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Genetic Dissection of Signal Transduction Mediated by the Sevenless Receptor Tyrosine Kinase in Drosophila

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Genetic dissection of signal transduction mediated by the sevenless receptor tyrosine kinase in *Drosophila*

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SUMMARY

The specification of the R7 photoreceptor cell fate in the developing eye of *Drosophila* depends on the local activation of the sevenless (Sev) receptor tyrosine kinase by Boss, a protein expressed on the membrane of the neighbouring R8 cell. Constitutive activation of the Sev receptor results in a dosage-dependent increase in the number of R7 cells per ommatidium. Genetic screens have been used to identify mutations that alter the efficiency of signal transduction. Subsequent molecular characterization of the corresponding genes has led to the identification of a number of proteins involved in transducing the signal from the receptor to the nucleus. In contrast to the receptor and its ligand, these components are shared between different signal transduction pathways not only in *Drosophila* but are also homologous to components involved in signal transduction in other organisms.

1. INTRODUCTION

Cell–cell interactions are important for the specification of cell fate, the control of cell growth and for pattern formation during the development of multicellular organisms. Hence the understanding of the mechanisms of cell–cell communication, in particular the identification of the signals, receptors and modes of intracellular signal transduction are prerequisites for the understanding of these developmental processes. In organisms such as *Drosophila* and *Caenorhabditis elegans* the identification of mutations which result in the misspecification of cells has led to the identification of genes encoding receptors and signals involved in inductive interactions (Horvitz & Sternberg 1991; Dickson & Hafen 1992). In particular, receptor tyrosine kinases have been shown to play key roles in a variety of inductive interactions. In *C. elegans* the let-23 gene encodes an EGF-receptor homologue that receives and transmits an inductive signal emanating from the anchor cell that specifies the vulva (Aroian *et al.* 1990). In *Drosophila*, the Torso receptor tyrosine kinase is expressed on all blastoderm cells but is only locally activated by a ligand released at the two poles of the embryo (Sprenger *et al.* 1989; Casanova & Struhl 1989; Sprenger & Nüsslein-Volhard 1992). Torso activity results in the specification of the terminal structures. Activation of the Sev protein, another receptor tyrosine kinase, in the ommatidial precursors results in the specification of photoreceptor cell fate (Hafen *et al.* 1987; Basler & Hafen 1988; Bowtell *et al.* 1988). Although these different processes are mediated by different receptors the characterization of mutations affecting the transduction of these different signals intracellularly indicates that seemingly different inductive signals are transmitted by a set of common signal transduction components which also function in vertebrates. Here we will summarize recent results on the genetic identification of signal transduction components in the Sev pathway and will compare these results with other signal transduction pathways in *Drosophila*, *C. elegans*, and vertebrates.

2. SPECIFICATION OF THE R7 PHOTORECEPTOR CELL: A MODEL FOR INDUCTIVE SIGNALLING

The compound eye of *Drosophila* consists of 800 identical unit eyes or ommatidia. Each ommatidium is composed of eight photoreceptor cells and 12 accessory cells that are arranged in a pseudocrystalline array (Ready *et al.* 1976). This highly regular pattern is established during the last larval period from an initially unpatterned epithelium, the eye imaginal disc (Tomlinson & Ready 1987*a*). Each ommatidial unit assembles independent of cell lineage restrictions and it has been proposed that cells which have already started to differentiate send signals to their undetermined neighbours inducing them to adopt a specific fate (Lawrence & Green 1979; Tomlinson & Ready 1987*a*; Wolff & Ready 1992).

The specification of the fate of the R7 photoreceptor is best understood. Because this cell is responsible for the positive phototactic behaviour of flies towards UV light it has been possible to isolate mutations that affect the development of this particular photoreceptor cell subtype by a behavioral assay. Perhaps somewhat fortuitously, the genes encoding both the signalling molecule and its receptor, *bride of sevenless* (*boss*) and *sevenless* (*sev*) respectively, seem to be exclusively required for this single decision, and so they could both be readily identified in screens for viable mutations producing aberrant phototactic

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behaviour (Harris *et al.* 1976; Reinke & Zipursky 1988).

The phenotypes produced by loss of function mutations in the genes *boss* and *sevenless* are identical: the R7 precursor cell does not initiate neuronal development, but instead adopts an alternative cell fate, that of a cone cell (Cagan *et al.* 1992; Tomlinson & Ready 1986). Mosaic analysis has shown, however, that the two genes are required in different cells for the proper recruitment of the R7 cell. Whereas sev^+ activity is required only in the R7 precursor itself, $boss^+$ function is required only in a neighbouring cell, the differentiating R8 cell (Campos-Ortega *et al.* 1979; Tomlinson *et al.* 1987*b*; Reinke & Zipursky 1988).

Cloning of these two genes revealed that both encode cell-surface proteins. *boss* encodes a 100 kDa glycoprotein containing a large N-terminal extracellular domain, seven putative transmembrane domains, and a smaller C-terminal cytoplasmic domain (Hart *et al.* 1990). Boss protein is ultimately expressed by all photoreceptor cells, but significantly, at the time of R7 specification, only the oldest photoreceptor cell, the R8 cell, has begun to express Boss (Krämer *et al.* 1991). Although required only in the R7 precursor, the Sev receptor tyrosine kinase is located on the apical surfaces of a number of ommatidial precursor cells: the precursors of the R3, R4, R7 and the cone cells, as well as the mystery cells (Tomlinson *et al.* 1987). Expression is only transient in each cell type, peaking prior to any detectable cellular differentiation. The phenotype of *boss* mutants and the molecular analysis of the *boss* gene suggested that it encodes the ligand for Sev (Hart *et al.* 1990). Compelling evidence for a direct interaction between the two proteins has been provided by cell culture experiments in which Boss-expressing S2 cells were shown to specifically aggregate with S2 cells expressing the Sev receptor (Krämer *et al.* 1991). In these aggregates, Boss protein could also be detected within the Sev-expressing cells. Evidence for a similar interaction *in vivo* comes from the observation that the entire Boss protein is also internalized in a Sev-dependant manner within the R7 precursor cell itself (Krämer *et al.* 1991; Cagan *et al.* 1992).

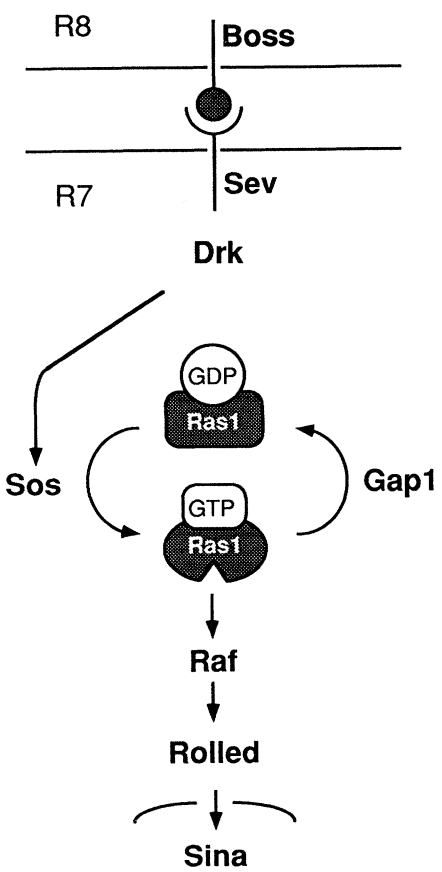

Figure 1. Components of the Sev signalling pathway. The hierarchy between the different components has been established by studying genetical interactions. In the case of Boss and Sev as well as in the case of Sev, Drk and Sos it has been shown biochemically that these interactions are direct. The other arrows do not neccessarily mean that these proteins interact directly.

Although the analysis of loss of function mutants of either *sev* or *boss* has indicated that both genes are essential for the specification of R7 cells it was unclear whether the activation of the Sev receptor is also sufficient to induce R7 development. To test this we created gain of function mutations in *sev* that result in the constitutive, ligand-independent activation of the Sev kinase in all cells that express *sev* in the wild-type. Indeed the constitutive activation of the Sev kinase led to the formation of not only one but multiple R7 cells per ommatidium (Basler *et al.* 1991; Dickson *et al.* 1992*a*). These additional R7 cells developed from the cone cell precursors which normally express the Sev receptor but as they do not contact the ligand-presenting R8 cell, the receptor is not activated in these cells in the wild-type. Similar results were obtained when the *boss* gene was expressed ubiquitously under the control of a heat shock promoter (Van Vactor *et al.* 1991). These results demonstrate that the activation of Sev but not its presence alone is sufficient to specify R7 development not only in the R7 precursor but also in at least some other ommatidial cells.

Experiments with an inducible constitutively activated Sev protein indicate that the competence of ommatidial cells to respond to the inducing signal is both spatially and temporally limited within the eye imaginal disc. First, not all cells can be induced to develop as R7 cells. Second, even those that can assume an R7 cell fate, are able to respond to Sev activity only during a relatively narrow time window. Once these cells have started to differentiate they were refractory the presence of the activated Sev (Dickson *et al.* 1992*a*). Therefore it appear that, in addition to the restriction imposed by the distribution of the Boss protein, other factors acting downstream from the receptor also regulate the competence of cells to respond to this ligand.

3. THE Sev SIGNAL IS TRANSMITTED THROUGH A CASCADE OF GENERAL SIGNAL TRANSDUCTION COMPONENTS

Despite extensive screens for recessive mutations that effect the development of the R7 cells, mutations in

only three genes *boss*, *sev*, and *seven-in-absentia* (*sina*), were found (Baker *et al.* 1992). Sina encodes a nuclear protein that might be involved in the interpretation of the inducing signal in the nucleus (Carthew & Rubin 1990). But how is the signal transmitted from the activated receptor to the nucleus? If components involved in intracellular signal transduction are required also in other pathways during development, loss of function mutations in the corresponding genes would be lethal and hence would not reveal their involvement in the Sev pathway in the homozygous condition. If the dosis of such a gene can be made rate limiting in some developmental process, the removal of one functional copy of the gene may produce a dominant, haploinsufficient phenotype. In the case of intracellular signal transduction, this can be acheived by modifying the flux through the pathway. Simon *et al.* (1991) generated transformant flies expressing a temperature-sensitive *sev* allele, and raised these flies at a culture temperature at which the level of signalling was just above the threshold required for efficient R7 development. In this background they screened for recessive loss-of-function mutations that, in the heterozygous state, reduced the level of signalling below this threshold, and so produced a dominant, R7-minus phenotype. Of the seven *Enhancer of sevenless*, *E(sev)*, loci identified in this screen, one corresponded to the *Ras1* gene, implicating it as an essential component of the signal transduction pathway activated by the Sev kinase (Simon *et al.* 1991). *ras* genes were first identified as human oncogenes, and have since been shown to play a pivotal role in the intracellular signal transduction in response to the activation of many different RTKs (review in Bourne *et al.* 1991). Furthermore, mutations in *Sos*, a gene encoding a putative guanidine nucleotide releasing factor which is proposed to act as activators of Ras as well as a homologue of GAP (GTPase activating protein) proposed to act as an inactivator of Ras have also been isolated in this and similar screens (Simon *et al.* 1991; Rogge *et al.* 1991, 1992; Bonfini *et al.* 1992; Gaul *et al.* 1992).

If activation of Ras is a primary consequence of Sev activation how is the signal transmitted from Sev to Ras? It has been proposed that Sev might act by stimulating Sos and by inhibiting GAP thereby exhibiting a dual control over Ras activity (Gaul *et al.* 1992). Recently it has become apparent that proteins with SH2 (src homology region 2) domains bind to activated receptors and might serve as a link between the receptor and Sos and or GAP (Pawson & Gish 1992). Such a protein of the structure SH2-SH3-SH2 has been identified as an essential component in the signal transduction cascade controlled by the let-23 receptor tyrosine kinase during *C. elegans* vulval development (Clark *et al.* 1992). A *Drosophila* homologue of Sem-5, *Drk* (*Downstream of receptor kinases*), was identified and shown to correspond to a dominant suppressor of the activated Sev. Drk appears to act between Sev and Ras1 in the Sev signal transduction pathway: a reduction in *Drk* gene dosage impairs signalling by an activated Sev kinase, but does not affect signalling by an activated Ras1 protein (Olivier *et al.* 1993; Simon *et al.* 1993). The presence of an SH2 domain further suggests that it interacts directly with Sev, and perhaps functions as a 'molecular glue', binding to the activated Sev kinase through its SH2 domain, and also to either Sos or Gap1 via one or both of the SH3 domains. Because Ras proteins are attached to the plasma membrane, this immediately suggests a model in which activation of the Sev kinase leads to the recruitment, via Drk, of Sos and/or Gap1 to the membrane, from which point they are able to switch Ras1 to the 'on' GTP-bound state. *In vitro* binding studies have indeed shown that the Drk protein can bind via its SH2 domains to the activated receptor tyrosine kinases and via its SH3 domain to the Sos protein (Olivier *et al.* 1993; Simon *et al.* 1993).

4. THE Raf SERINE/THREONINE KINASE IS AN EFFECTOR OF Ras FUNCTION

Another component of mammalian RTK signal transduction for which a wealth of biochemical data is available is the Raf-1 serine/threonine kinase. Raf-1 has been implicated in the pathways activated by a number of different RTKs: RTK activation induces the relocation of Raf-1 to the plasma membrane and the stimulation of its kinase activity, concomitant with hyperphosphorylation on serine, threonine and possibly also tyrosine residues (for a review, see Li *et al.* (1991)). The *Drosophila* homologue of Raf-1 is encoded by the *raf* gene, also known as *l(1)polehole*, *Draf-1* and *Draf*, and likewise appears to act in several different RTK pathways, including the sev pathway (Nishida *et al.* 1988; Ambrosio *et al.* 1989; Dickson *et al.* 1992*b*). Strong loss-of-function raf mutations block cell proliferation (Nishida *et al.* 1988), but a less severe loss of *raf* function permits hemizygous flies to survive to adulthood, and in such flies R7 cell specification is clearly impaired (Dickson *et al.* 1992*b*). This is true regardless of whether R7 development is triggered via the normal activation of Sev in a wild-type background or the ectopic activation of Ras1 in a *sev* background. Thus, normal Raf activity is required for efficient signalling from Ras1, suggesting that it acts downstream of Ras1.

As with Ras1, ectopic activation of the Raf kinase in the R7 precursor is also sufficient to trigger its neuronal development, bypassing the normal requirement for Sev activation (Dickson *et al.* 1992*b*). It is not yet known whether it also eliminates the need for *Ras1* function, as would be expected of a downstream component. However, the fact that signalling by Sev, but not Raf, is affected by changes in *Ras1* gene dosage is consistent with this hypothesis. Furthermore, there is convincing evidence that Raf proteins act downstream from Ras proteins in mammalian cells, where it has been shown that hyperphosphorylation (and presumably activation) of Raf-1 in response to activation of the *trk* RTK requires the product of the *c-ras* protooncogene (Wood *et al.* 1992). Thus, the Raf kinase seems likely to act, directly or indirectly, as an effector of Ras1 function.

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5. *rolled*: A FURTHER STEP TOWARDS THE NUCLEUS

Another critical step in Sev intracellular signal transduction is probably performed by the product of the *rolled* (*rl*) locus. Complete loss of *rl* function causes larval lethality due to defects in cell proliferation or cell maintenance, but flies homozygous for weak *rl* mutations survive to adulthood (Hilliker 1976; Dimitri 1991). These flies display a number of defects, including a *sev*-like phenotype in which most ommatidia lack the R7 cell. The fact that a dominant mutation at this locus, *Sevenmaker*, permits R7 development in the absence of *boss* and *sev* function suggests that, like Ras1 and Raf, modification of the Rolled protein may also be sufficient to direct the R7 precursor towards a neuronal fate (D. Brunner, unpublished results). *rl* has not yet been characterized molecularly, and it is perhaps premature to speculate on its role in the Sev pathway. However, the fact that a 50% reduction in *rl* gene dosage impairs signalling not only by Sev, but also by activated forms of both the Ras1 and Raf proteins, points to a role downstream from Raf.

6. THE Sev PATHWAY: A COMMON SIGNAL TRANSDUCTION PATHWAY

It may seem rather excessive that so many genes are required for the determination of a single cell fate in the developing *Drosophila* eye. In fact, probably only two of these, *boss* and *sev*, are dedicated to this decision. On the other hand, there is quite good evidence that the entire intracellular signal transduction cascade is also used by the other photoreceptor cells for their decision to adopt a neuronal fate. In flies homozygous for viable mutations in *raf* or *rolled* not only R7, but also some of the outer photoreceptor cells are frequently missing, and in clones of *Ras1*, *Sos* or *Drk*, no photoreceptor cells at all are formed (Simon *et al.* 1991; Dickson *et al.* 1992*b*; D. Brunner, unpublished results). In these cells, however, the signal transduction cascade must be activated by a different mechanism, since they develop normally in *boss* and *sev* mutants. Most likely, as the involvement of *Drk* would seem to indicate, this is achieved by other tyrosine kinases. Other RTKs are known that are expressed in the eye disc, and it has been shown that, within the R7 precursor itself, ectopic activation of other RTKs can activate the pathway in place of Sev (B. Dickson, unpublished results).

Utilization of the Sev signal transduction pathway is not limited to eye development. The *torso* RTK clearly uses at least some of these components for its signal transduction within the syncytial blastoderm (Ambrosio *et al.* 1989; Doyle & Bishop 1993; Lu *et al.* 1993). Indeed, ectopic expression of an activated Sev kinase in the embryo, or of an activated Torso kinase in the R7 precursor, activates the pathway to induce the appropriate context- (not kinase-) specific response (B. Dickson, unpublished results). The *Drosophila* homologue of the mammalian EGF receptor, *DER*, is involved in a number of intercellular signalling events, some (and perhaps all?) of which are also affected by mutations in *Ras1*, *Sos*, *Dos*, *raf* and *rolled* (Baker & Rubin 1989; Price *et al.* 1989; Rogge *et al.*

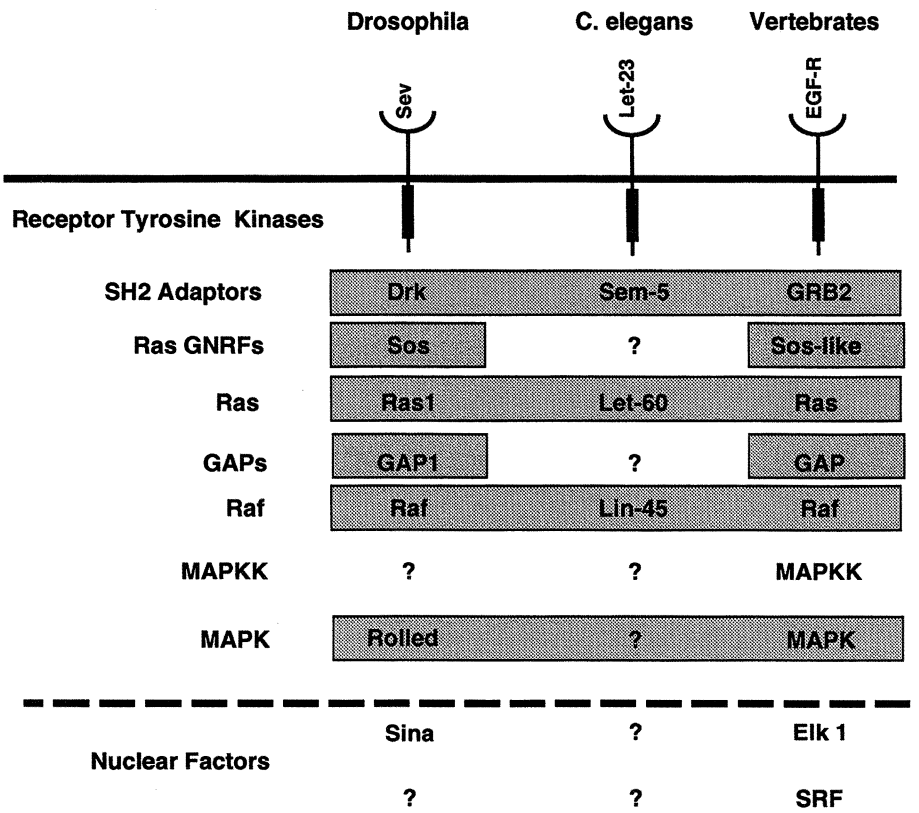

Figure 2. Comparison of the signal transduction pathways mediated by receptor tyrosine kinases in different organisms. The shaded boxes indicate that the proteins are homologous.

1991; Simon *et al.* 1991; Olivier *et al.* 1993; Dickson *et al.* 1992b; D. Brunner, unpublished results). Ectopic activation of the DER kinase in the R7 precursor also triggers its neuronal development, presumably via the same pathway (B. Dickson, unpublished results).

Extending the comparisons even further afield, similar pathways appear to be used for intracellular signal transduction in *C. elegans* and mammals. In particular, during vulval development in *C. elegans*, an inductive signal is transmitted from the anchor cell to the vulval cell precursors (Horvitz & Sternberg 1991). Genetic and biochemical evidence suggest that the receptor for this signal is the RTK encoded by the *let-23* gene, which bears significant homology to *DER* and the mammalian EGF receptor (Aroian *et al.* 1990). Intracellular transduction of this signal then proceeds via the products of *sem-5*, a protein consisting of SH2 and SH3 domains; *let-60*, a Ras protein; and *lin-45*, a serine/threonine kinase homologous to Raf (Beitel *et al.* 1990; Clark *et al.* 1992; P. Sternberg, personal communication).

We still have a lot to learn about the Sev signal transduction pathway. Clearly, not all components of the pathway have been identified yet. In particular, four *E(sev)* loci, as well as *rolled*, still await molecular analysis. However, with such a multitude of entry points into the pathway now available, and the ease of isolating dominant enhancer and suppressor mutations, it seems reasonable to predict that most, if not all, remaining components will be identified within a relatively short time. Furthermore the high degree of overlap in the signal transduction components identified by genetic means in *Drosophila* and *C. elegans*, and by biochemical criteria in cell culture underscores the importance of the combining the two approaches. A good example for the power of the combined approach is the identification of SH3-SH2-SH3 domain protein Drk. Drk was isolated on one hand biochemically from an expression library by virtue of its binding to activated receptors and on the other hand its function was identified genetically in screens for modifiers of the strength of the Sev signal transduction pathway (Olivier *et al.* 1993). The ability to assess the function of the individual components *in vivo*, as well as investigating their biochemical properties *in vitro*, provides the exciting prospect of obtaining a very detailed understanding of a signal transduction pathway that is used not only for the specification of the R7 cell fate, but also for other cell fate decisions in *Drosophila*, in *C. elegans* and in mammals.

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