Receptor signalling: **To Sevenless, a daughter**

Lindsay K. MacDougall* and Michael D. Waterfield*†

Genetic studies with *Drosophila* identified what appeared to be a linear signalling cassette connecting extracellular signals to nuclear responses. But the discovery of a substrate for the Sevenless receptor indicates that the concept of a single, linear pathway may be an oversimplification.

Addresses: *Ludwig Institute for Cancer Research, University College Branch, 91 Riding House Street, London W1P 8BT, UK. †Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.

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Most cell-surface receptors can be classified into one of a few major groups, distinguished by their signal transduction mechanism. The receptor tyrosine kinases are one such group — ligand stimulation of such receptors characteristically results in the autophosphorylation of multiple tyrosine residues within the receptor's cytoplasmic domain. These residues then act as docking sites for a diverse array of signalling molecules that contain domains that recognize specific phosphotyrosine motifs — either Src homology 2 (SH2) or phosphotyrosine-binding (PTB) domains [1]. Activation of the platelet-derived growth factor (PDGF) receptor, for example, results in autophosphorylation at least eight sites, which can recruit a wide variety of downstream molecules: adaptor proteins, such as Grb2, Shc and Nck; enzymes, such as phospholipase Cγ and phosphoinositide 3-kinase; cytoplasmic tyrosine kinases of the Src family; and the Ras GTPase-activating protein. A variation on this theme is provided by the insulin-like growth factor receptor and its relatives, where the activated receptor phosphorylates distinct molecules that provide the phosphoacceptor sites for the docking of downstream molecules.

Events triggered after docking are as complex as the array of molecules recruited, and both biochemical and genetic approaches have been used to assemble the jigsaw of modular signalling proteins into pathways which can potentially link receptors to nuclear effectors. Thus, the recruitment of a preassembled complex of the adaptor Grb2 and the guanine nucleotide-exchange protein Sos promotes activation of Ras at the plasma membrane. The activation of Ras is, in turn, transduced by a conserved cytoplasmic cascade of the kinases Raf, MEK and, finally, mitogen-activated protein kinase (MAPK), which translocates to the nucleus and phosphorylates target proteins. Further complexity is generated by the ability of Grb2's SH2 domain to interact with receptors either directly or indirectly *via* a distinct docking protein, such as the SH2-containing tyrosine phosphatase Syp. In the case of mammalian receptor receptor tyrosine kinases, mutagenesis studies have implied that the 'Grb2/Ras/MAPK cassette' is just one pathway, if the best understood, out of many that may be activated in response to receptor stimulation (reviewed in [2]). In the case of the fruitfly *Drosophila*, until recently it seemed that the linear Grb2/Ras/MAPK cassette was sufficient to transduce all the information downstream of receptor tyrosine kinases, but recent results suggest that this view may be too simplistic.

A linear pathway?

In *Drosophila*, the Grb2/Ras/MAPK cassette has been identified downstream of a number of receptors responsible for cell specification in development (reviewed in [3,4]). These include the Sevenless receptor, which determines the fate of a single cell, the R7 photoreceptor in the compound eye, Torso, which is structurally similar to the mammalian PDGF receptor and mediates specification in the embryo of the terminal structures ('head and tail'), and the *Drosophila* EGF receptor (DER), which has multiple roles including the differentiation of wing veins and the control of cell size. For each of these pathways, it seems that the different receptors use a common set of intracellular components; the specificity of the response to the different receptor tyrosine kinases presumably arises through the modulation of distinct nuclear targets.

The notion that, in *Drosophila*, a single transduction cassette transduces the signal from different receptor tyrosine kinases in different cell types is supported by the observation that activated downstream components can mimic the effect of an ectopically activated receptor. The *Sevenless* gene is expressed in a number of cells in the *Drosophila* eye, but is normally activated only within the R7 photoreceptor. Constitutively activated versions of the *Drosophila* Ras (Ras1) and Raf homologues, when expressed using regulatory elements from the *Sevenless* gene, have been shown to convert non-neuronal cells in the eye to the R7 fate (reviewed in [4]). Furthermore, a mutant allele of the *Rolled* gene that encodes a constitutively active MAPK has pleiotropic effects with similarities to the phenotypes associated with gain-of-function mutations of the *Sevenless* (extra R7 cells), *Torso* (enlarged terminal structures) and *DER* (extra wing veins) genes [5].

These results have suggested that, in *Drosophila*, the Grb2/Ras/MAPK pathway is not only necessary but sufficient to transduce the signal from multiple receptor tyrosine kinases. Indeed, it has been shown that expression of

a constitutively activated (ligand-independent) version of Torso in the developing eye can, like a gain-of-function *Sevenless* mutation, produce extra R7 cells [6]. So, it appears to be the case that, as long as the time and the place are right, any activated receptor tyrosine kinase will do. Implicit to this argument is that the link between the receptor and the next element downstream must be conserved between receptors. So are all the effects of Sevenless (or of Torso or DER) signalling mediated through the activation of Ras1 by the recruitment of the Grb2 homologue, Drk, and of Sos?

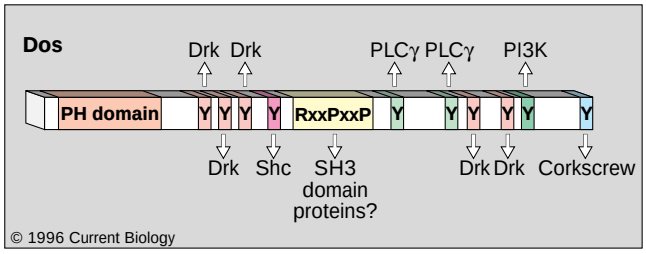
The only site within the Sevenless receptor that fulfils the criteria for recruitment of a phosphotyrosine-binding domain is that around tyrosine 2546 near the receptor's carboxyl terminus, where the SH2 domain of Drk can bind [7]. Mutation of tyrosine 2546 abolishes detectable binding of Drk to the receptor but, *in vivo*, this mutant receptor is still able to induce R7 cell development, albeit at a much reduced frequency [7]. This indicates that a component of the Sevenless signalling pathway might act independently of a direct interaction of Drk with the receptor. Additional genes have been isolated encoding proteins that appear to play a role in transducing signals between the receptor and nucleus. These include the Syp homologue, Corkscrew, which was initially identified, by its mutant phenotype of a twisted cuticle, as functioning downstream of Torso [8], but which has been shown also to act in the Sevenless pathway [9].

A step towards the resolution of this apparent paradox appears to be provided by two recent papers from Raabe *et al.* [10] and Herbst *et al.* [11], which report the identification of a novel protein, Daughter of Sevenless (Dos), the sequence of which predicts that it functions both as a receptor tyrosine kinase substrate and as a docking protein for a number of SH2- and SH3-containing proteins (Fig. 1). The genetic placement of Dos, like its brother Sos, between Sevenless and Ras1, is consistent with such a role. Furthermore, the biochemical identification of Dos as a substrate for the SH2-containing protein Corkscrew helps to explain how Corkscrew functions in Sevenless signalling, an interaction which had been difficult to envisage within a linear Ras/MAPK cassette.

A new addition to the receptor substrate family

In mammals, a number of receptor substrates have been identified that provide docking sites for downstream elements [1,2]. The simplest of these, such as Shc, contain only a single site for interaction with SH2 domains and would thus be expected to link to only one pathway. Other examples of multisite adaptor proteins include those found in the cytoskeleton, such as paxillin and p130^{cas}. They are best exemplified, however, by the family of proteins originally identified as components of the insulin, cytokine and EGF receptor signalling pathways — the docking proteins

Figure 1



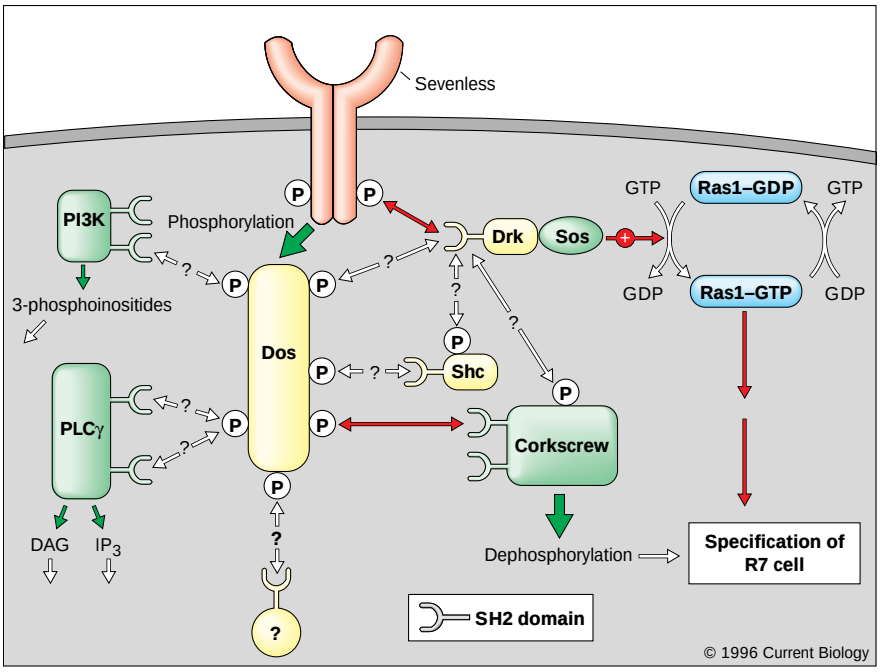
Functional interactions predicted by the Dos sequence. The tyrosine residues (Y) in Dos that are within sequence motifs predicted to interact with SH2 domains of various signal-transducing proteins are indicated. *Drosophila* homologues of Shc, phosphoinositide 3-kinase and phospholipase Cγ have been identified. PH, pleckstrin homology domain; PLCγ, phospholipase Cγ; PI3K, phosphoinositide 3-kinase.

IRS-1, IRS-2 and Gab1. This family has now been extended to include Dos. Functional homology between these proteins is indicated by the conservation of an amino-terminal pleckstrin homology (PH) domain, and motifs for interaction with SH3 and, particularly, SH2 domains.

The amino-acid sequence of Dos includes ten potential tyrosine phosphorylation sites, suggesting that — as in the case of mammalian receptor tyrosine kinases — multiple pathways may operate downstream of the Sevenless receptor [10] (Fig. 2). These sequences include potential docking sites for the adaptor proteins Drk and Shc, the phosphoinositide 3-kinase-specific regulatory subunit, and the enzymes phospholipase Cγ and Corkscrew. Dos may interact with other proteins *via* a polyproline region that is predicted to bind SH3 domains and the PH domain mentioned above. PH domains appear to mediate the transient membrane association that is required for the functioning of many signalling proteins — to get them to the right place at the right time. In developing photoreceptor cells, the PH domain may be responsible for the observed colocalization of Dos with the activated Sevenless receptor [10].

Genetic analyses have shown that, like the components of the Grb2/Ras/MAPK cassette and Dos itself, the tyrosine phosphatase Corkscrew is involved in multiple developmental pathways. As is the case for other protein phosphatases, it has been difficult to pinpoint Corkscrew's site of action precisely, possibly because it interacts with multiple components both within and between pathways. Biochemical associations have also been difficult to demonstrate because of the transience of interactions between such enzymes and their substrates. But studies using a catalytically inactive mutant Corkscrew have now shown that Dos is both a major binding protein and a substrate for Corkscrew [11]. Indeed, Dos can be phosphorylated by a constitutively activated version of Sevenless on sites that allow interaction with Corkscrew, presumably *via* one of its SH2 domains, demonstrating that at least

Figure 2



Sevenless signalling *via* Dos. Although Sevenless contains only a single site for direct interaction with SH2 domains, its substrate Dos contains multiple potential SH2-binding motifs through which it may promote the formation of multienzyme signalling complexes. Interactions which have been shown to occur are indicated with red arrows; potential interactions between Dos and downstream elements are indicated with a question mark. Yellow objects represent adaptor proteins; green objects and arrows represent enzymes and their activities (except for the Sevenless receptor, which is orange); the Ras1 GTP-dependent switch protein is blue. PLCγ, phospholipase Cγ; PI3K, phosphoinositide 3-kinase; DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate.

some of the docking sites predicted by sequence homology are likely to be functional. Although Dos is also a daughter for Torso and DER [10], it does not appear to be a target for the *Drosophila* insulin receptor homologue [11]. Interestingly, in the variations on the theme of receptors which Nature has provided, the tail of the *Drosophila* insulin receptor appears to the docking sites that in the mammalian receptor are provided by intermediary substrate molecules [12].

As well as being a substrate for receptors that directly activate the Grb2/Ras/MAPK cassette, Dos may provide an indirect route to the same downstream targets as it has potential binding sites for Drk, the *Drosophila* Grb2 homologue. In combination with a *Sevenless* mutation that prevents the receptor from contacting Drk directly, loss-of-function mutations in Drk and Ras1, as well as in Corkscrew and Dos, were found further to reduce R7 cell formation [10]. This indicates that Dos may also signal by activating Ras1 *via* Drk. But the potential for the parallel operation of distinct pathways suggested by the predicted SH2-binding sites on Dos suggests that many new relations of Sevenless will be found to function in divergent signalling pathways downstream of this receptor.

The positioning of Dos on signalling pathways by genetic means, and the identification of homologous domains and binding motifs in its sequence, have suggested how this molecule may function in receptor tyrosine kinase signalling. The missing piece of the jigsaw is now the biochemical demonstration that the predicted interactions really occur *in vivo*. The approach of testing the effects of substituting specific receptor tyrosine residues, pursued so avidly in studies of mammalian receptors, may now be used in *Drosophila* to define individual signalling pathways. While the new results for the time being complicate our view of receptor signalling in fruitflies, in the long run this may help us understand multiple signalling pathways in both fruitflies and mammals.

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