Mutational Analysis of the SRC Homology 2 Domain Protein-tyrosine Phosphatase Corkscrew*

John D. Allard‡, Ronald Herbst§, Pamela M. Carroll, and Michael A. Simon

From the Department of Biological Sciences, Stanford University, Stanford, California 94305-5020

Receptor tyrosine kinases (RTKs) regulate cellular growth and differentiation in response to extracellular signals. The binding of a specific ligand to the extracellular domain of an RTK leads to a well-characterized series of biochemical events, including RTK dimerization, activation of the cytoplasmic tyrosine kinase domain, and phosphorylation of the RTK on specific tyrosine residues (reviewed in Ref. 1). The activated and phosphorylated RTK then regulates key cellular target proteins. Frequently, these targets contain either SRC homology 2 (SH2) domains or phosphotyrosine binding domains that bind to specific phosphorylated tyrosine residues of the RTK. The recruitment of such SH2- and phosphotyrosine binding domain-containing proteins regulates their function and leads to the activation of important intracellular signaling pathways. For example, the interaction of the SH2 domain-containing protein GRB2 with activated RTKs leads to the stimulation of the RAS pathways (2, 3).

Among the key signaling proteins, the function of which is required during RTK signaling, is a class of protein-tyrosine phosphatases that includes SHP2 in mammals and Corkscrew (CSW) in Drosophila (reviewed in Ref. 4). In addition to their protein-tyrosine phosphatase (PTP) domains, these proteins are characterized by the presence of two SH2 domains at their amino terminus and a poorly conserved carboxy-terminal “tail” region. In contrast, CSW contains a domain of approximately 150 amino acids that interrupts its PTP domain and is not present in SHP2 (5). The first evidence that SHP2/CSW proteins were important for RTK signaling came from the identification of CSW as a maternal contributor, positively acting component during signaling by the Drosophila TORSO RTK (5). Subsequent studies have implicated CSW in signaling by several other Drosophila RTKs including SEV and the Drosophila epidermal growth factor receptor (6, 7). In vertebrates, SHP2 has been shown to participate in signaling by the epidermal growth factor, PDGF, fibroblast growth factor, and insulin receptors (reviewed in Refs. 4 and 8).

Two potential mechanisms for the action of SHP2/CSW during RTK signaling have been proposed. In the case of certain RTKs, such as the PDGF receptor, RTK autophosphorylation creates binding sites for the SH2 domains of SHP2 (9, 10). Once bound to receptor, SHP2 becomes phosphorylated at a particular tyrosine residue in its tail region. Phosphorylated SHP2 can then be bound by the SH2 domain of GRB2. The recruitment of GRB2 to the SHP2/RTK complex is proposed to lead to the activation of the RAS signaling pathway. Studies of signaling by the SEV RTK in Drosophila have led to an additional model for CSW action during RTK signaling (6, 11). The activation of SEV induces neuronal differentiation of the R7 photoreceptor precursor cell (for reviews, see Refs. 12–14). In the R7 cell, CSW acts as a positive component of the signaling pathway initiated by SEV (6). Genetic epistasis experiments have demonstrated that CSW function is required in the developing R7 cell even when constitutively activated forms of RAS and RAF are used to initiate the SEV signaling cascade. These experiments have suggested that although CSW may be involved in RAS activation, CSW must also play a role either downstream of RAS/RAF activation or in a parallel signaling pathway that acts in conjunction with the RAS/MAPK pathway.

One approach to understanding the role of CSW/SHP2 during RTK signaling has been to identify substrates for their phosphatase activity. A CSW substrate, the Daughter of Sev-
enless protein) (DOS), was identified as a tyrosine-phosphorylated protein trapped in a complex with a catalytically inactive version of CSW (11). DOS contains an amino-terminal pleckstrin homology domain and is most similar to the family of multiadapter proteins that includes Gab-1 and IRS-1 (15). The analysis of mutations that inactivate the dos gene has indicated that DOS is a positive component of the SEV pathway (11, 15). Together, these biochemical and genetic studies have led to the proposal that DOS must be dephosphorylated by CSW during SEV signaling. Searches for SHP2 substrates in mammalian cells have also led to the identification of a number of potential substrates. At present, these include the transmembrane protein SHPS1/SIRPα1s, IRS-1, the PDGFR, and several tyrosine-phosphorylated proteins with sizes ranging from 80 to 120 kDa (reviewed in Refs. 4 and 8).

In order to define further the role of CSW/SHP2 during RTK signaling, we have mutated each of the domains of CSW and investigated their effects on SEV signaling and Drosophila development. In this report, we provide evidence that the the PTP domain and at least one functional SH2 domain are essential for CSW function. Analysis of the ability of either mutant or wild type CSW to interact with DOS and SEV indicates that the association in vivo of CSW with SEV is not dependent on either the tyrosine phosphorylation of SEV or the presence of functional SH2 domains in CSW. In contrast, the association of DOS and CSW requires functional CSW SH2 domains. These results suggest that the role of the SH2 domains is not in binding to SEV but is instead in the recognition of substrates, such as DOS. In addition, we found that a deletion of either the unique CSW PTP insert region or the conserved GRB2 binding site-containing carboxyl terminus of CSW does not appreciably reduce CSW function during signaling by SEV, TORSO, and perhaps all RTKs.

MATERIALS AND METHODS

Genetics and Histology—Drosophila culture and crosses were carried out using standard procedures. The csw13–87 and csw1114 allele used in this study have been characterized as genetic nulls, whereas the csw6 allele is a partial activity. All three alleles are recessive lethal (5). Tissue sectioning and scanning electron microscopy was performed as described (6).

Generation of csw Mutants and P-element Transformation—Mutations in csw were generated using oligonucleotide mutagenesis as described previously (6). The Arg to Lys substitutions in the SH2 domains of CSW were generated using oligonucleotide 5′-AGGAGAAGAGCTT-GCCGAGGAAC-3′ for cswR32K and oligonucleotide 5′-TCTGAGATTC-CTTGACGAGAAAC-3′ for cswR137K. The cswR32K/R137K construct was made by subcloning fragments from the constructs containing the single point mutations. Deletions of the CSW PTP domain insert (amino acids 290–444) and the carboxyl terminus (amino acids 551–841) were generated using the oligonucleotides 5′-GATGATGCTTAAAC-CAATGCGTGCCTGGCGGGCCAGC-3′ and 5′-GACCTGAAATTCATTGTGACGGCAGCTGGGCTTATATA-3′, respectively. The csw construct encoding only the SH2 domains (cswSH2SH2) was made by placing a stop codon after residue 231 in the csw cDNA. The csw mutant cDNAs were subcloned into the transformation vector pKb267, which contains a hybrid promoter (SE) consisting of sevenless enhancer and the hsp70 promoter (16). This vector directs general expression in a subset of cells in the Drosophila eye, including the photoreceptor R7, R3, and R4 precursor cells and all four of the cone cell precursors. In addition, the SE cassette also provides pulses of ubiquitous expression when the flies are grown at 37 °C for short periods.

The mutated SE-csw constructs were introduced into the genome by P element-mediated germline transformation and assayed for function. In order to control for possible variation in transcription due to P element insertions at different sites in the genome, several different insertions were analyzed for each construct. In each case, equivalent results were obtained. The function of the mutated CSW proteins was tested in three ways. The first assay took advantage of a particular allele of csw that we had previously isolated in a genetic screen for mutations that attenuate signaling by SEV (18). Although in vitro analysis of the CSW[547E] protein indicates that it retains the ability to dephosphorylate model substrates such as p-nitrophenyl phosphate (data not shown), the mutant protein in vivo lacks normal CSW function and inhibits the functioning of wild type CSW (6). The expression of CSW[547E] in the developing eye under SE transcriptional control blocks development of the R3, R4, and R7 photoreceptors and leads to flies with small eyes that are rough and disordered (6) (see Fig. 3). Because expression of wild type CSW under SE control suppresses the effects of SE-csw[G547E], we assayed the signaling ability of mutant CSW proteins by determining whether their expression in the sevenless transcriptional pattern could also suppress the SE-csw[G547E] phenotype.

The second assay took advantage of previous observations that animals that lack zygotic CSW function die during pupation due to severe developmental abnormalities in many of their tissues (5, 7). Heat shock promoter-driven expression was therefore used to determine whether the mutant proteins could

RESULTS

Our approach to investigating the functional role of each of the domains of CSW was to introduce domain-specific mutations into a construct that contained a csw cDNA expressed under the control of a hybrid sevenless enhancer/heat shock promoter transcriptional control element (16). This transcription unit (SE) directs constitutive expression in a subset of cells in the Drosophila eye, including the photoreceptor R7, R3, and R4 precursor cells and all four of the cone cell precursors. In addition, the SE cassette also provides pulses of ubiquitous expression when the flies are grown at 37 °C for short periods.

The mutated SE-csw constructs were introduced into the genome by P element-mediated germline transformation and assayed for function. In order to control for possible variation in transcription due to P element insertions at different sites in the genome, several different insertions were analyzed for each construct. In each case, equivalent results were obtained. The function of the mutated CSW proteins was tested in three ways. The first assay took advantage of a particular allele of csw that we had previously isolated in a genetic screen for mutations that attenuate signaling by SEV (18). Although in vitro analysis of the CSW[547E] protein indicates that it retains the ability to dephosphorylate model substrates such as p-nitrophenyl phosphate (data not shown), the mutant protein in vivo lacks normal CSW function and inhibits the functioning of wild type CSW (6). The expression of CSW[547E] in the developing eye under SE transcriptional control blocks development of the R3, R4, and R7 photoreceptors and leads to flies with small eyes that are rough and disordered (6) (see Fig. 3). Because expression of wild type CSW under SE control suppresses the effects of SE-csw[G547E], we assayed the signaling ability of mutant CSW proteins by determining whether their expression in the sevenless transcriptional pattern could also suppress the SE-csw[G547E] phenotype.

The second assay took advantage of previous observations that animals that lack zygotic CSW function die during pupation due to severe developmental abnormalities in many of their tissues (5, 7). Heat shock promoter-driven expression was therefore used to determine whether the mutant proteins could
provide sufficient CSW function to rescue these defects in animals that otherwise lacked CSW function. The test was conducted by crossing flies carrying the SE-csw transgene to females heterozygous for a loss-of-function csw allele in order to generate males that were hemizygous for the defective csw allele but carried the SE-csw transgene. For these assays, we generally used both the csw13–87 and cswC114 alleles. Each of these alleles has been genetically characterized as a loss-of-function allele (5).

The third assay was applicable only to the cases in which expression of the mutated protein was able to rescue the lethality of csw animals. In these cases, the resulting csw13–87 males carrying the SE-csw transgene were crossed to csw13–87/FM7c females in order to generate females that were homozygous for the csw13–87 allele but were alive due to rescue by the expression of the mutant CSW. These females were then tested for their fertility. Since maternal contribution of CSW is essential for TORSO signaling and proper embryonic development, the fertility of these females is a sensitive assay for the ability of the mutant CSW to function during TORSO signaling (5).

SH2 Domain Function Is Essential for CSW Action—SH2 domains possess an invariant arginine residue that is contained in a highly conserved motif (FLVRES) (19). Mutagenesis and crystallography studies have shown that this arginine residue is crucial for the interaction between an SH2 domain and the phosphotyrosine of its target binding sequence (20). We therefore tested the importance of the CSW SH2 domains by mutating the corresponding arginines of each SH2 domain (Arg32 and Arg137) to lysines and assaying the ability of the mutant protein to function during TORSO signaling (5). A construct containing mutations in both SH2 domains was inactivated. Similar results were obtained with both CSWR32K and CSWR137K. In each case, the mutant protein was capable of providing sufficient CSW function to rescue the lethality of male flies (csw13–87/Y) otherwise lacking CSW function and of providing maternal CSW function in eggs derived from homozygous csw13–87 females (Fig. 1). Furthermore, the rescued male flies had smooth eyes with normally constructed ommatidia (data not shown). These results indicated that each of the individual SH2 mutant proteins was capable of providing sufficient CSW function to fulfill the normal roles of CSW and suggest that the two SH2 domains may perform redundant functions.

In contrast to their ability to function as the sole maternal or zygotic source of CSW function during normal development, neither of the single SH2 domain mutated proteins was effective at suppressing the rough-eyed phenotype of SE-cswG547E flies (Table I). This may indicate that expression of CSWG547E interferes with normal CSW function by using both of its SH2 domains to bind to an essential regulator or target of CSW. The CSWR32K and the CSWR137K proteins may be less effective competitors than wild type CSW because of the reduced binding affinity caused by the loss of one of the SH2 domain interactions.

The SH2 Domains Function in Substrate Recognition during SEV Signaling—One possible role for the SH2 domains of CSW during SEV signaling is to allow CSW to bind to activated SEV. We first sought to identify whether the SH2 domains of CSW might be required to allow CSW to interact with activated SEV. We first sought to

![FIG. 1. csw constructs and their ability to provide CSW function. Shown is a schematic representation of the mutant constructs generated in this study. Open boxes, CSW SH2 domains; shaded boxes, CSW PTP domain; black box, first 90 amino acids from SRC64, which contain the myristylation site. Point mutations generated by in vitro mutagenesis are indicated by asterisks. The constructs were introduced into the germ line and expressed from the hybrid SE casette. Zygotic CSW function was scored by the ability of hemizygous csw13–87/Y males to survive in the presence of the csw transgene. A + indicates >50% of the expected class survived. A - indicates <1% of the expected class survived. Maternal CSW function was scored by the ability of zygotically rescued csw13–87 females to produce wild type progeny. A + indicates >50% of the progeny were wild type. More than 200 animals were scored in each experiment. nd, not determined.](Image 254x527 to 554x729)
Fig. 2. CSW interaction with SEV and DOS. A, lysates from SL2 cells expressing SEV<sup>ST1KM</sup> and CSW were immunoprecipitated with anti-SEV antibodies and immunoblotted with anti-CSW-CT serum (upper panel). IP, immunoprecipitation. Total cell lysates were immunoblotted with anti-SEV as a control (lower panel). B, equal amounts of glutathione S-transferase-CSW SH2 domain constructs were incubated with cell lysates prepared from pervanadate-treated SL2-DOS cells. Precipitates were subjected to immunoblot with anti-DOS-1. SH2<sup>SH2</sup>, R32K mutant; SH2<sup>SH2</sup>, R137K mutant; SH2<sup>SH2</sup>, R32K/R137K mutant.

The PTP Activity Is Important for CSW Function—Our previous studies had suggested that the PTP activity of CSW is essential for CSW function during photoreceptor development (6, 11). In those studies, we had shown that expression of either of two CSW proteins carrying mutations in the PTP domain (CSW<sup>G547E</sup> and CSW<sup>C683S</sup>) inhibit development of each of the photoreceptors in which they are expressed. Because each mu-
tation affects the PTP domain of CSW, these results implied that CSW PTP activity is important for CSW function during photoreceptor development. However, these experiments did not directly test whether the catalytic activity of CSW was absolutely required for all CSW function. In order to verify that the catalytic activity of CSW is crucial for function, these mutant proteins were tested for their ability to rescue the lethality of male animals hemizygous for the null csw<sup>13-87</sup> allele. We also tested an additional version of CSW (CSW<sup>SH2SH2</sup>) in which the entire PTP domain and tail region were deleted (Fig. 1). SE expression of the CSW<sup>SH2SH2</sup> protein yielded roughened eyes and photoreceptor defects that were similar to those seen in flies expressing either CSWG547E or CSWC583S (data not shown). We found that none of the three mutant proteins was able to rescue the viability of any csw<sup>13-87</sup> individuals (Fig. 1). These results indicate that CSW PTP domain function is crucial for CSW action.

**CSW<sup>C583S</sup> Retains Partial Function in the Absence of Catalytic Activity**—Despite this strong evidence that CSW PTP activity is needed for CSW action, one feature of our previous results was inconsistent with the idea that CSW function absolutely requires catalytic activity. As described above, the csw<sup>G547E</sup>, csw<sup>SH2SH2</sup>, and csw<sup>C583S</sup> alleles each encode a protein that interferes with the function of wild type CSW. A probable explanation for these inhibitory effects is that the mutant proteins use their SH2 domains to compete with wild type CSW for binding to the CSW substrate DOS but then fail to dephosphorylate DOS. Surprisingly, expression of the CSW<sup>C583S</sup> protein has a much less profound inhibitory effect on photoreceptor development than does the expression of either the CSW<sup>G547E</sup> or CSW<sup>SH2SH2</sup> proteins (Table I and Fig. 3). These results were puzzling because the csw<sup>C583S</sup> mutation changes an evolutionarily conserved cysteine residue that is absolutely required for the PTP activity (4, 24). Thus, CSW<sup>C583S</sup> entirely lacks catalytic activity (data not shown), and hence, its expression might have been expected to yield the most severe phenotype.

These results suggested that CSW<sup>C583S</sup> might retain some of its signaling abilities despite its lack of catalytic activity. However, another possible explanation for these results was that the CSW<sup>C583S</sup> protein was merely expressed at lower levels than either the CSW<sup>G547E</sup> or the CSW<sup>SH2SH2</sup> protein. We tested this possibility by generating flies that carried both the P[SE-csw<sup>G547E</sup>] and the P[SE-csw<sup>C583S</sup>] transgenes. If the difference between the phenotypes caused by the two transgenes was merely a result of differences in the expression or stability of the two mutant CSW proteins, then the combined phenotype of both transgenes would be expected to be at least as severe as the P[SE-csw<sup>G547E</sup>] phenotype alone. The eyes of the P[SE-csw<sup>G547E</sup>], P[SE-csw<sup>C583S</sup>] flies were less severely roughened than those of P[SE-csw<sup>G547E</sup>] flies and more similar to those of P[SE-csw<sup>C583S</sup>] flies (Fig. 3). These results suggested that the CSW<sup>C583S</sup> protein retains some of its function and can thus partially rescue the SE-csw<sup>G547E</sup> inhibitory phenotype.

Since the effect of expression of CSW<sup>C583S</sup> on the P[SE-csw<sup>G547E</sup>] phenotype was relatively weak, we sought further confirmation of the positive signaling ability of the CSW<sup>C583S</sup> protein. Our approach was to create a version of CSW<sup>C583S</sup> that might possess greater function. Since our previous experiments had indicated that wild type CSW function could be enhanced by constitutive localization to the plasma membrane, we targeted CSW<sup>C583S</sup> to the plasma membrane with the myristylation sequences at the amino terminus of the SRC64 protein. The resulting protein, CSW<sup>C583Ssrc90</sup>, was then placed under the control of the SE expression cassette, and the resulting P element (P[SE-csw<sup>C583Ssrc90</sup>]) was inserted into the genome by P element-mediated transformation. The first apparent difference between the effects of the P[SE-csw<sup>C583Ssrc90</sup>] element and either the P[SE-csw<sup>C583S</sup>] or P[SE-csw<sup>G547E</sup>] elements was that presence of the P[SE-csw<sup>C583Ssrc90</sup>] element did not yield a rough eye phenotype (Fig. 3). Sectioning of the eyes of the P[SE-csw<sup>C583Ssrc90</sup>] flies did reveal minor abnormalities, usually the absence of the R7 cell, in approximately 10% of the ommatidia.

We then generated flies carrying both P[SE-csw<sup>C583Ssrc90</sup>] and the P[SE-csw<sup>G547E</sup>] elements. The resulting flies were entirely smooth-eyed and showed few defects in their photoreceptor development (Fig. 3). These results indicated that the abil-
ity of the CSW<sup>C583Sarc90</sup> protein to rescue the P[SE-csw<sup>G547E</sup>] phenotype was nearly comparable to that of wild type CSW and thus supported the idea that some of the functions of CSW can be provided by a catalytically inactive CSW. We further investigated the ability of CSW<sup>C583Sarc90</sup> to function by examining whether the P[SE-csw<sup>C583Sarc90</sup>] element could rescue the lethality of animals hemizygous for either the null csw<sup>11–87</sup> allele or for the hypomorphic csw<sup>6</sup> allele (Table II). csw<sup>6</sup> is a weak allele of csw that supports development to a later stage than loss-of-function alleles, such as csw<sup>11–87</sup> (7). CSW<sup>C583Sarc90</sup> expression was unable to restore the viability of the csw<sup>11–87</sup> individuals. In contrast, CSW<sup>C583Sarc90</sup> expression was able to efficiently rescue the lethality of csw<sup>6</sup> individuals. These results demonstrated that a catalytically inactive CSW protein retains a significant level of CSW function.

The PTP Insert and the Carboxyl-terminal Tail of CSW Are Not Required for CSW Function—One difference between the structures of CSW and its vertebrate homolog SHP2 is that CSW contains an insert of approximately 150 amino acids in the amino-terminal region of the PTP domain (5). In order to determine whether the insert performs any crucial function, an allele of csw was constructed in which this region, amino acids 290–444, was deleted. The mutated CSW protein (CSW<sup>Δ290–444</sup>) was expressed under SE control and assayed for function in each of the three assays. In each case, the action of CSW<sup>Δ290–444</sup> was expressed under

| Construct | csw<sup>11–87</sup> | csw<sup>6</sup> |
|-----------|-----------------|-----------------|
| P[SE-csw] | +               | +               |
| P[SE-csw<sup>G547E</sup>] | -               | -               |
| P[SE-csw<sup>C583Sarc90</sup>] | -               | +               |

A catalytically inactive version of CSW can rescue a weak loss of function csw allele

Table II
A catalytically inactive version of CSW can rescue a weak loss of function csw allele

Rescue of CSW function was scored by the ability of hemizygous csw<sup>11–87</sup> or csw<sup>6</sup> males to survive in the presence of the transgene as described under "Materials and Methods."

We have investigated the functional contribution of each of the domains of CSW by generating CSW proteins with domain-specific mutations and evaluating the function of these proteins. Our results indicate that SH2 domain function is required for in vivo CSW function but that this requirement can be met by either SH2 SH2 domain. Furthermore, the ability of either SH2 domain to support CSW function implies that the crucial binding partner(s) for the CSW SH2 domain is likely to possess binding sites for both SH2 domains. Candidates for such binding partners during SEV signaling included SEV itself and DOS, a putative multi-adaptor protein and CSW substrate. In this report, we have shown that although SEV and CSW can be co-immunoprecipitated from Drosophila cells, their association is independent of SH2 domain function and SEV tyrosine phosphorylation. Thus, SEV is unlikely to be a binding partner for the CSW SH2 domains. We cannot presently evaluate whether the observed phosphotyrosine-independent association of CSW and SEV occurs in the developing R7 cell or is important for SEV signaling. In contrast to SEV, DOS does possess binding sites for each of the CSW SH2 domains and thus is a good candidate to be an important target for the CSW SH2 domains.

The ability of either SH2 domain to support CSW function suggests that the two domains have similar roles during CSW action. This finding contrasts with biochemical studies of SHP1, a mammalian SH2 domain PTP closely related to CSW and SHP2 that negatively regulates signaling by many cytokine receptors and RTKs (27, 28). These studies showed that deletions of the amino-terminal SH2 domain of SHP1 led to the activation of PTP activity, whereas deletion of the carboxyl-terminal SH2 domain did not markedly effect PTP activity. These results have led to the proposal that the activity of SHP1 can be regulated in two ways in response to the activation of different receptors. Receptors, such as the erythropoietin receptor, which contain a binding site for the amino-terminal SH2 domain of SHP1, could both recruit and activate SHP1, thus leading to the rapid termination of receptor signaling. In contrast, receptors possessing only binding sites for the carboxyl-terminal SH2 domain would be expected to recruit but not activate SHP1. This might result in a slower termination of signaling. Although it is not known whether such differential regulation of CSW PTP activity occurs, several studies have shown that the binding of CSW to phosphotyrosyl peptide ligands can increase the PTP activity of SHP2 (29–33). However, our results suggest that whether or not such regulation of CSW occurs, neither the binding of CSW to ligands possessing...
only one CSW SH2 domain binding site nor the activation of CSW PTP activity by occupancy of its amino-terminal SH2 domain is necessary for sufficient CSW function to support normal development.

Two lines of evidence suggest that the activity of the PTP domain is also important for CSW function. First, the expression of CSW proteins that either contain mutations in the catalytic domain (CSW\textsuperscript{GS47E} or CSW\textsuperscript{CS8S}) or lack the entire PTP domain (CSW\textsuperscript{SH2SH2}) inhibits the action of wild type CSW. Second, none of these three proteins is able to support the development of animals that otherwise lack CSW function. The ability of CSW\textsuperscript{SH2SH2} to act as an inhibitor suggests that the mechanism of inhibition is competition with wild type CSW for SH2 domain binding partners, such as DOS. Presumably, these PTP domain mutant proteins then fail to dephosphorylate the target molecules and thus fail to support effective RTK signaling.

Despite this clear evidence supporting a crucial role for PTP activity in CSW function, our results have indicated that CSW proteins that lack catalytic activity can display significant CSW function. In particular, we have shown that expression of the CSW\textsuperscript{CS8S} protein can partially rescue the inhibitory effects of the CSW\textsuperscript{GS47E} protein. The ability of CSW carrying the CS8S substitution to function became even more apparent when the protein was targeted to the plasma membrane with SRC64 myristylation sequences. CSW\textsuperscript{CS8Ssrc90} almost completely eliminated the dominant inhibitory effects of CSW\textsuperscript{GS47E} expression and also supported the development of animals that would normally fail to survive due to the presence of the hypomorphic csu\textsuperscript{0} allele.

Since no evidence for CSW function was detected with expression of either of the other PTP domain mutant CSW proteins, the ability of CSW\textsuperscript{CS8S} proteins to signal is likely due to a unique property of the CS8S mutation. Previous crystallographic and enzymatic studies have indicated that one unique property of PTPs in which the catalytic cysteine is mutated to serine is that such molecules can still bind to substrates with high affinity. For instance, our previous work has shown that CSW\textsuperscript{CS8S} can form a stable complex with tyrosine-phosphorylated DOS (11). One possible explanation for the ability of CSW\textsuperscript{CS8S} and CSW\textsuperscript{CS8Ssrc90} to function may be that these proteins can partially mimic the effects of substrate dephosphorylation by merely binding to the site of phosphorylation. For example, if DOS is normally maintained in an inactive state by an intramolecular interaction involving a phosphorylated tyrosine residue, then either dephosphorylation by wild type CSW or binding by an inactive CSW might relieve the inhibitory effect. However, for such a model to be consistent with our finding that expression of CSW\textsuperscript{CS8S} causes a mildly inhibitory phenotype in an otherwise wild type animal, one must postulate that binding by the catalytically inactive CSW is less effective than dephosphorylation by wild type CSW.

Since this work was submitted, a study examining the functional importance of the various domains of SHP2 during fibroblast growth factor-induced mesoderm induction in Xenopus has been published (34). Mutated SHP2 proteins were assayed for their ability to overcome the inhibitory effects on fibroblast growth factor signaling caused by an SHP2 carrying a deletion within the PTP domain. This test was similar to our assay of mutated CSW function in the presence of the inhibitory CSW\textsuperscript{GS47E} protein and yielded similar results. In each case, the mutation of either SH2 domain dramatically reduced SHP2/CSW function. However, the Xenopus studies were able to further show that whereas amino-terminal SH2 domain mutations abolished SHP2 function, SHP2 lacking carboxy-terminal SH2 domain function retained partial function that was apparent when the protein was expressed at elevated levels. Our inability to readily vary the level of CSW expression in the developing eye has prevented us from similarly determining whether CSW proteins lacking carboxy-terminal SH2 domain function also retain a low level of activity in our assay. However, our studies were able to show that the function of each of the SH2 domains was dispensable when assayed by the ability to provide the sole source of CSW rather than by the ability to overcome the effects of an inhibiting protein.

Acknowledgment—We thank members of our laboratory for critically reading the manuscript.

REFERENCES
1. van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) Annu. Rev. Cell Biol. 10, 251–337
2. McCormick, F. (1994) Curr. Opin. Genet. Dev. 4, 71–76
3. Egan, S. E., and Weinberg, R. A. (1993) Nature 365, 781–783
4. Neel, B. G., and Tonks, N. K. (1997) Curr. Opin. Cell Biol. 9, 193–204
5. Perkins, L. A., Larsen, I., and Perrimon, N. (1992) Cell 70, 225–236
6. Allard, J. D., Chang, H. C., Herbst, R., McNeill, H., and Simon, M. A. (1996) Development 122, 1137–1146
7. Perkins, L. A., Johnson, M. R., Melnick, M. B., and Perrimon, N. (1996) Dev. Biol. 180, 63–81
8. Streuli, M. (1996) Curr. Opin. Cell Biol. 8, 182–188
9. Bennett, A. M., Tang, T. L., Sugimoto, S., Walsh, C. T., and Neel, B. G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7385–7389
10. Li, W., Nishimura, R., Kashishian, A., Batzer, A. G., Kim, W. J., Cooper, J. A., and Simon, M. A. (1992) Nature 358, 701–716
11. Herbst, R., Carroll, P. M., Allard, J. D., Schilling, J., Raabe, T., and Simon, M. A. (1996) Cell 85, 899–909
12. Hafen, E., Dickson, B., Raabe, T., Brunner, D., Oellers, N., and van der Straaten, A. (1993) Dev. Suppl. 41–46
13. Simon, M. A. (1994) Dev. Biol. 166, 431–442
14. Zipursky, S. L., and Rubin, G. M. (1994) Annu. Rev. Neurosci. 17, 373–397
15. Raabe, T., Riesgo-Escovar, J., Li, X., Bausewine, B. S., Deak, P., Maroy, P., and Hafen, E. (1996) Cell 85, 911–920
16. Basler, K., Siegrist, P., and Hafen, E. (1989) EMBO J. 8, 2381–2386
17. Simon, M. A., Dodson, G. S., and Rubin, G. M. (1993) Cell 73, 169–177
18. Simon, M. A., Bowtell, D. D., Dodson, G. S., Laverty, T. R., and Rubin, G. M. (1991) Cell 67, 701–716
19. Koch, C. A., Anderson, D., Moran, M. F., Ellis, C., and Pawson, T. (1991) Science 252, 668–674
20. Schaffhausen, B. (1990) Biochem. Biophys. Acta 1242, 81–75
21. Vogel, W., Lammers, R., Huang, J., and Ullrich, A. (1993) Science 259, 1611–1614
22. Feng, G. S., and Pawson, T. (1994) Trends Genet. 10, 84–58
23. Lechleider, R. J., Freeman, R. M., Jr., and Neel, B. G. (1995) J. Biol. Chem. 268, 13434–13438
24. Denu, J. M., Stuecky, J. A., Saper, M. A., and Dixon, J. E. (1996) Cell 87, 361–364
25. Clegon, V., Gayko, U., Copeland, T. D., Perkins, L. A., Perrimon, N., and Morrison, D. K. (1996) Genes Dev. 10, 566–577
26. Bennett, A. M., Hauseroff, S. F., O’Reilly, A. M., Freeman, R. M., and Neel, B. G. (1996) Mol. Cell Biol. 16, 1189–1202
27. Pregel, M. J., Shen, S. H., and Storer, A. C. (1995) Protein Eng. 8, 1309–1316
28. Pei, D., Wang, J., and Walsh, C. T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1141–1145
29. Dechert, U., Adam, M., Harder, K. W., Clark-Lewis, I., and Jirik, F. (1994) J. Biol. Chem. 269, 5692–5691
30. Dechert, U., Affolter, M., Harder, K. W., Matthews, J., Owen, P., Clark-Lewis, I., Thomas, M. L., Aebersold, R., and Jirik, F. (1995) Eur. J. Biochem. 231, 673–681
31. Lechleider, R. J., Sugimoto, S., Bennett, A. M., Kashishian, A. S., Cooper, J. A., Shoelson, S. E., Walsh, C. T., and Neel, B. G. (1993) J. Biol. Chem. 268, 21478–21483
32. Plunsey, S., Wandless, T. J., Walsh, C. T., and Shoelson, S. E. (1995) J. Biol. Chem. 270, 2897–2900
33. Sugimoto, S., Wandless, T. J., Shoelson, S. E., Neel, B. G., and Walsh, C. T. (1994) J. Biol. Chem. 269, 13614–13622
34. O’Reilly, A. M., and Neel, B. G. (1998) Mol. Cell. Biol. 18, 161–177