d2}/*sev^{d2}*; TM3, P[*sev^{B4}*]/+	0.95
E(sev)1A^{e0P}, *sev^{d2}*/*sev^{d2}*; TM3, P[*sev^{B4}*]/+	0.01
Df(1)JA52, *sev^{d2}*/*sev^{d2}*; TM3, P[*sev^{B4}*]/+	0.97
csw^{C114}, *sev^{d2}*/*sev^{d2}*; TM3, P[*sev^{B4}*]/+	0.95
csw$^{13\text{-}87}$, *sev^{d2}*/*sev^{d2}*; TM3, P[*sev^{B4}*]/+	0.93

The presence of the R7 cell was scored in sectioned eyes and is represented as the fraction of ommatidia that contained an R7 cell. In each case, greater than 350 ommatidia were scored. *sev^{d2}* is an amorphic allele for *sevenless*, while *sev^{B4}* is a temperature sensitive allele placed on the TM3 balancer chromosome by P element mediated transposition (Simon et al., 1991). The genotypes are indicated only with respect to the genetic markers that affect R7 development.

The complementation and rescue experiments demonstrated that the chromosome carrying the *E(sev)1A^{e0P}* mutation is defective for *corkscrew*. However, the possibility remained that the *E(sev)1A^{e0P}* mutant chromosome actually carried both a *corkscrew* mutation and a second mutation in a closely linked gene that was actually responsible for the effect on sev signaling. Since our previous studies had shown that the effect of the *E(sev)1A^{e0P}* mutation on signaling by sev is autonomous to the R7 cell, we determined whether expression of wild-type csw in the R7 cell precursor could suppress the attenuation of sev signaling caused by the *E(sev)1A^{e0P}* mutation (Simon et al., 1991). We found that the increased levels of wild-type csw, expressed in the *sevenless* transcriptional pattern strongly suppressed the effect of the *E(sev)1A^{e0P}* mutation on sev signaling (Table 1). This result indicates that the effect of the *E(sev)1A^{e0P}* mutation on sev signaling is due to a reduction of csw activity.

E(sev)1A^{e0P} produces an inhibitory form of csw

The results described above support the conclusion that the *E(sev)1A^{e0P}* mutation is a mutant allele of *corkscrew* which causes a reduction of *corkscrew* function. We next sought to determine whether the *E(sev)1A^{e0P}* allele merely lacks *corkscrew* function. If the *E(sev)1A^{e0P}* allele is nonfunctional, then other *corkscrew* null alleles should act to attenuate sev signaling in the same assay by which the *E(sev)1A^{e0P}* allele was identified. This prediction was tested by assaying the *csw^{C114}*, *csw$^{13\text{-}87}$* and Df(1)JA52 alleles for their ability to act as *Enhancers of sevenless*. None of these alleles had any significant effect on sev signaling. These results demonstrate that the *E(sev)1A^{e0P}* mutation is not simply a loss of function allele of *corkscrew* and instead imply that the *E(sev)1A^{e0P}* mutation is a dominant allele of *corkscrew* (Table 1). Taken together with the ability of increased expression of wild-type csw to

suppress the effect of the *E(sev)1A*^{e0P} mutation on sev signaling, these results support the hypothesis that the *E(sev)1A*^{e0P} allele produces a form of csw which both lacks function and inhibits the action of wild-type protein.

If csw function is required for R7 development and the *E(sev)1A*^{e0P} allele does encode a dominantly inhibiting form of csw, then expression of elevated levels of the *E(sev)1A*^{e0P}-encoded csw in the R7 precursor cell might be expected to produce a dominant R7-minus phenotype. In order to test this prediction, the mutagenic change in the *E(sev)1A*^{e0P} allele of *corkscrew* was identified. Comparison of *corkscrew* genomic DNA sequences isolated from the *E(sev)1A*^{e0P} chromosome with the previously reported *corkscrew* cDNA sequence revealed a single coding difference which changes the codon 547 from glycine (GGA) to glutamate (GAA) (Fig. 1). Flies were generated that carried a P element, P[*sevhs-csw*^{G547E}], in which a *csw* cDNA carrying the glu-547 mutation was placed under the control of the *sevenless*/heat shock expression cassette. The flies carrying P[*sevhs-csw*^{G547E}] exhibited a strong rough-eye phenotype that was never observed in P[*sevhs-csw*] flies (Fig. 2). Examination of sectioned eyes from the P[*sevhs-csw*^{G547E}] flies revealed that all of their ommatidia were malformed and contained fewer than the normal complement of eight photoreceptors. The most common arrangement was the presence of one photoreceptor with a small centrally located rhabdomere and four photoreceptors with large rhabdomeres. In wild-type ommatidia, there are two photoreceptors, R8 and R7, which produce small central rhabdomeres and 6 photoreceptors, R1-6, which produce large diameter outer rhabdomeres. Since the *sevenless* enhancer directs strong expression in the developing R3, R4 and R7 photoreceptors, we suspected that these were the missing photoreceptor cells in the ommatidia of P[*sevhs-csw*^{G547E}] flies. In order to explore this hypothesis further, we examined P[*sevhs-csw*^{G547E}] eye discs for expression of the neuron-specific elav protein (Fig. 3; Robinow and White, 1988). In a wild-type eye disc, staining for neural antigens such as elav is first detectable in photoreceptors R8, R2 and R5, then in R3 and R4. Several more cells are then added to the developing cluster and staining becomes apparent in R1 and R6, and then finally in R7 (Wolff and Ready, 1995). When eye discs from P[*sevhs-csw*^{G547E}] flies were examined, elav expression appeared to be normal at the stage when R8, R2 and R5 express elav. However, subsequent elav staining was abnormal. Staining of cells occuppying the normal position of R3 and R4 was only rarely observed. Elav expression was often observed in two cells that are properly placed to be the precursors of photoreceptors R1 and R6. No staining was observed in any cells occupying the R7 precursor position. While the identification of cells within abnormally developing ommatidia is difficult, these results are most consistent with the idea that the ommatidia of P[*sevhs-csw*^{G547E}] generally lack R3, R4 and R7 photoreceptors.

These results indicate that overexpression of csw^{G547E} can cause a dominant phenotype by inhibiting the ability of cells to differentiate as photoreceptors. However, these results did not definitively address whether the expression of csw^{G547E} causes this phenotype by inhibiting the action of wild-type csw rather than some other protein whose function is crucial to photoreceptor development. If csw^{G547E} does interfere with csw function only, then the P[*sevhs-csw*^{G547E}] phenotype should be suppressed when additional wild-type csw is present and enhanced when the level of wild-type csw is reduced. The effect of additional wild-type csw was examined by generating flies that carried both P[*sevhs-csw*] and P[*sevhs-csw*^{G547E}].

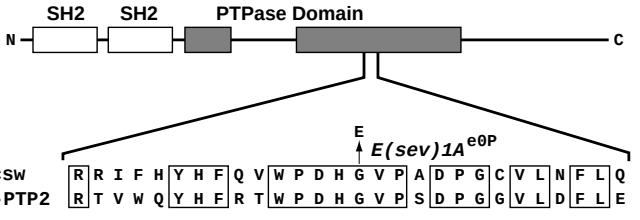

Fig. 1. The *E(sev)1A*^{e0P} mutation changes a single residue within the *corkscrew* PTP domain. The structure of the *corkscrew* protein is shown above (Perkins et al., 1992). Open rectangles represent SH2 domains, while the shaded rectangle represents the PTP domain. A comparison of amino acid sequences within the PTP domain of csw and its human homolog SH-PTP2 is shown below (Freeman et al., 1992; Vogel et al., 1993). Residues that are identical are boxed. The change (gly-547 to glu) which is present in the *E(sev)1A*^{e0P} allele of *corkscrew* is shown above the sequences.

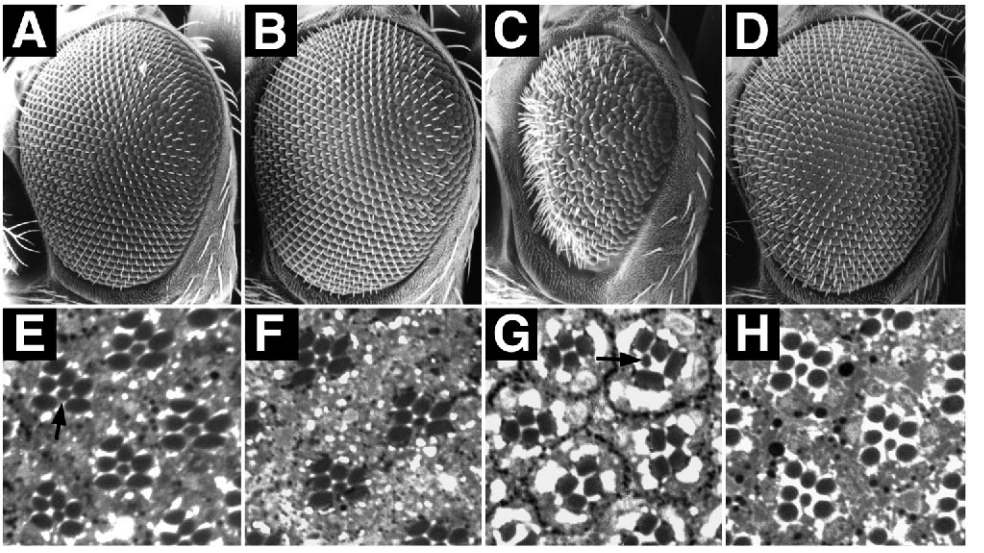

Fig. 2. Expression of *sevhs-csw*^{G547E} inhibits the function of wild-type csw. (A-D) Scanning electron micrographs of adult eyes. (E-H) Photomicrographs of apical sections of adult eyes. All of the flies were homozygous for the *w*^{1118} allele. The remainder of the genotypes were: (A,E) wild type; (B,F) P[*sevhs-csw*]/+; (C,G) P[*sevhs-csw*^{G547E}]/+; (D,H) P[*sevhs-csw*]/P[*sevhs-csw*^{G547E}]. The arrow in E points to the rhabdomere of an R7 cell. The arrow in G points to the rhabdomere of an R8 cell. R8 rhabdomeres normally are present in the basal portion of the retina beneath the R7 rhabdomere. However, when the R7 photoreceptor is absent, the R8 rhabdomere occasionally extends into the apical region.

The presence of P[*sevhs-csw*] strongly suppressed the P[*sevhs-csw^{G547E}*] phenotype (Fig. 2). The effect of decreasing the level of wild-type csw protein in flies was examined in P[*sevhs-csw^{G547E}*] flies that were also heterozygous for the loss of function *csw$^{13-87}$* allele. Heterozygosity for the *csw$^{13-87}$* allele led to an enhancement of the P[*sevhs-csw^{G547E}*] eye phenotype (data not shown). These results strongly support the idea that csw^{G547E} produced by the *E(sev)1A^{e0P}* allele of *corkscrew*, blocks photoreceptor and R7 development by antagonizing the function of wild-type csw. Consistent with this hypothesis, expression of additional drk in the R7 cell had no significant effect on the dominant phenotype induced by the presence of P[*sevhs-csw^{G547E}*] (data not shown). Furthermore, since *sevenless* transcription only slightly precedes the time that the R7 precursor cell commits to photoreceptor development in response to boss, the ability of *sevenless*-driven csw^{G547E} to block R7 development implies that csw must function during the same developmental period during which sev signals.

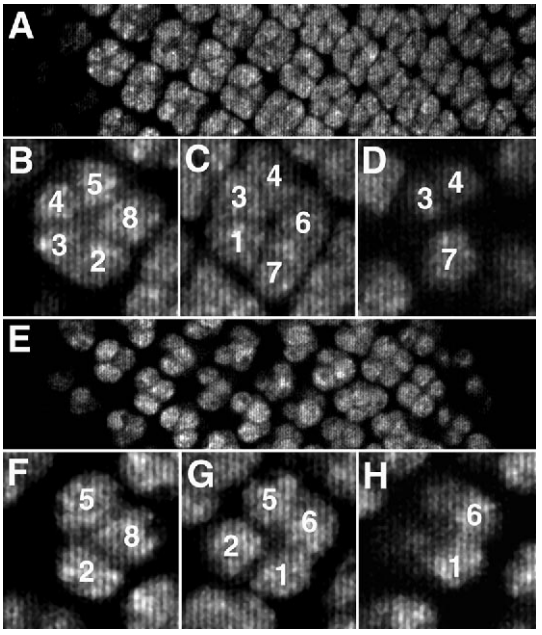

Fig. 3. Expression of csw^{G457E} blocks neural development of photoreceptor precursor cells. Each of the panels is a micrograph section of a confocal image of a third instar larval eye imaginal disc stained with anti-elav rat monoclonal antibody. elav protein is present in the nucleus of developing and mature neurons. Anterior is to the left. The morphogenetic furrow in A and E is near the left edge of the image. (A-D) Images of a *w^{1118}* eye disc. This disc shows the normal development of ommatidial clusters in which the order of differentiation is R8, R2 and R5, R3 and R4, R1 and R6, and finally R7. (B) The precluster stage when photoreceptors R8, R2, R5, R3 and R4 have commenced neural development. C and D show a later stage when R1, R6 and R7 have begun to express elav. D is a more apical section than C. In these images, R8, R2, and R5 are not visible because their nuclei have descended to a more basal position in the eye disc monolayer epithelium. (E-H) Images of a *w^{1118}*; P[*sevhs-csw^{G547E}*]/+ eye disc. F shows staining at the precluster stage. Normal staining is observed in R8, R2, and R5 cells but not in R3 or R4 precursors. G and H show a later stage. H is a more apical section than G. Staining is apparent in cells occupying the position of R1 and R6 cells, but no staining of a cell in the R7 position was detectable.

csw is required for the neuronal development of all photoreceptors

The ability of csw^{G547E} to prevent the presumptive R3 and R4 precursor cells from differentiating as photoreceptors suggested that csw might play an essential role in the commitment of all photoreceptor precursor cells to neuronal development. In order to examine the effect of removing all csw function from developing photoreceptors, X-ray induced mitotic recombination was used to generate marked clones of homozygous *csw^{C114}* or *csw$^{13-87}$* cells in heterozygous animals. The homozygous *corkscrew* cells were marked by the absence of *white* gene function and could therefore be recognized by the absence of pigment granules which are normally present in both photoreceptor and pigment cells. When clones of homozygous *corkscrew* cells were induced during the first larval instar period, well before any commitment of eye disc cells to any particular ommatidial cell fate has occurred, we found that cells which lacked csw function were only very rarely able to successfully differentiate as photoreceptors (Fig. 4A). This result suggests an autonomous requirement for csw function during the development of all photoreceptors including the R7 cell. In order to further examine the developmental defect of eye disc cells lacking csw function, we examined the expression of neuron-specific antigens in third larval instar eye imaginal discs from *csw$^{13-87}$* males before their death at the onset of pupation. The *csw$^{13-87}$* animals showed extremely abnormal photoreceptor development in which only isolated cells commenced photoreceptor development as assayed by staining with the neuronal-specific monoclonal antibody mAb BP104 (Fig. 4B; Hortsch et al., 1990). We could not follow the fate of the mAb BP104 staining cells further due to the pupal death of *csw$^{13-87}$* animals, but the absence of mAb BP104 staining cells at the posterior margin of the *csw$^{13-87}$* eye discs suggests that these cells may either die or be unable to maintain their neuronal fate in the absence of csw function.

Membrane localized csw can bypass sev activation

The experiments described above indicated that csw function is required both early in ommatidial development and at the time that the R7 precursor cell commits to photoreceptor development. However, these results do not fully address whether csw is actively involved in signaling by sev instead of being a component of another pathway that is merely permissive for R7 photoreceptor differentiation. One way to distinguish between these possibilities would be to determine whether a constitutively signaling version of csw could bypass the requirement for sev activity in the R7 precursor cell. Previous studies have demonstrated that the expression under *sevenless* transcriptional control of constitutively signaling forms of other components of the sev signaling pathway such as Ras1 (Ras1^{V12}) and Raf (Raf^{tor4021}) can bypass the requirement for sev function in the R7 precursor cell (Fortini et al., 1992; Dickson et al., 1992). In addition, these activated proteins can also induce cone cell precursors, which strongly express sev, to differentiate as R7 cells.

Our approach for generating a constitutively signaling form of csw was based on previous studies of the action of csw homologs in mammalian cells. Since these closely related proteins can bind to activated RTKs, we suspected that one possible mechanism of csw regulation might be translocation of csw to the plasma

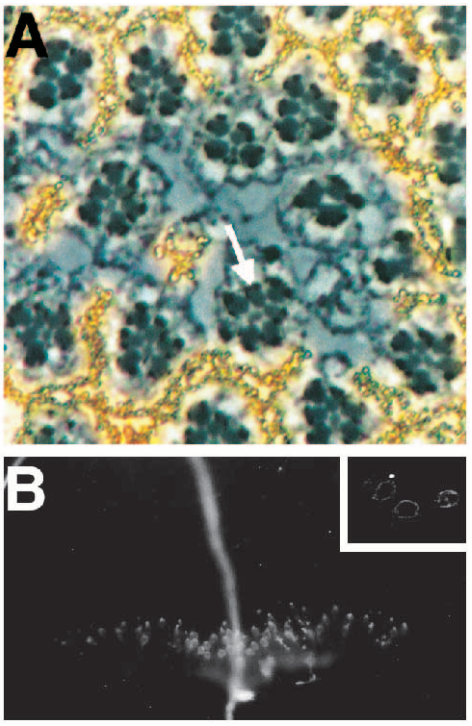

Fig. 4. csw is required in all photoreceptor cells. (A) A photomicrograph of a section of a *csw*^{C114}, *w*^{1118}/+ fly in which a clone of cells that is homozygous for both the *w*^{1118} and the *csw*^{C114} mutations has been generated by mitotic recombination. The homozygous cells can be identified by the lack of pigment granules indicating the absence of a functional *white* gene. The pigment granules of the photoreceptors are visible as small dark structures at the base of each rhabdomere (see arrow). The pigment granules within the pigment cells, surrounding each ommatidial cluster, are orange. If csw function was not required during photoreceptor development, many unpigmented photoreceptors would be observed. Instead, very few photoreceptors homozygous for the *csw*^{C114} allele were observed suggesting that csw is autonomously required for the development of each class of photoreceptors. We were unable to unambiguously establish the identity of these rare *csw* null photoreceptor cells (9/945 in 135 mosaic ommatidia) since they were located in highly disorganized ommatidial clusters. (B) A third instar eye imaginal disc of a *csw*$^{13\text{-}87}$, *w*^{1118}/Y male that has been stained with monoclonal antibody mAb BP104 which stains the plasma membranes of neurons. Ommatidial spacing is abnormal and each cluster appears to contain only a single cell that has commenced neuronal differentiation (see insert).

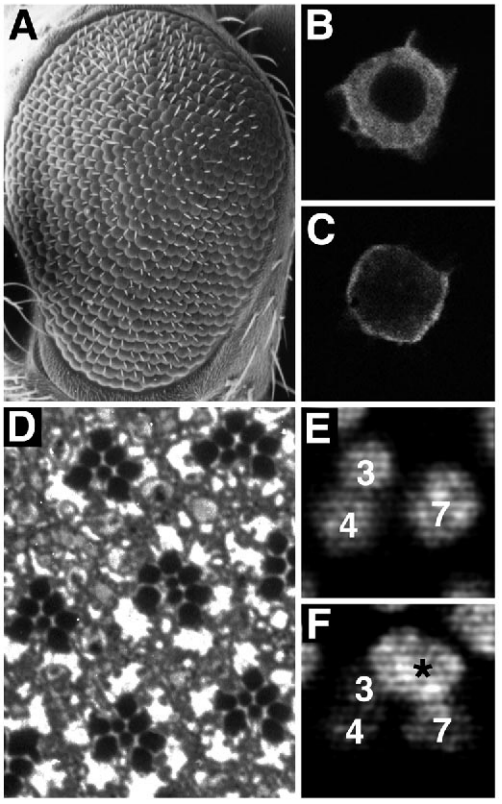

Fig. 5. Expression of a plasma membrane localized csw can induce R7 development. (A) A scanning electron micrograph of an eye from a *w*^{1118}/Y; P[*sevhs-csw*^{src90}] fly. The eye is slightly roughened compared to wild type (compare to Fig. 2A). (B,C) Confocal images of *Drosophila* tissue culture cells (SL2 line) transfected with constructs that express either (B) wild-type csw or (C) csw^{src90} and stained with rat antiserum raised against a fusion protein containing the carboxy-terminal 160 amino acids of csw. The location of wild-type csw is cytoplasmic while csw^{src90} is found at the plasma membrane. (D) A photomicrograph of an apical section of an eye from a *w*^{1118}/Y; P[*sevhs-csw*^{src90}]/+ fly. The extra R7-type cells can be seen by the presence of an additional small central rhabdomere. (E,F) Confocal images of anti-elav staining of *w*^{1118} and *w*^{1118}/Y; P[*sevhs-csw*^{src90}]/+ eye discs, respectively. In F, an additional nucleus contains the elav protein. This nucleus lies in the position of the nucleus of the anterior cone cell (*).

membrane after binding to activated sev (Vogel et al., 1993; Feng and Pawson, 1994; Lechleider et al., 1993b). In order to mimic this putative step in signaling, we constructed a cDNA that encodes a csw protein (csw^{src90}) in which the first 90 amino acids of the *Drosophila Src1* protein were fused to the amino terminus of csw (Simon et al., 1985). This region of Src1 contains the sequences neccesary for the myristylation and plasma membrane localization of the *Src1* protein. When expressed in cultured *Drosophila* cells, csw^{src90} was directed to the plasma membrane rather than the cytoplasm (Fig. 5B,C). Flies were then generated that carried a P element, P[*sevhs-csw*^{src90}] in which csw^{src90} was expressed under the control of the *sevenless*/heat shock expression cassette. In addition, flies were also generated that carried an equivalent P element, P[*sevhs-csw*^{src90G2A}], in which the myristylation sequence of Src1 was disrupted by changing the second codon from glycine to alanine. The P[*sevhs-csw*^{src90}] flies exhibited mildly roughened eyes while the P[*sevhs-csw*^{src90G2A}] flies did not (Fig. 5A and data not shown). Analysis of the P[*sevhs-csw*^{src90}] eyes showed that the roughness was due to the recruitment of cone cell precursors to the R7 fate (Fig. 5D-F). In addition, P[*sevhs-csw*^{src90}] was also able to induce R7 development in ommatidia that entirely lacked sev function (Fig. 6E). These phenotypes are very similar, though not quite as severe, as those caused by the expression of activated sev, Ras1 or Raf under the same transcriptional control. This similarity of phenotypes strongly supports the placement of csw directly in the sev signaling pathway rather than in a permissive pathway for R7 development.

Epastasis of csw, Ras1 and Raf

We next sought to place the action of csw during sev signaling

by examining both the effect of removing csw function during signaling by activated Ras1 and Raf and the effect of removing Ras1 function during signaling by csw^{src90}. Since *csw* and *Ras1* mutations each affect ommatidial development prior to sev action, we relied on the expression under the control of the *sevenless*/heat shock promoter cassette of dominantly inhibiting alleles to limit csw and Ras1 function in the R7 and cone cell precursors specifically at the stage when sev normally can function. In addition, we performed these studies in flies that lack all normal sev function in order to eliminate the possibility that any observed changes in the efficiency of R7 induction might be caused by inhibiting sev signaling rather than signaling by the particular activated protein. The inhibiting *Ras1* allele that we used had a single coding change that changed amino acid 17 from serine to asparagine. *ras* proteins with this change have been shown to act as strong inhibitors of *ras* function in mammalian cells (Feig and Cooper, 1988; Farnsworth and Feig, 1991). The ability of Ras1^{N17} to interfere with wild-type Ras1 action was verified by the observation that sixty percent of the ommatidia of flies carrying P[*sevhs-Ras1^{N17}*] lacked an R7 cell. In addition many ommatidia of P[*sevhs-Ras1^{N17}*] flies also lacked one or both of photoreceptors R3 and R4 (data not shown).

In order to determine whether Ras1 activity is needed during signaling by csw^{src90}, we compared the number of R7 cells per ommatidia in the eyes of *sev^{d2}*; P[*sevhs-csw^{src90}*] flies to that of eyes from *sev^{d2}*; P[*sevhs-csw^{src90}*] flies which also carried P[*sevhs-Ras1^{N17}*] (Fig. 6E,F). The presence of the inhibiting Ras1 protein completely blocked the ability of csw^{src90} to induce R7 development thus demonstrating that Ras1 activity is required during signaling by csw^{src90}. Similarly, we compared the number of R7 cells per ommatidium in the eyes of *sev^{d2}* flies that carried either P[*sev-Ras1^{V12}*] or P[*sevhs-Raf^{tor4021}*] flies to that of eyes from flies which also carried P[*sevhs-csw^{G547E}*] (Fig. 6A-D). The presence of csw^{G547E} strongly suppressed the induction of R7 cells by each of the activated proteins. The suppression was strongest for signaling by Raf^{tor4021}. In this case, R7-type cells were only rarely observed despite the normal ability of Raf^{tor4021} to efficiently induce R7 development. In addition, activated Raf did not effectively bypass the block on R3 and R4 photoreceptor development caused by the presence of csw^{G547E} in these cells. The effect of csw^{G547E} on signaling by Ras1^{V12} was slightly less extreme but still significant. In this case, the presence of P[*sevhs-csw^{G547E}*] resulted in a reduction in the average number of R7-type cells in each ommatidium from 2.3 to 1.0. Together, these results show that csw^{G547E} can block signaling by activated Ras1 and Raf. Since csw^{G547E} acts by limiting the ability of wild-type csw to signal, this result implies that csw function is required either downstream of Ras1/Raf activation or else in another pathway that acts in conjunction with the signaling cascade downstream of Ras1/Raf activation. A possible explanation for the milder effect of csw^{G547E} expression on the Ras1^{V12} phenotype than on the Raf^{tor4021} phenotype may be the difference in the routes by which the activated proteins reach the plasma membrane. Raf^{tor4021} is secreted to the plasma membrane while Ras1^{V12} is targeted to the plasma membrane by fatty acylation in the cytoplasm. Since the activated Ras1 and Raf proteins and csw^{G547E} are expressed simultaneously, the delay in appearance of Raf^{tor4021} at the plasma membrane caused by transit through the Golgi apparatus may allow csw^{G547E} to accumulate to higher levels before signaling by Raf^{tor4021} commences and therefore be a more effective antagonist.

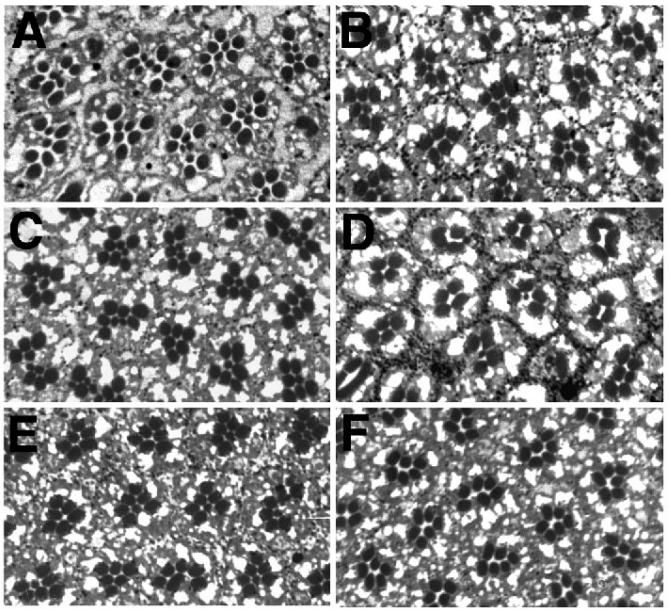

Fig. 6. Epastasis of csw, Ras1 and Raf during R7 development. Each panel is a photomicrograph of an apical section of an adult fly eye. The genotypes are: (A) *w^{1118}*, *sev^{d2}*/Y; P[*ry^{+}*, *sev-Ras1*^{V12}]2/+, (B) *w^{1118}*, *sev^{d2}*/Y; P[*sevhs-csw^{G547E}*]/+; P[*ry^{+}*, *sev-Ras1*^{V12}]2/+, (C) *w^{1118}*, *sev^{d2}*/Y; *SE-Raf^{tor4021}*/+, (D) *w^{1118}*, *sev^{d2}*/Y; *SE-Raf^{tor4021}*/+; P[*sevhs-csw^{G547E}*]/+, (E) *w^{1118}*, *sev^{d2}*/Y; P[*sevhs-csw^{src90}*]/+, and (F) *w^{1118}*, *sev^{d2}*/Y; P[*sevhs-csw^{src90}*]/P[*sevhs-Ras1^{N17}*]. Ras1^{V12} (A), and Raf^{tor4021} are able to efficiently induce cells to differentiate as R7-type photoreceptors. In each case, the coexpression of csw^{G547E} reduces the number of R7-type cells that are produced (B,D). csw^{src90} can also induce R7 development (E), but this ability is blocked by the expression of Ras1^{N17} (F).

DISCUSSION

Genetic screens for mutations which disrupt R7 photoreceptor development have identified several genes whose products are components of the sev signal transduction pathway (Simon et al., 1991; Rogge et al., 1991; Olivier et al., 1993). In this study, we have sought to identify an additional component of the sev signaling pathway by characterizing the product of one of these genes, *E(sev)1A*. We have shown that the *E(sev)1A^{e0P}* mutation is a dominantly inhibiting allele of *corkscrew*. Previous studies have shown that *corkscrew* encodes a PTP that contains two amino-terminal SH2 domains (Perkins et al., 1992). Our analysis indicates that the *E(sev)1A^{e0P}* allele of *corkscrew* changes a single residue within the PTP catalytic domain. Two lines of evidence indicate that the product of the *E(sev)1A^{e0P}* allele of *csw*, *csw^{G547E}*, has its effects on photoreceptor development by interfering with the function of wild-type csw. First, the effects caused by the *E(sev)1A^{e0P}* allele on sev signaling in the sev^{ts} assay can be strongly suppressed by the expression of additional wild-type csw protein in the R7 cell. Second, the effects of overexpressing csw^{G547E} can be entirely suppressed by overexpression of wild-type csw and significantly enhanced

by the inactivation of one of the normal chromosomal alleles of *csw*. While these results cannot entirely eliminate the possibility that a small portion of csw^{G547E} action is due to a poisoning effect on the ability of some other component of the sev signaling pathway to signal, they strongly suggest that the major effect of csw^{G547E} is to block wild-type csw function.

csw is a component of the sev signaling pathway

The ability of a mutation which limits csw function to attenuate sev^{ts} signaling indicates that csw has an important role during R7 development. Several lines of evidence suggest that csw performs this role by directly participating in the sev signaling pathway. First, our previous analysis of genetic mosaics for the *E(sev)1A^{e0P}* mutation demonstrated that the effect of the *E(sev)1A^{e0P}* mutation on sev^{ts} signaling is within the R7 cell itself rather than in any other cell within the developing ommatidium that might signal to the R7 precursor cell (Simon et al., 1991). The ability of *sevenless* enhancer-driven csw to suppress the effect of the *csw^{G547E}* mutation on sev signaling indicates that csw activity is crucial during the period when sev normally signals. Furthermore, the severe reduction of effective csw function caused by overexpression of csw^{G547E} in the R7 precursor cell yields a phenotype that is consistent with the R7 precursor cell defect in *sev* flies. In each case, the R7 precursor is unable to initiate neuronal development as assayed by the induction of early neuron specific antigens (Tomlinson and Ready, 1987). Finally, the ability of csw^{src90} to drive R7 development in the absence of sev function suggests that csw, like Ras1 and Raf, can act downstream of sev in the R7 photoreceptor determination pathway.

Genetic placement of csw function during sev signaling

The characterization of signaling and inhibiting forms of csw has permitted genetic experiments that ask where csw acts in the sev signaling pathway. The crucial result is that the reduction of csw function caused by the expression of csw^{G547E} can markedly suppress the ability of activated Ras1 or Raf to induce R7 photoreceptor development. These results suggest that at least a portion of csw function must act either downstream of Ras1/Raf activation, in conjuction with activated Ras1/Raf, or in an activation pathway that these constitutively signaling proteins still require for full activity. Since ras^{V12} proteins have been extensively characterized and appear to be independent of upstream signaling events, we favor the placement of at least a portion of csw activity either downstream or in parallel to Ras1/Raf activation. However, it must be noted that these results do not argue against placement of some csw function between sev and Ras1 activation as has been proposed for a csw homolog during signaling by the PDGF receptor (Li et al., 1994; Bennett et al., 1994).

We favor the hypothesis that csw functions in parallel to Ras1/Raf activation during sev signaling for several reasons. The first is that reduction of Ras1 by expression of Ras1^{N17} blocks signaling by activated csw. This result suggests that csw function cannot be placed entirely after Ras1 activation. However, this result must be interpreted with caution because the mechanism of csw^{src90} signaling has not been extensively characterized. An additional reason for placing csw in parallel to rather than downstream of Ras1/Raf activation is that sev and csw are complexed together in *Drosophila* cells (R. Herbst, unpublished results). Previous studies have shown that the mammalian homolog of csw, SH-PTP2 (which is also known as PTP1D, Syp, PTP2C, and SHPTP3), physically interact with and are activated by RTKs (Freeman et al., 1992; Vogel et al., 1993; Ahmad et al., 1993; Lechleider et al., 1993a, b; Feng and Pawson, 1994). The ability of csw and sev to interact is consistent with the placement of csw action directly after sev in the pathway rather than downstream of Ras1/Raf activation.

Placement of csw action in parallel to Ras1/Raf activation would also explain why simple loss of function alleles of *csw* do not act as *Enhancers of sevenless* in the sev^{ts} assay. In the genetic screen for dominant *Enhancers of sevenless*, the level of signaling by the sev^{ts} protein was deliberately calibrated to a level where R7 development barely failed. This meant that the level of sev activation of at least one downstream signaling pathway was only barely adequate to support R7 development. The isolation of *drk*, *Sos*, and *Ras1* alleles in the screen indicates that the Ras1 pathway was the limiting pathway in these assays because halving the level of these activities strongly affected signaling. However, if a second cooperating pathway were actually required in conjuction with the Ras1 pathway to drive R7 differentiation, then that second pathway would not necessarily have been limited at the level of sev activity used for screening. Such a second cooperating pathway might only be discovered in the sev^{ts} genetic screen if its function were reduced by significantly more than fifty percent. Since mutations were screened in heterozygotes, such a pathway could only have been identified by a dominantly inhibiting allele. This is precisely what we have observed for *corkscrew*. Only a dominantly inhibiting allele can act as an *Enhancer of sevenless*, but complete inhibition of csw function abolishes signaling.

If csw does act in parallel to Ras1 during sev signaling, what could be the nature of its role? Any model must account for the ability of csw^{src90} and Ras1^{V12} to each drive R7 development in the absence of sev-induced activation of the other. An appealing possibility is that activation of csw directly regulates the ability of activated Ras1 and Raf to induce their downstream signaling pathway. For example, activation of csw might lead to inhibition of a component, perhaps a serine/threonine phosphatase, which normally counteracts the kinase cascade downstream of Ras1/Raf activation. In this model, the effect of csw activation would be to reduce the level of Ras1/Raf activity required for effective signaling. Thus, high levels of Ras1 activation might compensate for the lack of csw activity and high levels of csw activity might lower the threshold for Ras1 below the basal unstimulated level. Biochemical experiments which directly examine the extent of Raf, MEK, and MAPK activation in cells that express activated sev will be required to distinguish between these models. However, a number of studies of signaling by mammalian RTKs have indicated that inhibition of SH-PTP2 activity can affect the extent of MAPK activation in response to RTK activation (Li et al., 1994; Bennett et al., 1994; Milarski and Saltiel, 1994; Noguchi et al., 1994; Tang et al., 1995; Zhao et al., 1995).

Our results indicate that csw plays a positive role during signaling by sev. This positive function is consistent both with the role of csw during signaling by the torso RTK and with the role of SH-PTP2 during signaling by the insulin, PDGF and FGF receptors (Perkins et al., 1992; Milarski and Saltiel, 1994;

Xiao et al., 1994; Yamauchi et al., 1995; Noguchi et al., 1994; Tang et al., 1995; Rivard et al., 1995; Zhao et al., 1995). However, there are important distinctions between our data and previous work in these other signaling systems. We have demonstrated that signaling by activated Ras1 and Raf is still largely dependent on csw function. In contrast, earlier studies have not definitely placed any of the action of csw or SH-PTP2 downstream of ras action. For example, studies of csw function during torso signaling in the *Drosophila* egg have indicated the requirement for csw function can be bypassed by microinjection of activated mammalian H-ras (Lu et al., 1993). Experimental differences may account for some of the difference in the perceived role of csw in these two signaling pathways. For example, injection of the activated ras protein may have saturated the signaling response such that csw was no longer strongly required. Another possibility is that csw may actually perform a different function during these two developmental decisions. For example, the main function of csw in the torso pathway may be to bind to and become phosphorylated by activated torso and then act as a linker between torso and drk. This would be similar to the proposed role of SH-PTP2 during signaling by the PDGF receptor to which torso is closely related. We think that it is unlikely that csw performs a similar role during sev signaling because csw is unphosphorylated in *Drosophila* cells that abundantly express an activated form of sev (R. Herbst, unpublished results). Our experiments instead indicate a new function for csw that is distinct from catalyzing Ras1 activation and is still required in cells that express activated Ras1 or Raf. Biochemical analysis of both csw regulation and csw targets in each signaling system will be required in order to understand these differences.

We wish to thank members of our laboratory, G. Rubin and M. Cyert for their helpful suggestions regarding the manuscript. We also thank L. Perkins for helpful discussions and for providing the *csw* cDNA. We thank Josette Ungos and Fran Thomas for assistance with sequencing and scanning electron microscopy. The early stages of this work were conducted while M. A. S. was a postdoctoral fellow in the laboratory of G. Rubin. The contribution of H. C. to this work was conducted in the laboratory of G. Rubin, This work was supported in part by a National Institutes of Health grant (1RO1EY9845), a National Science Foundation Young Investigator Award (MCB-9357009), and a Searle Scholars Program/Chicago Community Trust Award to M. A. S. J. A. is supported by an American Cancer Society Postdoctoral Fellowship. M. A. S. is a Lucille P. Markey Scholar in Biomedical Sciences.

REFERENCES

Ahmad, S., Banville, D., Zhao, Z., Fischer, E. H. and Shen, S. H. (1993). A widely expressed human protein-tyrosine phosphatase containing src homology 2 domains. *Proc. Natl. Acad. Sci. USA* **90**, 2197-2201.

Ambrosio, L., Mahowald, A. P. and Perrimon, N. (1989). Requirement of the *Drosophila* raf homologue for torso function. *Nature* **342**, 288-291.

Basler, K., Siegrist, P. and Hafen, E. (1989). The spatial and temporal expression pattern of *sevenless* is exclusively controlled by gene-internal elements. *EMBO J.* **8**, 2381-2386.

Basler, K., Christen, B. and Hafen, E. (1991). Ligand-independent activation of the sevenless receptor tyrosine kinase changes the fate of cells in the developing *Drosophila* eye. *Cell* **64**, 1069-1081.

Bennett, A. M., Tang, T. L., Sugimoto, S., Walsh, C. T. and Neel, B. G. (1994). Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor beta to Ras. *Proc. Natl. Acad. Sci. USA* **91**, 7335-7339.

Brunner, D., Ducker, K., Oellers, N., Hafen, E., Scholz, H. and Klambt, C. (1994a). The ETS domain protein pointed-P2 is a target of MAP kinase in the sevenless signal transduction pathway. *Nature* **370**, 386-389.

Brunner, D., Oellers, N., Szabad, J., Biggs, W. H., Zipursky, S. L. and Hafen, E. (1994b). A gain of function mutation in *Drosophila* MAP kinase activates multiple receptor kinase signalling pathways. *Cell* **76**, 875-888.

Dickson, B., Sprenger, F., Morrison, D. and Hafen, E. (1992). Raf functions downstream of Ras1 in the Sevenless signal transduction pathway. *Nature* **360**, 600-603.

Farnsworth, C. L. and Feig, L. A. (1991). Dominant inhibitory mutations in the $Mg(^{2+})$-binding site of RasH prevent its activation by GTP. *Mol. Cell. Biol.* **11**, 4822-4829.

Feig, L. A. and Cooper, G. M. (1988). Inhibition of NIH 3T3 cell proliferation by a mutant ras protein with preferential affinity for GDP. *Mol. Cell. Biol.* **8**, 3235-3243.

Feng, G. S. and Pawson, T. (1994). Phosphotyrosine phosphatases with SH2 domains: regulators of signal transduction. *Trends. Genet.* **10**, 54-58.

Fortini, M. E., Simon, M. A. and Rubin, G. M. (1992). Signalling by the sevenless protein tyrosine kinase is mimicked by Ras1 activation. *Nature* **355**, 559-561.

Freeman, R. M., Jr., Plutzky, J. and Neel, B. G. (1992). Identification of a human src homology 2-containing protein-tyrosine-phosphatase: a putative homolog of *Drosophila* corkscrew. *Proc. Natl. Acad. Sci. USA* **89**, 11239-11243.

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

Guan, K. L. and Dixon, J. E. (1991). Eukaryotic proteins expressed in *Escherichia coli*: an improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. *Anal. Biochem.* **192**, 262-267.

Hortsch, M., Bieber, A. J., Patel, N. H. and Goodman, C. S. (1990). Differential splicing generates a nervous system-specific form of *Drosophila* neuroglian. *Neuron* **4**, 697-709.

Kimmel, B. E., Heberlein, U. and Rubin, G. M. (1990). The homeo domain protein rough is expressed in a subset of cells in the developing *Drosophila* eye where it can specify photoreceptor cell subtype. *Genes Dev.* **4**, 712-727.

Kunkel, T. A. (1985). Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA* **82**, 488-492.

Lechleider, R. J., Freeman, R. M.,Jr. and Neel, B. G. (1993a). Tyrosyl phosphorylation and growth factor receptor association of the human corkscrew homologue, SH-PTP2. *J. Biol. Chem.* **268**, 13434-13438.

Lechleider, R. J., Sugimoto, S., Bennett, A. M., Kashishian, A. S., Cooper, J. A., Shoelson, S. E., Walsh, C. T. and Neel, B. G. (1993b). Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor. *J. Biol. Chem.* **268**, 21478-21481.

Li, W., Nishimura, R., Kashishian, A., Batzer, A. G., Kim, W. J., Cooper, J. A. and Schlessinger, J. (1994). A new function for a phosphotyrosine phosphatase: linking GRB2-Sos to a receptor tyrosine kinase. *Mol. Cell Biol.* **14**, 509-517.

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

Milarski, K. L. and Saltiel, A. R. (1994). Expression of catalytically inactive Syp phosphatase in 3T3 cells blocks stimulation of mitogen-activated protein kinase by insulin. *J. Biol. Chem.* **269**, 21239-21243.

Noguchi, T., Matozaki, T., Horita, K., Fujioka, Y. and Kasuga, M. (1994). Role of SH-PTP2, a protein-tyrosine phosphatase with Src homology 2 domains, in insulin-stimulated Ras activation. *Mol. Cell Biol.* **14**, 6674-6682.

O'Neill, E. M., Rebay, I., Tjian, R. and Rubin, G. M. (1994). The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. *Cell* **78**, 137-147.

Olivier, J. P., Raabe, T., Henkemeyer, M., Dickson, B., Mbamalu, G., Margolis, B., Schlessinger, J., Hafen, E. and Pawson, T. (1993). A *Drosophila* SH2-SH3 adaptor protein implicated in coupling the sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, Sos. *Cell* **73**, 179-191.

Perkins, L. A., Larsen, I. and Perrimon, N. (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.

Raabe, T., Olivier, J. P., Dickson, B., Liu, X., Gish, G. D., Pawson, T. and Hafen, E. (1995). Biochemical and genetic analysis of the Drk SH2/SH3 adaptor protein of *Drosophila*. *EMBO J.* **14**, 2509-2518.

Rebay, I. and Rubin, G. M. (1995). Yan functions as a general inhibitor of differentiation and is negatively regulated by activation of the Ras1/MAPK pathway. *Cell* **81**, 857-866.

Rivard, N., McKenzie, F. R., Brondello, J. M. and Pouyssegur, J. (1995). The phosphotyrosine phosphatase PTP1D, but not PTP1C, is an essential mediator of fibroblast proliferation induced by tyrosine kinase and G protein-coupled receptors. *J. Biol. Chem.* **270**, 11017-11024.

Robinow, S. and White, K. (1988). The locus *elav* of *Drosophila melanogaster* is expressed in neurons at all developmental stages. *Dev. Biol.* **126**, 294-303.

Rogge, R. D., Karlovich, C. A. and Banerjee, U. (1991). Genetic dissection of a neurodevelopmental pathway: *Son of sevenless* functions downstream of the *sevenless* and EGF receptor tyrosine kinases. *Cell* **64**, 39-48.

Rubin, G. M. and Spradling, A. C. (1982). Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**, 348-353.

Schlessinger, J. (1994). SH2/SH3 signaling proteins. *Curr. Opin. Genet. Dev.* **4**, 25-30.

Simon, M. A., Drees, B., Kornberg, T. and Bishop, J. M. (1985). The nucleotide sequence and the tissue-specific expression of *Drosophila c-src*. *Cell* **42**, 831-840.

Simon, M. A., Bowtell, D. D. and Rubin, G. M. (1989). Structure and activity of the sevenless protein: a protein tyrosine kinase receptor required for photoreceptor development in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **86**, 8333-8337.

Simon, M. A., Bowtell, D. D., Dodson, G. S., Laverty, T. R. and Rubin, G. M. (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., Dodson, G. S. and Rubin, G. M. (1993). An SH3-SH2-SH3 protein is required for p21Ras1 activation and binds to sevenless and Sos proteins in vitro. *Cell* **73**, 169-177.

Tang, T. L., Freeman, R. M., Jr., O'Reilly, A. M., Neel, B. G. and Sokol, S. Y. (1995). The SH2-containing protein-tyrosine phosphatase SH-PTP2 is required upstream of MAP kinase for early *Xenopus* development. *Cell* **80**, 473-483.

Tomlinson, A. and Ready, D. F. (1987). Neuronal differentiation in the *Drosophila* ommatidium. *Dev. Biol.* **120**, 366-376.

van der Geer, P., Hunter, T. and Lindberg, R. A. (1994). Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu. Rev. Cell. Biol.* **10**, 251-337.

Vogel, W., Lammers, R., Huang, J. and Ullrich, A. (1993). Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. *Science* **259**, 1611-1614.

Wolff, T. and Ready, D. F. (1995). Pattern Formation in the *Drosophila* Retina. In *The Development of Drosophila melanogaster*. (ed. M. Bate and A. Martinez Arias), pp. 1277-1326. Plainview: Cold Spring Harbor Laboratory Press.

Xiao, S., Rose, D. W., Sasaoka, T., Maegawa, H., Burke, T. R., Jr., Roller, P. P., Shoelson, S. E. and Olefsky, J. M. (1994). Syp (SH-PTP2) is a positive mediator of growth factor-stimulated mitogenic signal transduction. *J. Biol. Chem.* **269**, 21244-21248.

Yamauchi, K., Milarski, K. L., Saltiel, A. R. and Pessin, J. E. (1995). Protein-tyrosine-phosphatase SHPTP2 is a required positive effector for insulin downstream signaling. *Proc. Natl. Acad. Sci. USA* **92**, 664-668.

Zhao, Z., Tan, Z., Wright, J. H., Diltz, C. D., Shen, S. H., Krebs, E. G. and Fischer, E. H. (1995). Altered expression of protein-tyrosine phosphatase 2C in 293 cells affects protein tyrosine phosphorylation and mitogen-activated protein kinase activation. *J. Biol. Chem.* **270**, 11765-11769.

Zipursky, S. L. and Rubin, G. M. (1994). Determination of neuronal cell fate: lessons from the R7 neuron of *Drosophila*. *Annu. Rev. Neurosci.* **17**, 373-397.

(Accepted 3 January 1996)