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The Nonreceptor Protein Tyrosine Phosphatase Corkscrew Functions in Multiple Receptor Tyrosine Kinase Pathways in *Drosophila*

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***Corkscrew* (*csw*) encodes a nonreceptor protein tyrosine phosphatase (PTPase) that has been implicated in signaling from the Torso receptor tyrosine kinase (RTK). *csw* mutations, unlike *tor* mutations, are associated with zygotic lethality, indicating that Csw plays additional roles during development. We have conducted a detailed phenotypic analysis of *csw* mutations to identify these additional functions of Csw. Our results indicate that Csw operates positively downstream of other *Drosophila* RTKs such as the *Drosophila* epidermal growth factor receptor (DER), the fibroblast growth factor receptor (Breathless), and likely other RTKs. This model is substantiated by specific dosage interactions between *csw* and *DER*. It is proposed that Csw is part of the evolutionarily conserved "signaling cassette" that operates downstream of all RTKs. In support of this hypothesis, we demonstrate that SHP-2, a vertebrate PTPase similar to Csw and previously implicated in RTK signaling, encodes the functional vertebrate homologue of Csw.**

INTRODUCTION

Corkscrew (Csw) encodes a nonreceptor protein tyrosine phosphatase (PTPase) with two N-terminal SH2 domains and a C-terminal catalytic region (Perkins *et al.*, 1992). Genetic, developmental, and molecular studies have demonstrated that Csw functions in the Torso (Tor) receptor tyrosine kinase (RTK) signaling pathway which is required for the establishment of cell fate determination of embryonic terminal structures (Perkins *et al.*, 1992). Activation of the maternally provided Tor RTK at the poles of the early embryo triggers a signal transduction pathway that involves the SH3–SH2–SH3 adaptor protein Drk/Grb2, the GTPase Ras1, the guanine nucleotide releasing factor protein Son of Sevenless (Sos) and the GTPase activating protein Gap-1 which regulate Ras1 activity, and the kinases KSR, D-Raf (also known as l(1)pole hole), D-MEK (also known as DSor1), and the Rolled MAPK (Therrien *et al.*, 1996; see reviews by Perrimon, 1993; Lu *et al.*, 1993b; Duffy and Perrimon, 1994). The Tor pathway ultimately controls the spatial expression of the transcription factors *tailless* (*tll*) and *huckebein* (*hkb*) which in turn are responsible for activating developmental programs that specify formation of larval head and tail structures. Examination of embryos derived from female germlines lacking *csw* activity have defects in terminal structures similar to the defects affected by *tor*. However, Csw does not completely block signaling from Tor since loss of *csw* activity has a weaker terminal phenotype than *tor* loss-of-function mutations. Csw acts as a positive transducer in Tor signaling and genetic epistasis analyses have shown that Csw operates downstream of Tor since loss-of-function *csw* mutations suppress the dominant phenotype of a *tor* gain-of-function mutation (Perkins *et al.*, 1992).

The Drk, Sos, Gap-1, Ras1, KSR, D-Raf, MEK, and MAPK proteins have been shown to be essential components of the signaling pathways activated by other *Drosophila* RTKs such as the *Drosophila* epidermal growth factor receptor (DER) and Sevenless (Sev) (see review by Perrimon, 1993). Further, homologous proteins in *C. elegans* and vertebrates have also been shown to be required for RTK signaling. Altogether, these studies have established the existence of an evolutionarily conserved cassette of signal transducers that operate downstream of all RTKs (see reviews by van der Geer *et al.*, 1994; Perrimon, 1994; Dickson and Hafen,

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1994). Because Csw was originally identified as a transducer of the Tor signal (Perkins *et al.*, 1992), and more recently of the Sev signal (Allard *et al.*, 1996), we wished to determine whether Csw encodes a component specific to Tor and Sev signaling or whether, as is the case of other molecules such as Ras, Raf, MEK, and MAPK, it identifies a member of this evolutionarily conserved cassette.

csw mutations unlike Tor mutations are associated with zygotic lethality. Previous analyses have shown that *csw* encodes at least three developmentally regulated transcripts which are expressed throughout all developmental stages (Perkins *et al.*, 1992). Analyses of *csw* cDNAs and rescue analyses (Perkins *et al.*, 1992; Melnick *et al.*, in preparation) have shown that a full-length cDNA that corresponds to the 4.55-kb species, which encodes the Csw protein is sufficient to rescue all aspects of the *csw* mutant phenotypes to wild type. Because Csw is expressed throughout the life cycle and *csw* mutations are associated with zygotic lethality, we have examined in detail the phenotypes associated with *csw* mutations in order to identify developmental pathways, in addition to Tor signaling, in which Csw is involved. In analyzing these phenotypes we have focused our attention on those tissues where *Drosophila* RTKs have been previously shown to play a role. To date, the mutant phenotypes of five RTKs in addition to Tor have been reported; the *Drosophila* epidermal growth factor receptor (DER; aka Faint little ball and Torpedo) (Price *et al.*, 1989; Schejter and Shilo, 1989; Clifford and Schupbach, 1989), the fibroblast growth factor (FGF) receptor Breathless (Btl; aka DFGF-R1 and DFR2; Klambt *et al.*, 1992; Shishido *et al.*, 1993; Reichman-Fried *et al.*, 1994), Sevenless (Sev) (Tomlinson and Ready, 1986), Derailed (Drl; Callahan *et al.*, 1995) and the *Drosophila* insulin receptor (DIR; Fernandez *et al.*, 1995; Chen *et al.*, 1996). Our analyses suggest that Csw is required in RTK-initiated pathways other than Tor, further suggesting that Csw is a protein, like Ras1 and D-Raf, which is required in all *Drosophila* RTK signaling pathways. In support of this hypothesis genetic evidence is presented for a role of Csw downstream of DER; i.e., a reduction in *csw* activity enhances significantly a reduction in *DER* activity in follicle cells, as well as in the developing eye imaginal disc. We also demonstrate that SHP-2 (formerly known as SHPTP-2, SHPTP-3, Syp, PTP1D, or PTP2C, see Adachi *et al.*, 1996), a vertebrate PTPase structurally related to Csw and which acts *in vivo* as a positive transducer of a vertebrate RTK (Tang *et al.*, 1995), rescues *csw* mutant phenotypes. We propose that Csw/SHP-2 is an integral part of the evolutionarily conserved cassette of signal transducers that operate downstream of all *Drosophila* RTKs.

MATERIALS AND METHODS

Genetics and phenotypes of csw alleles. To date, 9 X-linked *csw* alleles have been characterized. They are all required for viability and exhibit similar fully penetrant maternal effect phenotypes on embryonic development (Table 1). *csw* mutant animals (csw^{-}/*Y*) derived from heterozygous females (csw^{-}/+) die during early pupal stages. However, all embryos derived from females that lack maternal activity for any of the *csw* alleles fail to hatch and we refer to these as *csw* mutant embryos.

Examination of *csw* embryos derived from homozygous germline clones (see below) allow us to classify the alleles with respect to their strength. All external cuticular structures are present; however, the various *csw* alleles display a range of cuticular phenotypes from U-shaped or severely twisted to slightly twisted or normal. When embryos from six of the nine *csw* alleles are scored for their cuticular phenotypes (Table 2) it is evident that the alleles define a phenotypic series which can be aligned as follows: $csw^{VA199} = csw^{LE120} > csw^{C114} = csw^{13-87} > csw^{e13d.3} > csw^{19-106} = csw^{6}$ with csw^{VA199} being the most severe allele and csw^{19-106} and csw^{6} being the weaker or more hypomorphic alleles. This analysis focuses on the phenotypic examination of the most severe (csw^{VA199} or csw^{LE120}) and least severe (csw^{6}) alleles. The phenotype of csw^{LE120} is indistinguishable from that of csw^{VA199} (data not shown).

While less severe csw^{6} mutant embryos have cuticular elements similar or indistinguishable from wild type, the cuticular elements of the most severe *csw* mutations, though present, fall into two phenotypic classes either U-shaped or twisted, ''corkscrew''-shaped (Perkins *et al.*, 1992). Although there is some overlap, the difference between the U-shaped and corkscrew-shaped embryos stems from the genetic contribution from the father; i.e., there is a slight paternal rescue of the germline clone phenotype (Table 3). This was shown when the recessive embryonic cuticular marker, *shavenbaby* (svb^{YP17b}) (Wieschaus *et al.*, 1984), was recombined onto the chromosome carrying the csw^{C114} allele to generate germline clones. *csw*/+ embryos, which receive a wild-type copy of *csw* from their father, progress further through embryogenesis, complete germ band shortening, often complete head involution, and exhibit the corkscrew shape; we refer to these as ''paternally rescued'' *csw* mutant embryos. *csw*/*Y* embryos, which do not receive a wild-type copy of *csw* from their father, die in the shape of a U when the germ band is fully extended or as it is shortening, often do not complete head involution and are not or only slightly corkscrew-shaped; we refer to these as ''null'' *csw* mutant embryos.

Production of csw germline mosaics. *csw* germline clones were generated using the ''dominant female sterile technique'' as previously described (Chou and Perrimon, 1992). The three *csw* chromosomes used to generate the mosaics are $y\ csw^{6}\ FRT^{101}$, $csw^{VA199}\ FRT^{101}$, and $y\ csw^{LE120}\ FRT^{101}$. csw^{6} corresponds to a weak allele and csw^{VA199} and csw^{LE120} correspond to severe, genetic null alleles. 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*) promotor. 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.

To analyze the genetic epistasis between *tor* and *csw* at blastoderm stages, embryos derived from csw^{VA199} germline clones, generated in tor^{RL3} homozygous mothers were produced as described above. We utilized the dominant, temperature-sensitive *tor* allele, tor^{RL3}, where two copies are required to produce the tor^{gof} phenotype (Klingler *et al.*, 1988; Strecker *et al.*, 1989). Females with

TABLE 1

The *csw* Alleles and Their Phenotypes

Alleles	Origin	Reference	LP	GLC analysis
csw^{VA199}	EMS	Perrimon *et al.* (1985)	P	MEL
csw^{C114}	X-ray	Perrimon *et al.* (1985)	P	MEL
csw^{13-87}	Spont.	Perkins *et al.* (1992)	P	MEL
$csw^{e13d.3}$	EMS	Perkins *et al.* (1992)	P	MEL
csw^{19-106}	Spont.	Perkins *et al.* (1992)	P	MEL
csw^{6}	EMS	Perkins *et al.* (1992)	P	MEL
csw^{KN27}	DCE	This report	P	MEL
csw^{LE120}	EMS	Provided by T. Schupbach	P	MEL
csw^{eOP}	EMS	Simon *et al.* (1991)	P	MEL

Note. Though all lethal phases are indicated as pupal lethal, in all cases a small fraction of mutant dead larvae are observed. The germ line clone analyses have revealed that all alleles have fully penetrant maternal effect lethal phenotypes. Abbreviations: DCE, 1,2 dichloroethane; EMS, ethylmethane sulfonate; GLC, germline clone; LP, lethal phase; MEL, maternal effect lethal; P, pupal lethal; Spont., spontaneous.

clones were crossed to wild-type males and raised at 25°C. Four-hour embryo collections were prepared for *in situ* hybridization as described below.

Examination of embryos. Enhancer trap lines used in this study to examine the *csw* embryonic phenotypes include: 1A121 (Perrimon *et al.*, 1991), which detects both anterior and posterior midgut invaginations and their derivatives, and *Sma*II (also known as *1-eve-1;* Perrimon *et al.*, 1991), which allows the lineage of the tracheal system to be followed from formation of the tracheal precursors.

In situ hybridization on whole-mount embryos using digoxygenin-labeled probes was performed according to Tautz and Pfeifle (1989). Single-stranded sense and anti-sense digoxigenin containing DNA probes were prepared by the PCR labeling technique (N. Patel, personal communication) using appropriate primers (Biolabs). Probes were prepared from plasmids containing the *tll* (Pignoni *et al.*, 1990, 1992) and *hkb* (Weigel *et al.*, 1990; Bronner and Jaeckle, 1991; Bronner *et al.*, 1994) cDNAs. For visualization, embryos were dehydrated through an ethanol series and mounted in Euparal (Carolina Biological Supply). Embryos were analyzed and photographed with a Zeiss Axiophot or Nikon FXA both equipped with Nomarski optics.

Immunocytochemistry was performed as described in Perkins *et al.* (1992). Embryos were dehydrated in ethanol and cleared in methyl salicylate. Antibodies were used at dilutions ranging from 1:250 to 1:2000. The *Fas*III ascites monoclonal antibody was obtained from N. Patel, αFkh was obtained from H. Jaeckle, $\alpha\beta$-Gal came from Boehringer Mannheim, and αHRP came from Jackson ImmunoResearch.

Larval cuticles were prepared in Hoyer's mountant as described by van der Meer (1977). Cuticles were examined using dark-field or phase illumination. The distance between the ventrolaterally positioned Keilin's organs in thoracic segment 3 was measured using the Image, Version 1.51, Program from the NIH.

Generation of zygotically rescued csw flies. To generate zygotically rescued *csw* mutant males (genotype *csw/Y;* [w^+ *hs-Y1229*]/+) transformed fly lines carrying the wild-type *csw* cDNA *Y1229* under the control of an inducible heat shock promoter, *hs-Y1229,* were used (Perkins *et al.*, 1992). In these experiments the severe *csw* alleles csw^{VA199} and csw^{LE120} were used. Following daily 1-hr heat shocks throughout larval and pupal stages, the zygotic pupal lethality of *csw* can be fully rescued; partial rescue can be achieved with one 1-hr heat shock during early larval stages. Partially zygotically rescued *csw* mutant adults were stored in 100% ethanol. Body parts were selected under a dissecting scope, separated from the carcass, and mounted for visualization in Hoyer's mountant.

Fully zygotically rescued adult males (genotype *csw/Y;* [w^+ *hs-Y1229*]/+) when crossed to *csw*/+; +/+ females, produce both male (*csw/Y;* [*hs-Y1229*]/+) and female (*csw/csw;* [w^+ *hs-Y1229*]/+) progeny; these latter females generate eggs with fused dorsal chorionic appendages. The enhancer trap line BB142 (kindly provided by T. Schupbach) was used to visualize the most dorsal follicle cells which are located near the oocyte nucleus during mid-oogenesis. Follicle cells of genotype *csw/csw;* [w^+ *hs-Y1229*]*/BB142* were visualized following X-Gal staining which was performed as described by Klambt *et al.* (1991).

Genetic interactions between csw and DER. *UAS-DER*^{DN} was a gift from Alan Michelson. It expresses a DER protein which contains a deletion of the cytoplasmic domain, including the tyrosine kinase domain, and as a result it acts as a dominant-negative DER (A. Michelson, personal communication). Two different insertions were used in this study (29.77 and 29-8-1); these lines are homozygous viable and located on the II and III chromosomes, respectively.

The two Gal4 lines used in this study, C253 and T155v, were identified by Beth Noll to generate specific phenotypes in combination with *UAS-DER*^{DN}. To examine the effects of *csw* mutations (both csw^6 and csw^{LE120}) on the phenotypes associated with a reduction in *DER* activity, the eye (Gal4 line C253) and egg (Gal4 line T155v) phenotypes associated with *csw*/+; *Gal4*/+; *UAS-DER*^{DN}/+ and their +/+; *Gal4*/+; *UAS-DER*^{DN}/+ siblings were compared. Crosses were performed at various temperatures (25 and 29°C) to allow different levels of expression of the UAS constructs (Brand *et al.*, 1994).

Rescue of csw mutations by SHP-2. cDNAs for SHP-2 and SHP-1 (kindly provided by Drs. B. Neel and S. H. Shen, respectively) were cloned into pCaSpeR-hs (Thummel, 1988; C. Thummel, personal communication) and genomic transformants obtained by standard protocols (Spradling, 1986). Four independent transformed lines per construct were tested for their abilities to rescue the zygotic lethality of *csw*. Utilized as controls were transformed lines carrying the wild-type *csw* cDNA *Y1229* which had previously been shown to rescue the zygotic lethality of *csw* (Perkins *et al.*, 1992). In these experiments the severe *csw* alleles csw^{VA199} and csw^{LE120} were used. Heterozygous *csw/FM7;* +/+ virgins were mated to transformed males homozygous for one of the insertions, [w^+ *hs-Y1229*], [w^+ *hs-SHP-1*], or [w^+ *hs-SHP-2*], each under the control of the inducible

TABLE 2

Cuticle Phenotypes of Embryos Derived from Mothers with Germline Clones

Alleles	% U-shaped or corkscrew	% Slightly twisted or normal
csw^{VA199}	97	3
csw^{C114}	74	26
csw^{13-87}	71	29
$csw^{e13d.3}$	56	44
csw^{19-106}	8	92
csw^{6}	6	94

TABLE 3
Paternal Rescue of *csw* Defects

Genotype	Embryos scored	Germband retracted	Head involuted
$csw^{C114}svb/Y$	33	21	6
$csw^{C114}svb/+$	38	37	37

Note. $csw^{C114}svb/Y$ represents null *csw* mutant embryos and $csw^{C114}svb/+$ represents paternally rescued *csw* mutant embryos.

heat shock promoter. Following hatching, larvae and pupae were heat shocked daily for 1 hr at 37°C until adults emerged. Adult males were counted and their phenotypes scored.

RESULTS

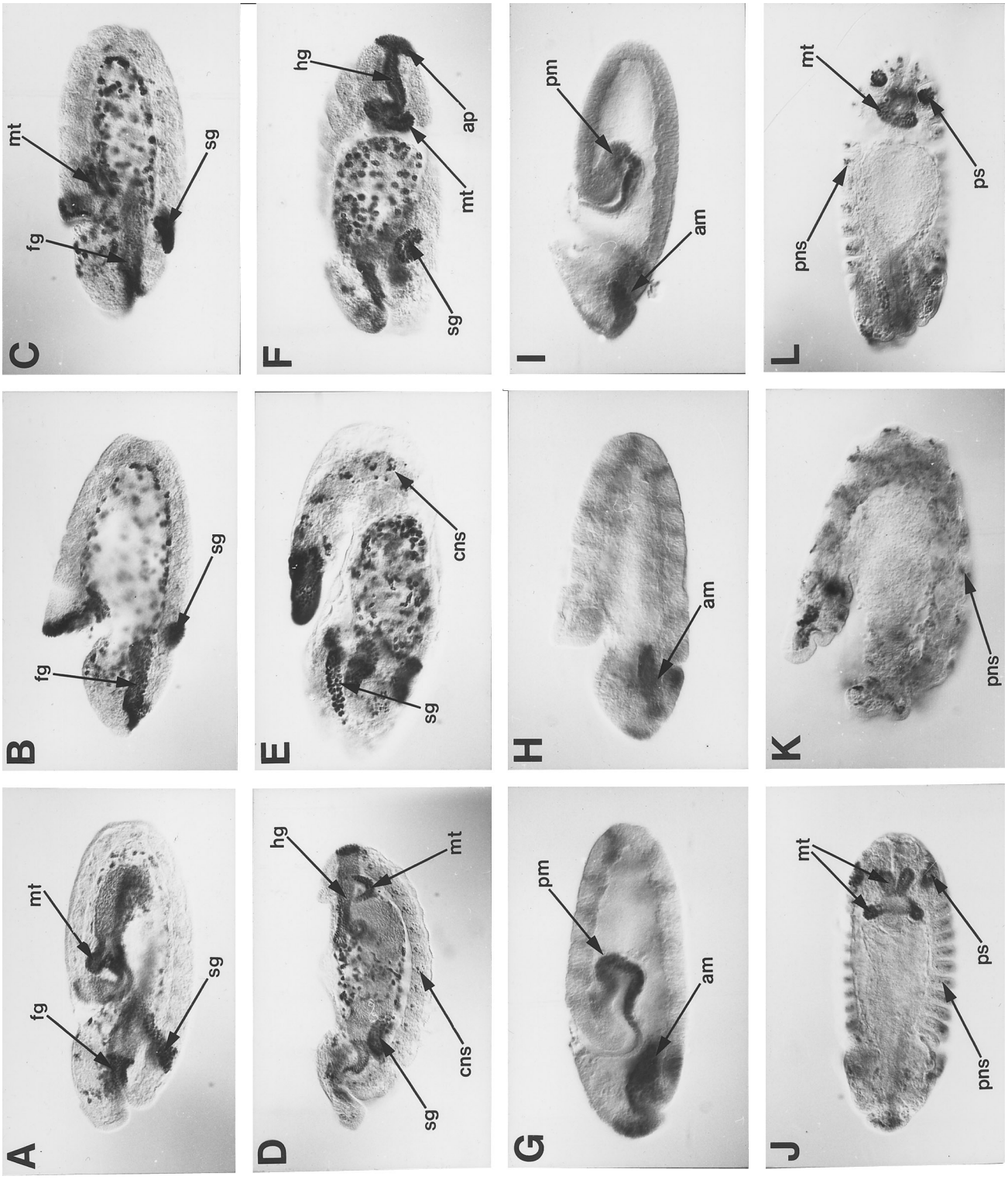
Csw Is Not Only Required in Tor Signaling

tor mutations were isolated in a screen for female sterile mutations associated with maternal effect lethal phenotypes (Schupbach and Wieschaus, 1986). Subsequent molecular analyses of *tor* (Sprenger *et al.*, 1989) revealed that the gene is expressed maternally and protein null alleles do not affect viability. Thus, the only evident function of Tor is during the establishment of embryonic terminal cell fates. *csw* mutations, however, were isolated in a screen for zygotic lethal mutations associated with specific maternal effect phenotypes (Perrimon *et al.*, 1989). The *csw* mutations define a phenotypic series (see Materials and Methods and Table 2) and they are all associated with zygotic lethality and a fully penetrant maternal effect phenotype (see Materials and Methods and Table 1). The zygotic lethality associated with *csw* mutations indicates that Csw, unlike Tor, is required during zygotic development. Additional evidence that *csw* is involved in developmental processes other than Tor signaling comes from the analysis of the *csw* maternal effect phenotype. Unlike *tor*, the *csw* maternal effect phenotype is partially paternally rescuable (see Materials and Methods and Table 3). This analysis focuses on the phenotypic examination of the most severe (csw^{VA199} and csw^{LE120}) and least severe (csw^{6}) *csw* alleles (see Materials and Methods). To determine more precisely the phenotypes of *csw* mutant embryos (i.e., embryos derived from homozygous *csw* mutant germlines) several molecular markers were selected which allowed the analysis of the terminal derivatives, ventral ectodermal tissues, central nervous system, and tracheal system, where development has been shown to involve RTK signaling pathways.

The Role of Csw in Terminal Development

Posteriorly $tor^{loss\text{-}of\text{-}function}$ (tor^{lof}) mutations delete all structures derived from 0 to 20% egg length (EL) or all structures posterior to abdominal segment 7, and including the 8th abdominal segment, anal pads, posterior spiracles, malpighian tubules, hindgut, and posterior midgut (see reviews by Perkins and Perrimon, 1991; Lu *et al.*, 1993b; Sprenger and Nusslein-Volhard, 1993; Duffy and Perrimon, 1994). To determine whether *csw* mutations affect internal posterior structures to the same extent as tor^{lof} mutations we utilized three molecular markers which specifically label structures deleted in tor^{lof} mutations. We used antibodies to the Fork head (Fkh) protein to examine the foregut, the hindgut, and the malpighian tubules (Weigel *et al.*, 1989), the enhancer trap line 1A121 to examine the anterior and posterior midguts (Perrimon *et al.*, 1991), and antibodies to the Cut protein to examine cells surrounding the terminally derived anterior and posterior spiracles and the malpighian tubules (Blochlinger *et al.*, 1990) in *csw* mutant embryos (Fig. 1). Collectively, these data indicate that in both paternally res-

FIG. 1. Csw is required to specify the terminal structures. Wild-type embryos (A, D, G, J), and null (B, E, H, K) and paternally rescued (C, F, I, L) csw^{VA199} mutant embryos are stained for molecular markers which label terminal structures. Throughout embryogenesis antibodies to the Fkh protein labels the foregut (fg) and its derivative the salivary gland (sg) as well as the hindgut (hg) and its derivative the malpighian tubules (mt). Late in embryogenesis Fkh protein is also expressed in a subset of cells in the central nervous system (cns) (A, D; Weigel *et al.*, 1989). At germ band elongation (A, B, C) anterior foregut and salivary gland staining appears normal; however, paternally rescued and null csw^{VA199} mutant embryos exhibit distinct Fkh staining patterns. Following germ band shortening (D, E, F) paternally rescued and null csw^{VA199} mutant embryos are easily identified. Relative to wild-type (D) paternally rescued csw^{VA199} embryos display a somewhat shortened, but internalized hindgut, and the malpighian tubule buds do not elongate into tubules (F). Null csw^{VA199} embryos remain U-shaped and posterior terminal structures are external and cannot be discerned (E). The enhancer trap line 1A121 (Perrimon *et al.*, 1991) allows visualization of the anterior and posterior midguts (am and pm, respectively; G). Paternally rescued csw^{VA199} mutant embryos are characterized by an internalized posterior midgut invagination which is reduced in size (I) and frequently malformed. Null csw^{VA199} embryos are U-shaped and exhibit no posterior midgut invagination or labeling with 1A121 at the extreme posterior end of the germ band (H). The anterior midgut invagination (am) appears normal in paternally rescued and null csw^{VA199} embryos. Antibodies to the Cut protein label cells surrounding the terminally derived anterior and posterior spiracles (ps), the malpighian tubules (mt), the peripheral nervous system (pns), and many cells in the central nervous system (not shown) (Blochlinger *et al.*, 1990; J). Early in embryogenesis both malpighian tubule buds and posterior spiracles are observed in all csw^{VA199} mutant embryos; however, later in embryogenesis paternally rescued csw^{VA199} embryos show internalized, but undifferentiated malpighian tubule buds (mt; L) and null, U-shaped csw^{VA199} embryos show only external staining (presumably the posterior spiracles) posteriorly (K). All embryos are oriented with anterior to the left. Abbreviations: am, anterior midgut; ap, anal pads; cns, central nervous system; fg, foregut; hg, hindgut; mt, malpighian tubules or buds; pm, posterior midgut; pns, peripheral nervous system; ps, posterior spiracles; sg, salivary gland invagination.



cued and null (for definitions see Materials and Methods) csw^{VA199} mutant embryos specific terminal structures are entirely deleted and/or malformed. The posterior midgut invagination, and hence the midgut, is entirely deleted in null embryos (Fig. 1H); whereas in paternally rescued embryos it is reduced in size and malformed (Fig. 1I). The null embryos which lack the posterior midgut invagination most frequently die with a U-shape at germ band elongation and in these embryos posterior structures are difficult or impossible to identify (Figs. 1B, 1E, 1H, and 1K). Posterior specific staining is more easily interpretable in paternally rescued csw^{VA199} embryos in which the midgut invaginates and death occurs sometime after germ band shortening. In these embryos it is apparent that the posterior midgut is variably deleted and malformed, the malpighian tubule buds form but do not elongate, and the hindgut is shortened (Figs. 1C, 1F, 1I, and 1L). These analyses illustrate that the most severe *csw* mutation, csw^{VA199}, affects only a subset of the structures which are entirely deleted in tor^{lof} mutations.

Casanova and Struhl (1989) have shown that different levels of active Tor protein are capable of ''instructing'' the development of different terminal structures, suggesting that activated Tor protein, or its phosphorylated substrates, may organize the terminalia by acting as gradient morphogens. *tll* is also known to act in a graded fashion since the formation of the seventh Ftz stripe depends on the dosage of *tll,* suggesting that a specific threshold of *tll* activity dictates the appearance of this stripe in wild type (Casanova, 1990). Posteriorly the information encoded by *csw* also appears to be utilized in a graded fashion (Perkins, unpublished results). In *csw* mutant embryos the extent to which *tll* is reduced depends upon the allele being examined; if in wild type *tll* is expressed from 0 to 15% EL, in the severe csw^{VA199} allele *tll* is expressed from 0 to 5.9% EL and in the weaker csw^{6} allele *tll* is expressed from 0 to 8.7% EL.

The posterior abnormalities we observe in *csw* mutant embryos correlate with the expression of the downstream zygotic genes *tll* and *hkb*. In wild type the posterior domains of expression of *tll* and *hkb* overlap (Figs. 2A and 2C) but their resulting developmental lesions are complementary. In *hkb* mutant embryos only the posterior midgut is deleted (Weigel *et al.,* 1990), which is the only posterior structure not completely deleted in *tll* embryos (Pignoni *et al.*, 1990). In csw^{VA199} mutant embryos posterior *tll* expression is decreased (Fig. 2B) and posterior *hkb* expression is entirely deleted (Fig. 2D). Significantly, as in *hkb* mutant embryos the only structure deleted in csw^{VA199} mutant embryos is the posterior midgut. We conclude that posterior structures deleted and/or malformed in *csw* mutant embryos correlates nicely with the extent of both *tll* and *hkb* expression.

Further, since it had previously been shown at the cuticular level that *csw* is epistatic to *tor* (Perkins *et al.*, 1992) we examined the epistatic relationship between *tor* and *csw* at blastoderm stages. Specifically, we tested whether the effect of a $tor^{gain\text{-}of\text{-}function}$ ($tor^{gof} = tor^{RL3}$) allele can be negated by a severe csw^{VA199} allele. The *hkb* expression pattern in blastoderm stage embryos derived from females mutant for the tor^{gof} mutation is expanded centrally from both termini (Fig. 2E); whereas *hkb* expression in blastoderm embryos doubly mutant for tor^{gof} and severe csw^{VA199} mutations (Fig. 2F) is indistinguishable from *hkb* expression in the same severe csw^{VA199} mutation alone (Fig. 2D). Therefore, a loss of *csw* function suppresses tor^{gof} activity at blastoderm stages.

The Role of Csw in Embryonic Ventral Cell Fate Development

While Tor is likely the first RTK to function during embryogenesis, other RTKs have been shown to function through mid and late embryogenesis. Using molecular markers specific for the ventral and lateral ectoderm, Raz and Shilo (1992, 1993) demonstrated that DER is required at 3 to 4.5 hr after egg laying for patterning of the ventral ectoderm. In *DER* mutants ventral cell fates are replaced by an expansion of more lateral cell fates. To analyze whether Csw is involved in this aspect of DER signaling we utilized a number of molecular markers (see Materials and Methods) to visualize the determination of ventral cell fates in *csw* mutant embryos.

Using an antibody to Fas III which identifies a subset of ventral ectodermal cells immediately adjacent to the ventral midline, we found that significantly fewer cells were stained in *csw* mutant embryos (8 to 10 cells per segment in weak csw^{6} mutant embryos, Fig. 3B; and 4 to 6 cells per segment in strong csw^{VA199} or csw^{LE120} mutant embryos, not shown) relative to wild type (16 to 20 cells per segment, Fig. 3A; Patel *et al.*, 1987; L. A. Perkins, unpublished results). To determine whether this apparent loss of ventral cell fates was accompanied by a concomitant expansion of lateral cell fates we examined the expression of an enhancer trap line that detects the laterally positioned cells of the tracheal pits (see Materials and Methods) in *csw* mutant embryos. In wild type the ventral most cells of the tracheal pits are 7 to 8 cells from the ventral midline (Fig. 3C), whereas in weak csw^{6} mutant embryos the ventral cells of the tracheal pits are 3 to 6 cells from the ventral midline (Fig. 3D). To further confirm that more lateral cell fates are shifted toward the ventral midline we measured the distance between the ventrolaterally positioned Keilin's organs in thoracic segment 3. In wild-type embryos the T3 Keilin's organs are separated by 76.3 μm (standard deviation (SD) 4.7; Figs. 3E and 3G); however, in weak csw^{6} mutant embryos they are separated by 60.4 μm or 79.1% that of wild type (SD 7.2; Figs. 3F and 3G) and in strong csw^{VA199} mutant embryos by 28.0 μm or 36.6% that of wild type (SD 11.1; Fig. 3G). Taken together we conclude that in *csw* mutant embryos ventral cells undergo cell fate changes to that of more lateral epidermal cell fates. Further, the phenotypes observed in the most severe allele of *csw*, csw^{VA199}, is similar to those of embryonic null alleles of *DER* (Raz and Shilo, 1993; Clifford and Schupbach, 1992), suggesting that Csw, as in the case of its role in Tor RTK signaling, is a positive transducer which operates in the DER signaling pathway. Further, the effect of loss of *csw* activity on lateral cell fate determination is not as severe

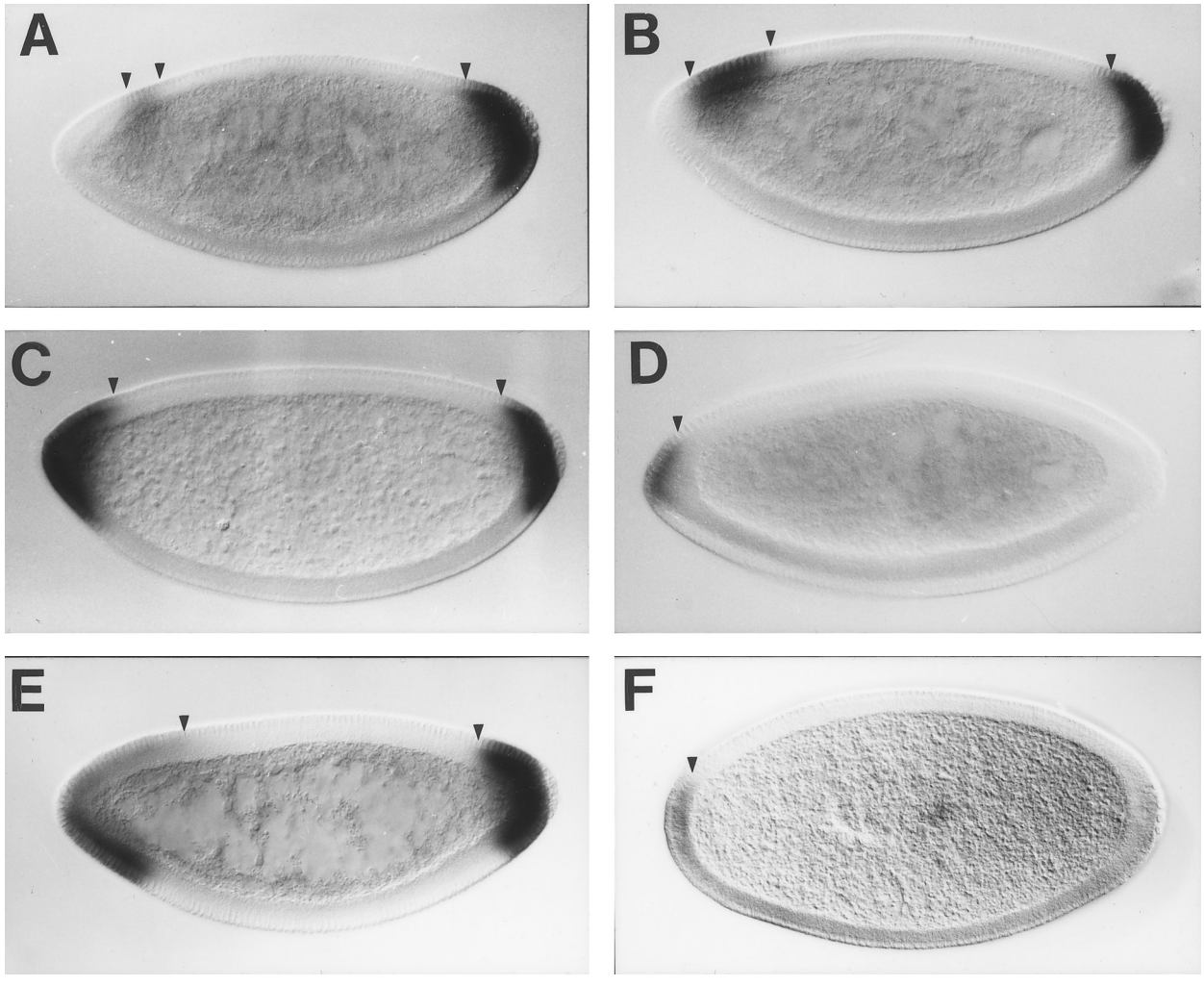


FIG. 2. Terminal structures are specified at blastoderm stages. Blastoderm stage embryos reveal *tll* (A, B) and *hkb* (C, D, E, F) RNA expression patterns (limits are denoted by arrowheads). In wild type, at the blastoderm stage, *tll* (A) and *hkb* (C) are expressed both anteriorly and posteriorly. In csw^{VA199} mutant embryos posterior *tll* expression (B) is reduced and anterior *tll* expression is expanded. In csw^{VA199} mutant embryos posterior *hkb* expression (D) is entirely deleted and anterior *hkb* expression is reduced. In tor^{gof} embryos (E) *hkb* expression is expanded from both termini centrally and in csw^{VA199}; tor^{gof} double mutant embryos (F) *hkb* expression is indistinguishable from csw^{VA199} mutant embryos (D). All embryos are oriented with anterior to the left and dorsal up.

as observed in *DER* mutants, suggesting that, as is the case in Tor signaling, Csw transduces only a partial increment of the DER signal (see Discussion).

The Role of Csw in Embryonic CNS Development

In addition to the ventral epidermis the ventral ectoderm also gives rise to the central nervous system (CNS) and like the ventral epidermis the formation of the CNS is known to require the activity of DER (Schejter and Shilo, 1989; Zak *et al.*, 1990). The two commissures per segment that extend across the ventral midline in wild type (Fig. 4A) are absent or fused in *DER* mutant embryos. Further, the longitudinal axonal tracts are often fused. To examine whether Csw plays a role in this function of DER, we examined the CNS phenotypes of *csw* mutant embryos.

As shown in Fig. 4, two classes of embryonic phenotypes were observed when *csw* embryos are immunostained with α-HRP (see Materials and Methods). In null csw^{VA199} embryos, which display the more severe phenotype, the horizontal commissures are collapsed (Fig. 4B); whereas in paternally rescued csw^{VA199} embryos, which display the less severe phenotype, the commissures are separated along the ventral midline (Fig. 4C). In both paternally rescued and null mutant embryos the longitudinal axon tracts are rudimentary and discontinuous. Similar phenotypes have been reported in embryos mutant for *DER* (Raz and Shilo, 1993) where severe *DER* mutant embryos exhibit collapsed hori-

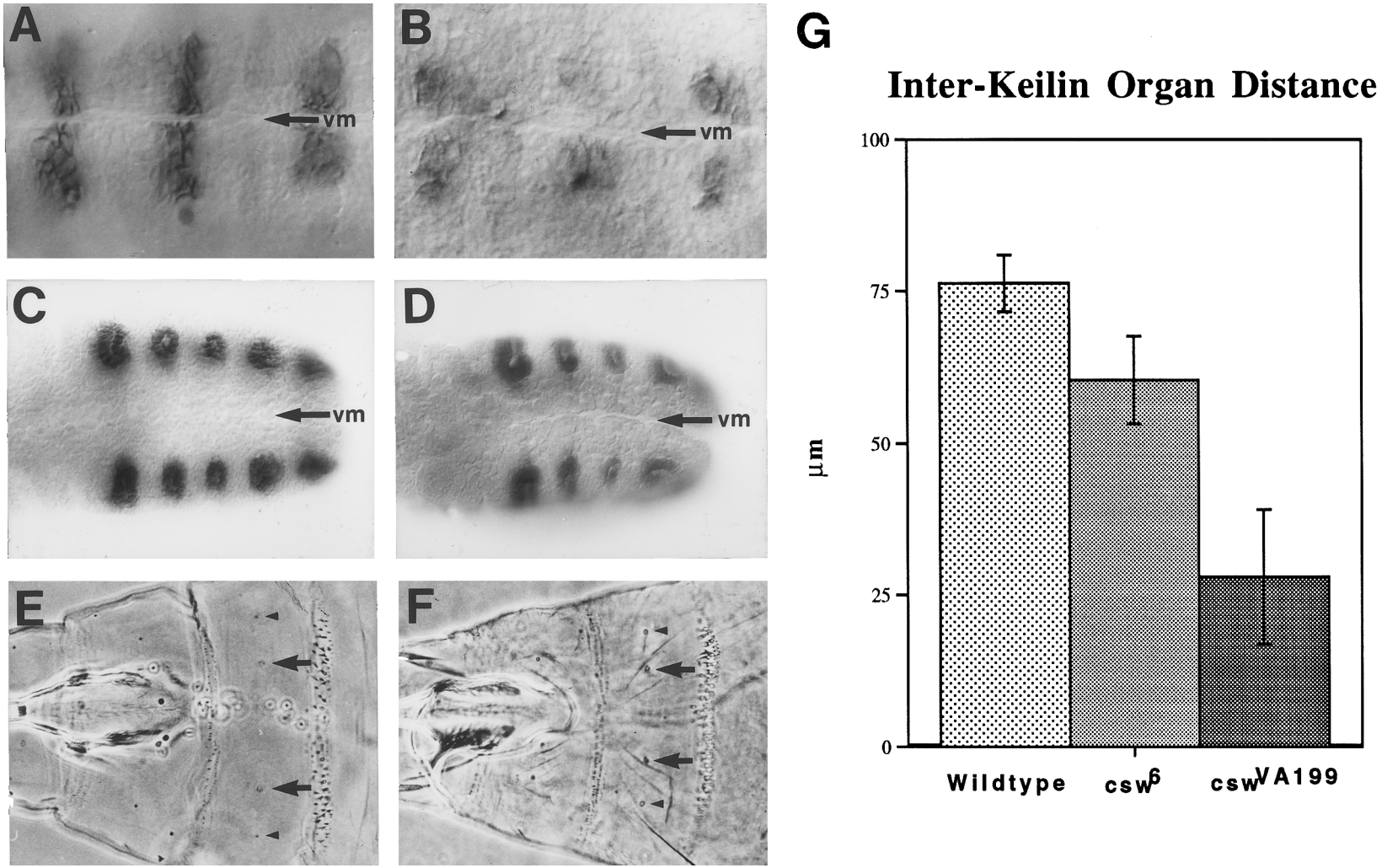


FIG. 3. Csw is required for cell fate specification of the ventral ectoderm. Wild-type embryos (A, C, E) and *csw* mutant embryos (B, D, F) are shown. αFas III, a molecular marker for the ventral epidermis (Patel *et al.*, 1987) reveals that fewer cells per segment express Fas III adjacent to the ventral midline (vm) of csw^{6} mutant embryos (B) relative to wild type (A). The enhancer trap line *Sma*II is a marker for the developing larval trachea (Perrimon *et al.*, 1991) which differentiates from lateral epidermal cells. Embryos at the segmentally arrayed tracheal pit stage reveal that fewer cells separate the ventral most cells of the tracheal pits from the ventral midline (vm) in csw^{6} mutant embryos (D) than in wild type (C), suggesting that ventral cells have assumed more lateral cell fates. Relative to wild type (E and G; $n = 51$), the distance between the ventrolateral Keilin's Organs, thoracic cuticular sensory organs (Campos-Ortega and Hartenstein, 1985) is reduced in both weak csw^{6} (F and G; $n = 27$) and severe csw^{VA199} (G; $n = 20$) mutant embryos. All embryos are ventral views with anterior to the left.

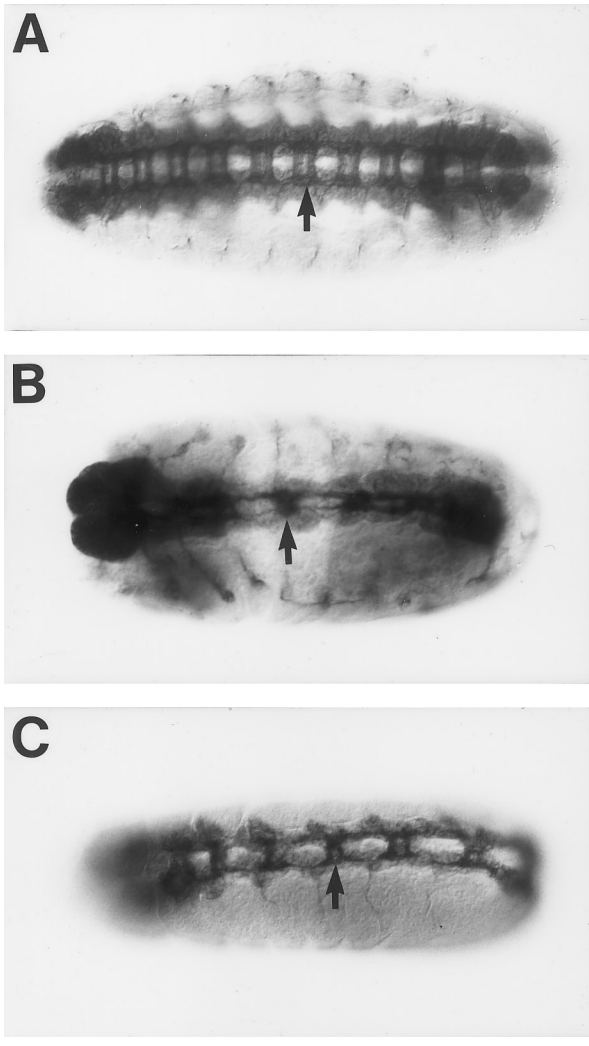


FIG. 4. Csw is required in the developing embryonic CNS. αHRP, a molecular marker specific for neurons and their processes (Jan and Jan, 1982), reveals the two commissural axonal tracts (arrows) per segment which cross the ventral midline and the longitudinal axon tracts which extend along the anteroposterior axis of the embryo. The commissures within each segment remain separate in wild-type (A) and paternally rescued csw^{VA199} mutant embryos (C), whereas in null csw^{VA199} mutant embryos (B) the commissures are collapsed. The anteroposterior longitudinal axon tracts are rudimentary in both weak and severe csw^{VA199} mutant embryos relative to wild type.

zontal commissures and discontinuous longitudinal axon tracts. These results suggest that Csw operates downstream of DER in the formation of the larval CNS.

The Role of Csw in Embryonic Tracheal Development

During embryogenesis the formation of the larval trachea requires the activity of a *Drosophila* homologue of the fibroblast growth factor (FGF) receptor encoded by the gene *btl* (Klambt *et al.*, 1992; Shishido *et al.*, 1993; Reichman-Fried *et al.*, 1994). In *btl* mutant embryos, the tracheal system is highly disorganized. The tracheal defects do not appear to arise from changes in cell fate, but rather a failure of the tracheal precursors to migrate along the stereotypic paths to form the invariant branches of the tracheal tree (Klambt *et al.*, 1992; Reichman-Fried *et al.*, 1994). To determine whether Csw plays a role in signaling from the Btl RTK, we examined the phenotypes of mutant *csw* embryos using a tracheal-specific marker, the enhancer trap line *Sma*II (Perrimon *et al.*, 1991; see Materials and Methods).

In weak csw^{6} and severe csw^{VA199} mutant embryos it is apparent that the tracheal cell precursors are produced normally (Figs. 5D and 5G). However, their subsequent migration, which generates the tracheal tree, appears defective (Figs. 5E and 5H) and an incomplete and disconnected system of tracheal branches is the final result (Figs. 5F and 5I). The degree to which migration is disrupted depends on the severity of the *csw* allele examined. Whereas the mature trachea present in weak csw^{6} embryos (Fig. 5F) shows only minimal tracheal defects (e.g., disruption of the major anterioposterior tracheal branch), the trachea of severe csw^{VA199} embryos (Fig. 5I) is barely recognizable as tracheal tissue. This effect is similar to the role of Btl during tracheal precursor migration (Klambt *et al.*, 1992; Reichman-Fried *et al.*, 1994) and suggests that Csw operates positively in Btl signaling for the formation of the mature larval trachea.

Csw Is Required for the Development of Adult Structures

Several *DER* alleles are homozygous viable and survive to adulthood; however, these mutations result in various cuticular phenotypes as well as female sterility (Garcia-Bellido, 1975; Clifford and Schupbach, 1989; Price *et al.*, 1989). Among the structures defective in *DER* mutant adults are the compound eyes and ocelli, the aristae, wing veins, tarsal claws, specific macrochaetae, male sex combs, and female genital disc derivatives. To determine whether Csw might function during imaginal development in the DER signaling pathway, partially zygotically rescued csw^{LE120} and csw^{VA199} mutant adults were generated using a wild-type *csw* transgene (see Materials and Methods).

Examination of mutant csw^{LE120} and csw^{VA199} adults reveals consistent defects including absence of one or both of the distal-most antennal segment, the aristae (Figs. 6B and 6C); lack of one or more of the distal-most leg segments, the tarsal claws (Fig. 6G); incomplete formation of distal portions of wing vein L5 (Fig. 6E) and less often L4; and eyes with reduced numbers of disorganized ommatidia and ommatidial bristles (data not shown). The phenotypes of *csw* mutant adults are similar to those reported for viable *DER* mutations (Clifford and Schupbach, 1989), suggesting that *csw* functions positively during imaginal development in the DER signaling pathway.

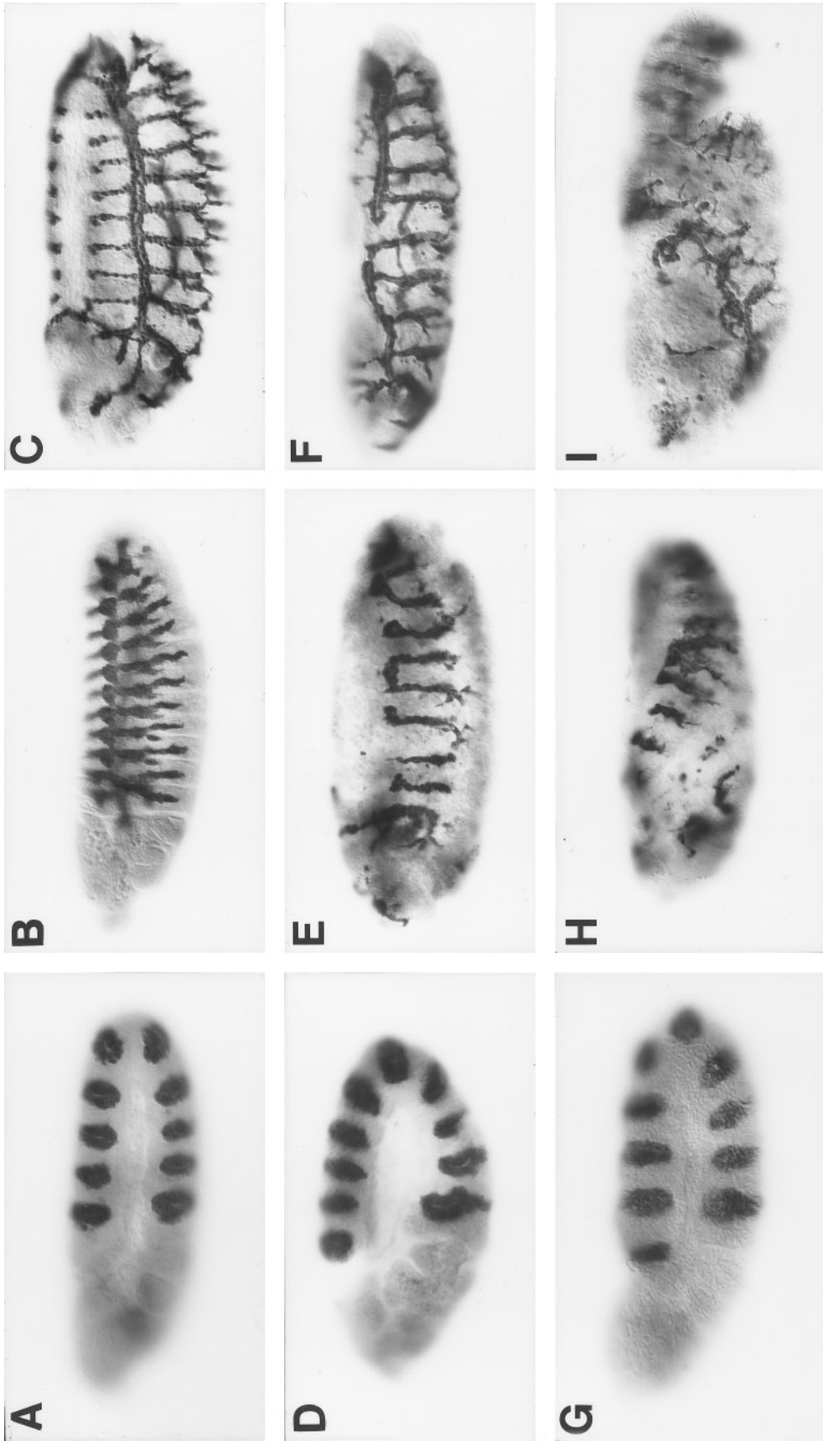


FIG. 5. Csw is required for tracheal cell migration. The enhancer trap line *SmaII* (Perrimon *et al.*, 1991) is a marker for the developing larval trachea and is used here to delineate tracheal development in wild-type (A, B, C) and both weak csw^{6} (D, E, F) and severe csw^{VA199} (G, H, I) mutant embryos. The larval trachea is produced by cell divisions within the ectoderm and organized into tracheal pits (A). The tracheal precursors migrate along stereotypic routes (B) and subsequently differentiate into the mature tracheal tree (C) (Campos-Ortega and Hartenstein, 1985; Manning and Krasnow, 1993). The segmental array of tracheal pits are present in both weak csw^{6} (D) and severe csw^{VA199} (G) mutant embryos; however, the subsequent migration of tracheal precursors is defective (E and H; respectively). Relative to wild type (C) the "mature" tracheal tree of weak csw^{6} mutant embryos is incomplete and disconnected (F), while in severe csw^{VA199} mutant embryos it is barely recognizable as tracheal tissue (I). All embryos are lateral views oriented with anterior to the left.

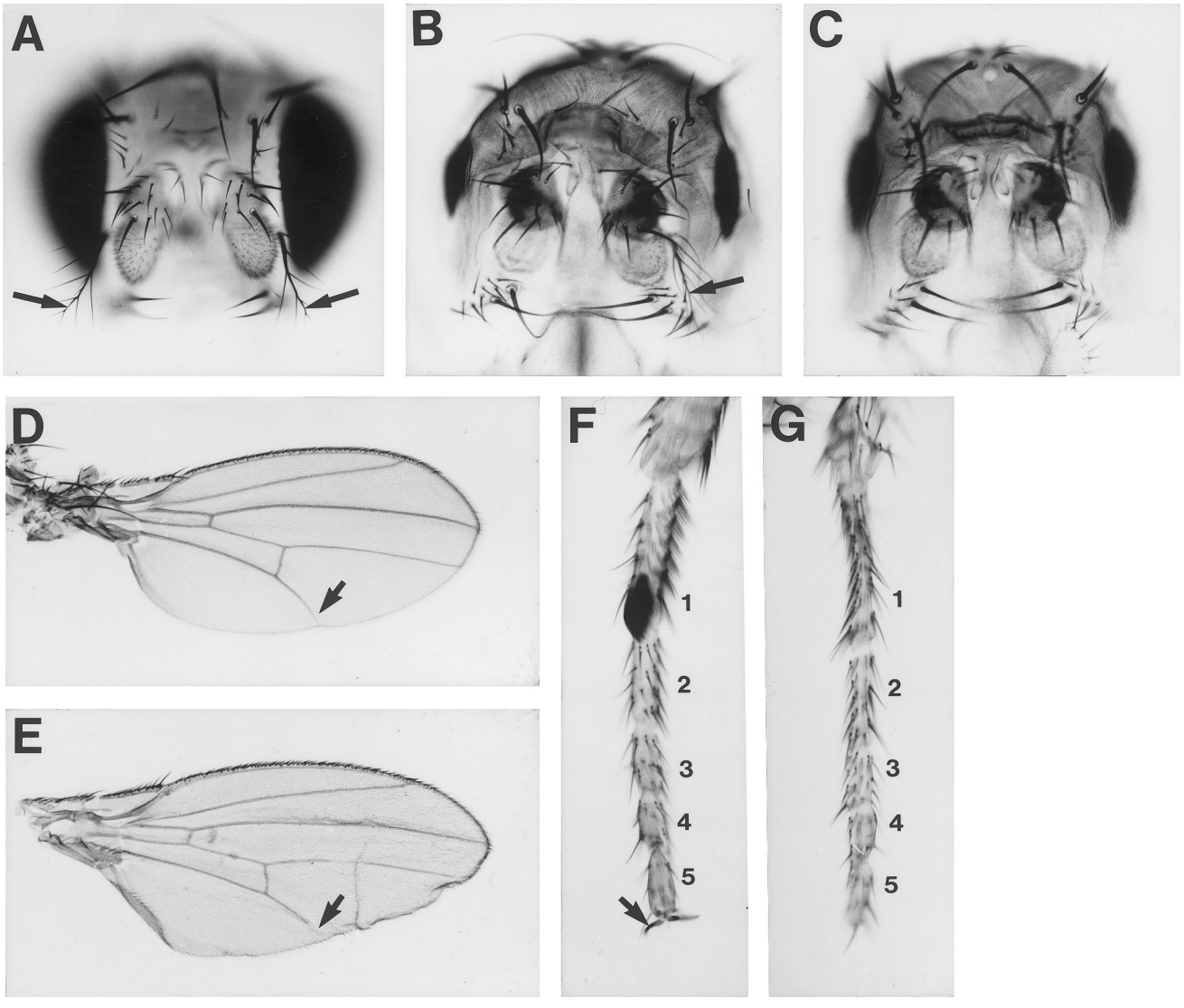


FIG. 6. Csw is required for formation of adult structures. Csw functions during imaginal development for proper formation of adult structures. Wild type (A) and csw^{LE120} mutant (B, C) adult heads reveal two prominant defects. The eyes of csw^{LE120} mutant adults are severely reduced in size and the distal-most antennal segment, the feather-like aristae (arrows) are frequently missing. The longitudinal, L5 wing vein which in wild type (arrow in D) extends to the distal wing margin, fails to reach the wing margin in csw^{LE120} mutant wings (arrow in E). The distal-most leg segment, the tarsal claw (denoted by an arrow in the male leg shown in F) is frequently missing from the legs of csw^{LE120} mutant adults (G, note a female leg is illustrated). In F and G tarsal segments are denoted by numbers.

Csw Is Required during Oogenesis in Follicle Cell Development

During oogenesis DER is involved in the establishment of dorsal follicle cell fates (Price *et al.*, 1989). An asymmetrically localized signal encoded by *gurken* originates from the oocyte to activate DER in the dorsal follicle cells (Neumann-Silberberg and Schupbach, 1993). Dorsal cell fates can be visualized by the expression of specific molecular markers and the production of dorsal chorionic filaments. To determine whether Csw plays a role in this DER-mediated signaling pathway, we examined the phenotypes of the eggs derived from partially zygotically rescued csw^{VA199} females (see Materials and Methods). Because females that possess germlines homozygous for the severe csw^{LE120} and csw^{VA199} mutations lay normally shaped eggs, we reasoned that structural egg phenotypes derived from partially zygotically rescued *csw* females will reflect defects in the somatic component of oogenesis.

Partially zygotically rescued csw^{LE120} and csw^{VA199} females lay eggs with fused dorsal appendages which correspond to an expansion of ventral chorionic cell fates at the expense of dorsal chorionic cell fates (Fig. 7). A molecular marker specific for the dorsal chorionic cells (T. Schupbach,

personal communication) reveals that egg chambers from these partially zygotically rescued *csw* females lack their dorsal chorionic cells starting from very early stages of oogenesis. These observations suggest that Csw is required for determination of dorsal chorionic cell fates and that in the absence of csw^{LE120} or csw^{VA199} function these cell fates undergo changes to those of more ventral chorionic cells. This effect is similar to the role of DER during oogenesis (Price *et al.*, 1989) and suggests that Csw operates downstream of DER in the establishment of dorsal follicle cell fates.

Csw Acts Positively in DER Signaling

The results presented above strongly suggest that Csw acts as a positive signal transducer downstream of DER. To obtain direct genetic evidence that the two genes operate in the same signaling pathway, we tested whether a reduction in *csw* gene activity enhances a phenotype associated with a reduction in *DER* activity. We used the Gal4-UAS system (Brand and Perrimon, 1993) to design a sensitized genetic background where *DER* activity is reduced. A number of Gal4 strains were identified that generate phenotypes when outcrossed to flies that carry a UAS construct driving a DER dominant negative protein ($UAS\text{-}DER^{DN}$, see Materials and Methods). Misexpression of $UAS\text{-}DER^{DN}$ in the *Gal4-C253* expression pattern generates flies with eyes reduced in size (Fig. 8B), most likely reflecting the role of DER in cell proliferation during imaginal disc development. In addition, females that carry both *Gal4-T155v* and $UAS\text{-}DER^{DN}$ lay partially to completely ventralized eggs (Fig. 9) reflecting a decrease of *DER* activity in follicle cells.

If Csw acts as a positive transducer of DER we expect that the severity of the phenotypes generated by the combination of $Gal4;\ UAS\text{-}DER^{DN}$ would be enhanced when *csw* activity is reduced. To test this model, flies of the appropriate genotype were generated. We found that a reduction in *csw* activity greatly enhanced the loss-of-function phenotype generated by the DER^{DN} sensitized background. Specifically, we found that for both csw^{LE120} and csw^{6}, the eyes of $csw/+,\ C253\text{-}DER^{DN}$ flies were smaller than their $+/+,\ C253\text{-}DER^{DN}$ siblings (Fig. 8) and the eggs derived from $csw/+,\ T155v\text{-}DER^{DN}$ mothers were more severely ventralized than their $+/+,\ T155v\text{-}DER^{DN}$ siblings (Fig. 9). The phenotypic differences observed were more pronounced at 29°C than at 25°C, suggesting that different levels of expression of the $UAS\text{-}DER^{DN}$ were achieved at the two temperatures. In conclusion, our results provide supporting genetic evidence for a role of Csw as a positive signal transducer that acts downstream of DER.

SHP-2 Encodes a Functional Vertebrate Homologue of Csw

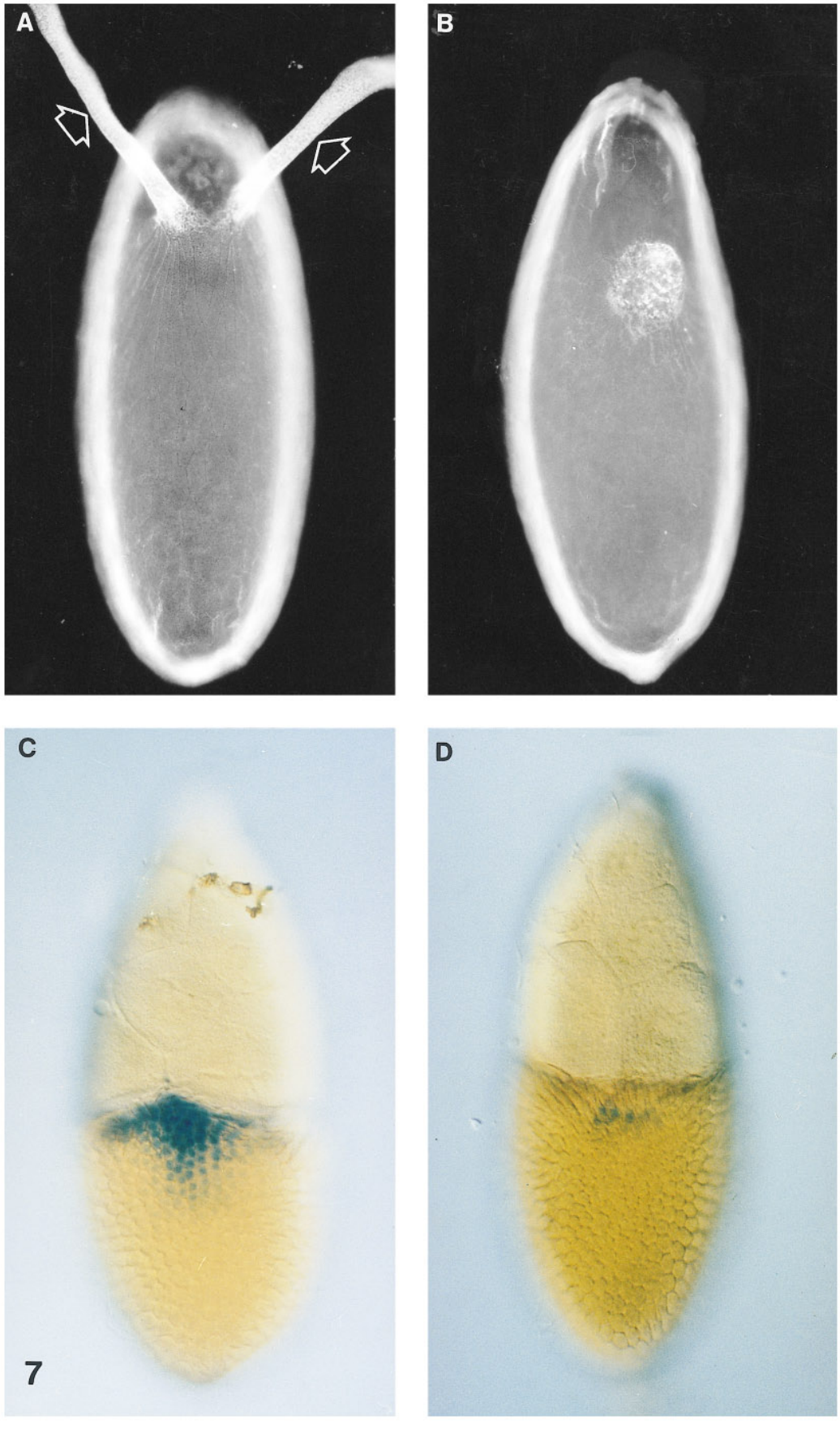
The overall structure of Csw is like that of the mammalian PTPases SHP-1 and SHP-2 (formerly known as PTP1C, SHPTP-1, HCP, SHP and SHPTP-2, SHPTP-3, Syp, PTP1D, PTP2C, respectively; see Adachi *et al.*, 1996) which feature tandem SH2 domains in their N-terminal noncatalytic regions. However, Csw distinguishes itself with a unique "PTPase insert" sequence that interrupts the catalytic domain (Perkins *et al.*, 1992). The overall sequence similarities between these molecules (reviewed in Neel, 1993) reveal that Csw is more similar to SHP-2 (63.2%) than Csw is to SHP-1 (50.1%). Further, SHP-2 is more similar to Csw than it is to SHP-1 (54.7%).

To determine whether SHP-1 or SHP-2, or perhaps both PTPases, represent functional vertebrate homologues of Csw, we expressed each mammalian PTPase in flies. We observed that expression of SHP-2, but not SHP-1, in both weak csw^{6} and severe csw^{LE120} and csw^{VA199} mutant larvae and pupae was able to rescue the pupal lethality associated with *csw* mutations (Table 4). Since both mammalian constructs are being overexpressed following heat shock treatment it could be argued that the functional overlap is nonspecific. However, this is unlikely since flies zygotically rescued with SHP-2 exhibit, under appropriate conditions, adult phenotypes similar to those of partially zygotically rescued *csw* mutant adults (see Fig. 6). Thus, SHP-2, but not SHP-1, represents a functional mammalian homologue of Csw.

DISCUSSION

In this study we have examined whether Csw operates downstream of *Drosophila* RTK signaling pathways other than Tor. Using a variety of molecular markers we provide evidence for the requirement of Csw in developmental pathways under the control of the RTKs DER and Btl, and possibly others. Our phenotypic analysis of *csw* mutants indicates that Csw is required to positively transduce signals from these RTKs. This model is further supported by results from genetic dosage experiments which indicate that a decrease in *csw* activity enhances the phenotype associated with a reduction in *DER* activity. Based on these observations, as well as the *csw*-rescuing activity of SHP-2, a verte-

FIG. 7. Csw is required during oogenesis. During oogenesis Csw function is required in the follicle cells for proper formation of the egg shell or chorion. In wild type (A) paired dorsal appendages (open arrows) extend from the anterodorsal surface of the chorion, whereas in csw^{VA199} mutant egg chambers (B) the dorsal appendages are rudimentary and fused. The enhancer trap line BB142 labels precursors of dorsal chorionic cells from mid oogenesis (C). In csw^{VA199} mutant egg chambers BB142 staining is largely absent (D), suggesting that dorsal chorionic cell fates are deleted from *csw* mutant egg chambers from as early as mid (stage 10) oogenesis. All eggs are dorsal views and anterior is up.


A
B
C
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7

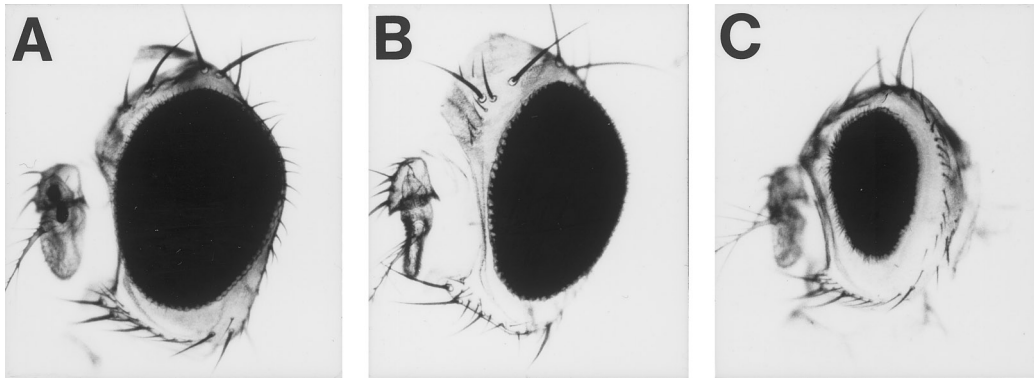

FIG. 8. Dosage interactions between Csw and DER in the eye. Relative to wild type (A) eyes are reduced in size when one copy of *UAS-DER^{DN}* is expressed in the *Gal4-C253* expression pattern (B; genotype +/+; *Gal4*/+; *UAS-DER^{DN}*/+). The severity of the phenotype shown in B is enhanced when the dosage of *csw* is reduced; i.e., eyes are further reduced in size (approximately by 30%) in flies of genotype *csw*/+; *Gal4*/+; *UAS-DER^{DN}*/+ (C). Two *csw* alleles that gave similar results, *csw^{6}* and *csw^{LE120}*, were used in this analysis. The size of the eyes of *csw*/+ adults is indistinguishable from wild type (data not shown). Left eyes are shown with dorsal up and one medially located antennae is shown on the left side of each eye.

brate PTPase similar to Csw, we propose that Csw/SHP-2 is part of the evolutionarily conserved cassette of signal transducers that operate downstream of all RTKs. Further examination of the function of Csw in the Tor and DER pathways establishes that signaling from these RTKs diverges at the level of Csw demonstrating that RTK signaling involves both a Csw-dependent and a Csw-independent pathway.

Csw Positively Transduces Signals from the DER and Btl RTKs

A number of findings suggest that Csw acts as a positive transducer of DER. During embryogenesis DER is involved in the establishment of ventral cell fates, survival of amnioserosa and ventral ectodermal cells, CNS development, production of embryonic cuticle, and germband retraction (Clifford and Schupbach, 1992; Raz and Shilo, 1993). Similarly, we find that in *csw* mutant embryos ventral ectodermal cell fates are missing, suggesting a defect in the determination of these cell fates. Frequently in null *csw* mutant embryos germband retraction fails and the horizontal commissures in the CNS collapse. During imaginal development DER is involved in a variety of developmental processes within the compound eyes and ocelli, the aristae, wing veins, tarsal claws, specific macrochaetae, male sex combs, and female genital disc derivatives (Clifford and Schupbach, 1989). We find that Csw also is required during imaginal development for specification of many of these structures, including the compound eye, aristae, tarsal claws, and wing veins. Further, we find that a reduction in *csw* activity in follicle cells is associated with production of eggs with a partially ventralized phenotype. This phenotype is reminiscent of the function of DER during oogenesis (Price *et al.*, 1989). Finally, we demonstrate that a reduction in *csw* activity enhances significantly a reduction in *DER* activity in follicle cells, as well as in the developing eye imaginal disc, providing more direct genetic evidence for a role of Csw downstream of DER.

During mid to late embryogenesis *csw* is required for proper tracheal morphogenesis, a developmental process known to be specified by the *Drosophila* FGF receptor Btl. In *csw* mutant embryos, as observed in *btl* mutants, tracheal precursor cells fail to migrate into their final positions. During embryogenesis Btl is expressed in invaginating endodermal, mesectodermal, and epidermal cells (Glazer and Shilo, 1991; Shishido *et al.*, 1993). Analysis of *btl* mutations (Klambt *et al.*, 1992; Reichman-Fried *et al.*, 1994) indicate a role in cell migration for this RTK since in *btl* mutant animals, subsets of glial cells fail to migrate to their proper positions during embryonic CNS formation. In addition, in *btl* mutant animals the embryonic tracheal tree does not differentiate properly and exhibits a phenotype which has been associated with defective tracheal cell migration, but not division and differentiation. These results suggest strongly that Csw is a positive transducer of Btl signaling.

Csw Is a Universal Component of RTK Signaling

Our results are consistent with the hypothesis that Csw is a positive signal transducer that acts downstream of three distinct RTKs, Tor, DER, and Btl. Further, Csw is also known to function in the Sevenless RTK signaling pathway (Allard *et al.*, 1996). As mutations in other RTK family members are characterized it might be possible to correlate additional *csw* mutant phenotypes and thereby implicate Csw in these pathways. In this regard, a novel RTK family member, encoded by the gene *derailed* (*drl*) has recently been found to be essential in the embryonic CNS for proper selection of axonal pathways by extending growth cones (Callahan *et al.*, 1995). At this time

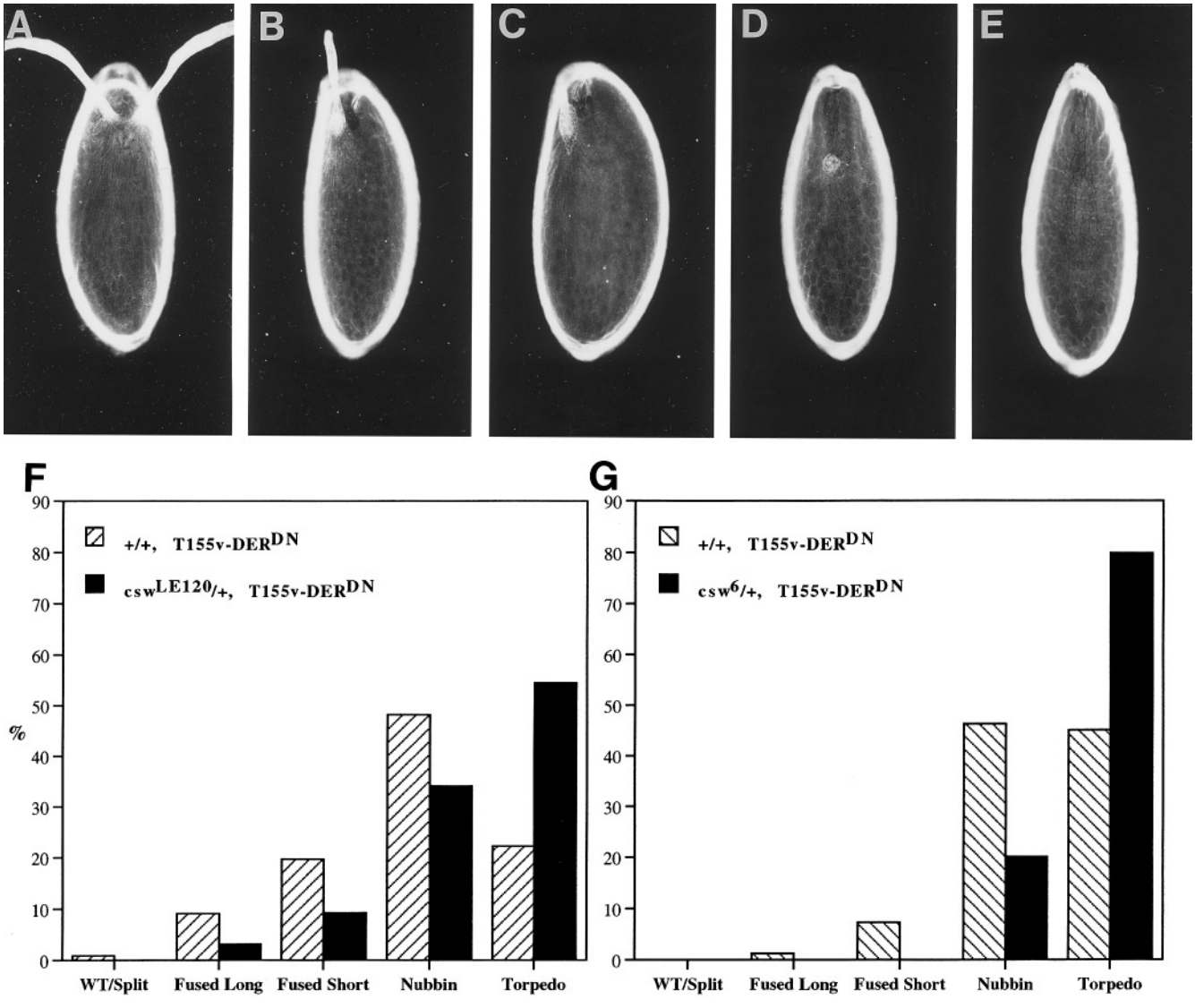

FIG. 9. Dosage interactions between Csw and DER during oogenesis. A series of phenotypes is observed when one copy of *UAS-DER*^{DN} is expressed in the *Gal4-T155v* expression pattern. With respect to the dorsal appendages the phenotypes range from wild type where the dorsal appendages are split (A), through varying degrees of fusion: fused and elongated beyond the anterior end of the egg (B; the fused long phenotype), fused but not elongated beyond the anterior end of the egg (C; the fused short phenotype), nubbin (D), and the most severe phenotype where the dorsal appendages are entirely deleted (E; the torpedo phenotype). F and G reveal the phenotypic distributions of chorions observed when one copy of *UAS-DER*^{DN} is expressed in the *Gal4-T155v* expression pattern (hatched), and in the same sensitized background when the dosage of *csw* is reduced by one half (solid). The experiment depicted in F was carried out at 25°C; the genotype of the chorions represented by the hatched bars is +/+, *T155v-DER*^{DN} (n = 446) and the genotype of the chorions represented by the solid black bars is *csw*^{LE120}/+, *T155v-DER*^{DN} (n = 427). The experiment depicted in G was carried out at 29°C; the genotype of the chorions represented by the hatched bars is +/+, *T155v-DER*^{DN} (n = 82), and the genotype of the chorions represented by the solid black bars is *csw*^{6}/+, *T155v-DER*^{DN} (n = 229). In both F and G a significant shift toward more severe phenotypes is observed when the dosage of *csw* is reduced by one-half. The chorions of eggs derived from *csw*/+ females are indistinguishable from wild type at both 25 and 29°C (data not shown). All eggs are near dorsal views with anterior up.

we cannot conclude that the CNS phenotype of Csw mutant embryos reflects a role in the Drl RTK pathway since the overall structure of the CNS, as assayed by α-HRP, of *drl* mutant embryos is indistinguishable from wild type (Callahan *et al.*, 1995). Finally, identification of additional *csw* phenotypes may identify other RTK pathways

TABLE 4
Functional Conservation of Csw with Similar Mammalian Molecules

	Rescued csw/Y males	Sibling FM7/Y males
Csw^{Y1229}	184 (64.6%)	285
SHP-2	125 (37.0%)	338
SHP-1	0	>300

Note. Equal numbers of males of each genotype are expected for full rescue; however, Csw^{Y1229}, the wild-type cDNA of *csw*, rescues 64.6% of the expected *csw/Y* males and SHP-2 rescues 37%.

that have not yet been described and/or identified. For example, recent studies of Csw in the developing embryonic muscle and heart precursors implicate Csw as a positive transducer of the DER pathway, as well as an undescribed signaling pathway (A. Michelson and L. Perkins, in preparation).

We conclude that like other known transducers of RTKs (e.g., Drk/Grb2, Ras, Raf, MEK, and MAPK) Csw is part of the evolutionarily conserved cassette of signal transducers that operate downstream of RTKs. To identify a functional mammalian homologue of Csw, we tested the rescuing activity of two PTPases, SHP-2 and SHP-1, that share substantial structural and sequence homologies with Csw (see Results). Interestingly, we found that SHP-2, but not SHP-1, rescues *csw* mutant phenotypes. Recently SHP-1, whose expression is predominantly in hematopoietic cells, has been implicated as a negative regulator of signaling by cytokine receptor family members (see review by Imboden and Koretsky, 1995). Specifically, proliferative signals, generated by binding of erythropoietin (EPO) to its receptor (EPOR), are terminated upon binding of SHP-1 to the EPOR (Klingmuller *et al.*, 1995). Thus, it may not be surprising that this PTPase does not rescue *csw* mutants since it probably cannot operate as a positive transducer in RTK signaling pathways. However, SHP-2 has been implicated in RTK-initiated pathways (reviewed in Sun and Tonks, 1994). The *Xenopus* homolog of SHP-2 has been cloned and, like Csw, XSHP-2 has been shown to be maternally expressed and functions as a positive transducer downstream of the FGF RTK which is required for mesoderm induction during early development (Tang *et al.*, 1995).

The Role of Csw in RTK Signaling

Two sets of observations suggest that in the absence of Csw activity, RTK signaling still operates. First, in the Tor pathway the most severe, genetically null *csw* alleles delete only a subset of the structures affected by genetically null *tor* mutations. This can be visualized at the blastoderm stage where posterior *tll* expression is reduced in *csw* mutant embryos but *tll* is entirely deleted in tor^{lof} embryos. Second, in the DER pathway for specification of ventral ectodermal cell fates, in severe, genetically null *csw* alleles the positional information between the ventrolateral Keilin's organs is 36.6% of wild type (see Results), while in genetically null *DER* alleles only 25% of the information is retained (Raz and Shilo, 1993). These observations are reminiscent to those made for Drk, Sos, and Ras1 (Hou *et al.*, 1995), which revealed the existence of parallel activities. Our studies suggest that in the case of Tor, DER, and possibly others, Csw operates in only one of these parallel pathways and therefore transduces only part of the RTK-generated signal.

The biochemical roles of the Csw/SHP-2 PTPases in RTK signaling is not yet understood, however, the presence of two SH2 domains in these enzymes suggests that they could directly bind activated RTKs. This model is supported by studies with SHP-2, which physically associates with the PDGF and EGF receptors (Feng *et al.*, 1993; Vogel *et al.*, 1993; Lechleider *et al.*, 1993) and the insulin receptor substrate IRS-1 (Kuhne *et al.*, 1993). Further, upon binding to the EGF or PDGF receptors, SHP-2 becomes tyrosine phosphorylated (Vogel *et al.*, 1993; Feng *et al.*, 1993; Lechleider *et al.*, 1993). One of the sites of tyrosine phosphorylation within SHP-2 provides a binding site for Grb2 (Bennett *et al.*, 1994; Li *et al.*, 1994). These findings suggest a mechanism whereby upon PDGF receptor activation SHP-2 is recruited to the receptor, becomes tyrosine phosphorylated which in turn recruits the Grb2/Sos complex to the membrane thereby activating Ras.

This model is not inconsistent with the function of Csw in *Drosophila* RTK signaling since Csw associates directly with the Tor RTK and becomes tyrosyl phosphorylated upon receptor activation (Cleghon *et al.*, 1996). Further, *csw* mutant phenotypes can be rescued by injection of v-ras proteins into precellular *csw* mutant embryos (Lu *et al.*, 1993a), suggesting that Csw could activate Ras by bringing the Grb2/SOS complex to the receptor. However, this model does not account for the PTPase catalytic activity of Csw.

The PTPase activity of XSHP-2 is essential since a deletion of the catalytic active site results in a dominant negative phenotype which can be rescued by coinjection of wild type along with the catalytic dead XSHP-2 (Tang *et al.*, 1995). Similarly, in *Drosophila* dominant negative phenotypes are observed when catalytic dead Csw proteins are expressed during embryogenesis (M. B. Melnick *et al.*, in preparation). Therefore, in the *Drosophila* and *Xenopus* developmental pathways in which Csw/SHP-2 is required, PTPase activity is essential for function. It should be kept in mind, however, that although the PTPase domain has been implicated in Csw/SHP-2 signaling, no role for this domain has yet been described. Further analyses are clearly required to determine the exact role of Csw in RTK signaling.

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