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The Fat and Warts signaling pathways: new insights into their regulation, mechanism and conservation

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A cassette of cytoplasmic *Drosophila* tumor suppressors, including the kinases Hippo and Warts, has recently been linked to the transmembrane tumor suppressor Fat. These proteins act within interconnected signaling pathways, the principal functions of which are to control the growth and polarity of developing tissues. Recent studies have enhanced our understanding of the basis for signal transduction by Fat and Warts pathways, including the identification of a DNA-binding protein at the end of the pathway, have established the conservation of Fat and Warts signaling from flies to mammals, and have given us new insights into their regulation and biological functions.

Introduction

Both the patterning and the proportions of different organs and tissues are strictly regulated during metazoan development. Much has been learned about the signaling pathways that regulate developmental patterning, but until recently the mechanisms responsible for developmental growth control have remained poorly understood. With the discovery and characterization of the Warts (or Hippo) and Fat pathways, this has begun to change, as these pathways form an interconnected signaling network that plays a major role in controlling growth (Fig. 1). A distinctive feature of Fat-Hippo-Warts signaling is that it can influence organ growth without affecting organ patterning, and indeed in *Drosophila* acts downstream of the Decapentaplegic morphogen gradient to influence wing growth (Rogulja et al., 2008).

fat encodes a large (>5000 amino acid) transmembrane protein with 34 cadherin domains in its extracellular region (Mahoney et al., 1991). Null alleles of *fat* are lethal, and mutants have overgrown imaginal discs. However, mutants with weak viable alleles exhibit a broadening of the abdomen (hence the name) and wing, and a reduction in the distance between the two wing cross-veins (Mohr, 1923; Waddington, 1940). A reduced distance between cross-veins is also characteristic of three other classical *Drosophila* mutants, *four-jointed* (*fj*), *dachsous* (*ds*) and *dachs* (*d*) (Bridges and Morgan, 1919; Waddington, 1940). Ds is a large (>3000 amino acid) transmembrane protein with 27 cadherin domains (Clark et al., 1995), Fj is a Golgi protein kinase (Ishikawa et al., 2008; Strutt et al., 2004; Villano and Katz, 1995) and Dachs is an unconventional myosin (Mao et al., 2006). A wealth of observations have now established that these four genes function together within a Fat signaling pathway that influences growth, gene expression and planar cell polarity (PCP) (Fig. 1).

Many components of the Warts pathway, including Warts (Wts), Hippo (Hpo), Salvador (Sav) and Mob-as-tumor suppressor (Mats), were first identified through genetic screens for *Drosophila* tumor suppressors (Harvey et al., 2003; Jia et al., 2003; Justice et al., 1995; Kango-Singh et al., 2002; Lai et al., 2005; Pantalacci et al., 2003; Tapon et al., 2002; Udan et al., 2003; Wu et al., 2003; Xu et al., 1995). When any one of these genes is mutant in a patch of cells in the body or head of the fly, an overgrowth phenotype can occur, and this is accompanied by a characteristic distortion and folding of the normally smooth cuticular surface. These mutant phenotypes identified an essential, normal function for these genes in limiting growth during the development of imaginal tissues in *Drosophila*, and this appears to be the principle function of Warts signaling. Nonetheless, it is now clear that theses genes can also regulate other cellular behaviors, which are just now beginning to be identified. It has become more popular over the past couple of years to refer to these genes as functioning within the Hippo signaling pathway, but we prefer (and will employ here) the term Warts signaling, reserving the term Hippo signaling for pathways that act exclusively through the regulation of Hpo. We make this distinction because some signaling through Wts is Hpo independent. This terminology also has the advantage of using the antecedent gene name, as *wts* was first discovered almost a decade before *hpo*.

Two years ago, our understanding of Fat and Warts signaling was greatly advanced by the realization that these pathways are interconnected, as Fat influences growth and gene expression through its effects on Warts. As will be described below, these and other recent studies have given us a framework of intertwined pathways, which extend from transmembrane receptors to DNA-binding transcription factors, and which influence growth, patterning and polarity. Although our understanding of these pathways continues to evolve, recent studies have clarified long-standing issues, including the identification of a DNA-binding protein at the end of the pathway, mechanisms by which the pathways are regulated and signals transduced, and the conservation of Fat and Warts signaling from flies to mammals. Here, we review our current understanding of Fat and Warts signaling, focusing on these most recent discoveries.

The Hippo kinase cassette in *Drosophila*

Genetic and biochemical studies have positioned Wts, Hpo, Sav and Mats at the center of Warts signaling, and have identified a series of positively reinforcing interactions among them. We will refer to these four proteins as the Hippo kinase cassette (Fig. 2). Hpo and Wts are both Ser/Thr kinases, and their activity is regulated by phosphorylation and by their association with Sav and Mats (Fig. 2A). Studies of mammalian homologues of Hpo (Mst1 and Mst2), have indicated that Hpo/Mst can be activated by intermolecular autophosphorylation (Glantschnig et al., 2002; Lee and Yonehara, 2002). Activated Hpo then phosphorylates Wts, Sav and Mats (Wei et al., 2007; Wu et al., 2003). The phosphorylation of Wts by Hpo is facilitated by Sav, which binds to both Hpo and Wts, thus acting as a scaffolding protein (Wu et al., 2003). The activation of Wts requires Mats, which acts as a co-factor (Lai et al., 2005), and the phosphorylation of Mats by Hpo promotes Mats-Wts binding (Wei

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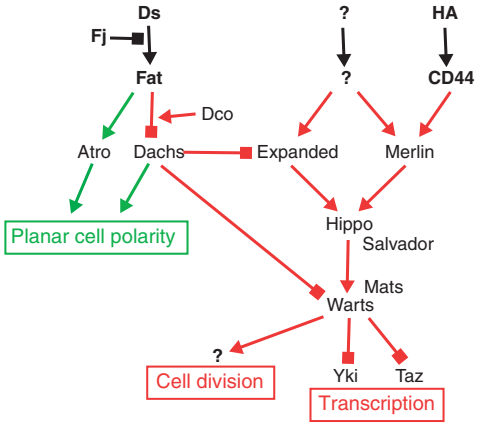

Fig. 1. The Fat-Warts signaling network. A regulatory network perspective of Fat-Warts signaling. Fat PCP signaling is indicated in green, Warts signaling pathways in red. *Drosophila* gene names are used, except for CD44 and Taz, which are only found in vertebrates. Regulatory inputs include Ds, a ligand for Fat, and hyaluraonate (HA), a ligand for CD44, but other regulators for Expanded and Merlin (?) remain to be identified. Pointed arrows indicate positive effects, block arrows indicate inhibitory effects. As discussed in the text, Warts is likely to have as yet unidentified substrates (?) involved in cell division. Abbreviations: Atro, Atrophin; Ds, Dachsous; Dco, Discs overgrown; Fj, four jointed; Mats, Mob-as-tumor suppressor; Taz, transcriptional co-activator with PDZ-binding motif; Yki, Yorkie.

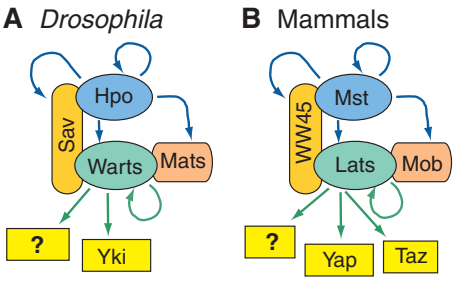

Fig. 2. The Hippo kinase cassette. A schematic of the physical associations and the kinase-substrate relationships among proteins in the Hippo (Hpo) kinase cassette in (**A**) *Drosophila* and (**B**) mammals. Colored arrows identify proteins phosphorylated by Hpo/Mst (blue) and Warts/Lats (green). Hpo and Mst autophosphorylate and then phosphorylate Sav/WW45, Warts/Lats and Mats/Mob. The phosphorylation of Warts by Hpo is facilitated by Sav, which interacts with both proteins. Warts autophosphorylates and phosphorylates downstream effectors, including Yki/Yap, Taz and presumably other substrates (?). Abbreviations: Hpo, Hippo; Mats, Mob-as-tumor suppressor; Sav, Salvador; Taz, transcriptional co-activator with PDZ-binding motif; Yap, Yes-associated protein; Yki, Yorkie.

et al., 2007). The activation of Wts is also associated with autophosphorylation (Wei et al., 2007). Once activated, Wts then phosphorylates and thereby inhibits the transcriptional co-activator Yorkie (Yki), which is the crucial substrate of Wts in transcriptional and growth regulation (Huang et al., 2005).

Although simplified presentations of the pathway sometimes present Hpo, Sav, Wts and Mats as co-equal partners, the mutant phenotypes of *wts* and *mats* appear to be more severe than *hpo*, whereas the *sav* mutant phenotype appears weaker than *hpo* (Cho et al., 2006; Lai et al., 2005; Wu et al., 2003). Comparisons of mutant phenotypes in clones can be complicated by uncertainties over whether particular alleles are null, and differences in perdurance of wild-type gene products, but the current biochemical understanding of the Hippo kinase cassette could explain these genetic differences. As Sav is required only for the phosphorylation of Wts by Hpo (Wu et al., 2003), and not for the phosphorylation of Mats (Wei et al., 2007), it makes sense that the *sav* mutant phenotype is weaker than the *wts* mutant phenotype. Moreover, as Hpo phosphorylation of Mats seems to work by promoting Mats-Wts binding, to the extent that some association between Mats and Wts occurs even when they are unphosphorylated (Wei et al., 2007), it could explain why *hpo* mutant phenotypes appear weaker than *wts* or *mats*.

The Hippo kinase cassette in mammals

It has been known for some time that homologues of the Hippo kinase cassette genes exist in mammals (Table 1). Indeed, in several cases it has been demonstrated that these mammalian genes can rescue the phenotypes of *Drosophila* mutants (Lai et al., 2005; Tao et al., 1999; Wu et al., 2003). However, only more recently has cellular and biochemical evidence appeared to establish that these mammalian proteins are linked in an analogous signaling cassette (Fig. 2B), and that, as in *Drosophila*, this signaling cassette plays a significant role in mammalian growth control. Several regulatory steps that were first characterized with *Drosophila* proteins have now been identified in their mammalian homologues (Fig. 2B, compare with Fig. 2A), including: phosphorylation of Lats (Wts), Mob (Mats) and WW45 (Sav) proteins by Mst (Hpo) kinases (Callus et al., 2006; Chan et al., 2005; Hirabayashi et al., 2008; Praskova et al., 2008); the association of WW45 with Mst and Lats (Callus et al., 2006; Lee et al., 2008); a requirement for WW45 for the phosphorylation of Lats (Lee et al., 2008); the association of Mob and Lats and the consequent promotion of Lats autophosphorylation (Praskova et al., 2008); and the phosphorylation of the Yki homologue Yes-associated protein (Yap) by Lats (Dong et al., 2007; Hao et al., 2008; Zhang et al., 2008a; Zhao et al., 2007). Studies of Mst in mammalian cells have identified autophosphorylation as being a crucial regulatory step for Hpo/Mst (Glantschnig et al., 2002; Lee and Yonehara, 2002), and have identified an association of Mst with Ras association domain family proteins (Praskova et al., 2004), which was subsequently also observed in *Drosophila* (Polesello et al., 2006). In mammalian cells, Mst proteins can also be activated by a caspase-mediated cleavage (Graves et al., 2001; Graves et al., 1998), which has not yet been observed in *Drosophila*. Studies using *Ww45* mutant mouse keratinocytes have also identified an unexpected influence of WW45 on Mst1 autophosphorylation (Lee et al., 2008), although evidence for a modest influence of Sav on Hpo autophosphorylation has also been reported in cultured *Drosophila* cells (Wei et al., 2007). Another feature that has been described in cultured mammalian cells, but which has not yet been documented in *Drosophila*, is nuclear-cytoplasmic shuttling of Mst (Lee et al., 2008; Lee and Yonehara, 2002). In parallel with *Drosophila* studies, genetic and cell culture studies in mammalian cells have also linked the Hpo kinase cassette to the phosphorylation of Yap and to the regulation of growth (Hao et al., 2008; Lee et al., 2008; Zhang et al., 2008a; Zhao et al., 2007).

Other substrates of the Hippo kinase cassette

Since the discovery of Yki and its role in Hpo-mediated growth regulation, the focus of the field has been on transcriptional regulation through Yki/Yap as mediators of the effects of the Hpo kinase cassette genes. However, in mammalian cells, Lats can also

Table 1. Components of Fat-Warts signaling in *Drosophila* and mouse

Drosophila name	Mouse name	Protein type
Dachsous (Ds)	Dsch1, Dsch2	Transmembrane ligand
Fat	Fat4	Transmembrane receptor
	CD44	Transmembrane receptor
Four-jointed (Fj)	Fjx1	Golgi Ser/Thr kinase
Discs overgrown (Dco)	CKIδ, CKIε	Casein kinase family, Ser/Thr kinase
Dachs		Unconventional myosin
Atrophin/Grunge	Atrophin	Transcriptional co-repressor
Expanded (Ex)	Ex1/Frmd6, Ex2	FERM-domain protein
Merlin (Mer)	Merlin	FERM-domain protein
Hippo (Hpo)	Mst1, Mst2	Sterile-20 family, Ser/Thr kinase
Salvador (sav)	WW45 (Sav1)	Scaffolding protein
Warts (Wts)	Lats1, Lats2	Nuclear Dbf2-related (NDR) family Ser/Thr kinase
Mob as tumor suppressor (Mats)	Mob1, Mob2	NDR kinase family co-factor
Yorkie (Yki)	Yes-associated protein (Yap)	Transcriptional co-activator
Scalloped (Sd)	Tead/Tef1-Tef4	DNA binding

regulate the activity of transcriptional co-activator with PDZ-binding motif (Taz) (Fig. 2), which shares sequence similarity to Yap and modulates mesenchymal differentiation (Lei et al., 2008). In addition, both Hpo/Mst and Wts/Lats may affect cell proliferation and survival through non-transcriptional processes. Hpo has been reported to phosphorylate *Drosophila* inhibitor of apoptosis protein 1 (Diap1), and this activity might influence Diap1 stability (Harvey et al., 2003; Pantalacci et al., 2003).

Studies of Warts/Lats also imply that it has substrates crucial for cell division (Fig. 1). In mammalian cells, Lats proteins are phosphorylated in a cell cycle-dependent manner, and negatively regulate Cdc2/Cyclin A (Tao et al., 1999; Toji et al., 2004). Lats1 has also been reported to act as a dynamic component of the mitotic apparatus and to promote mitotic exit (Bothos et al., 2005; Morisaki et al., 2002). Lats proteins localize to centrosomes during interphase, and to the mitotic spindle during metaphase (Nishiyama et al., 1999; Toji et al., 2004). In addition, Lats proteins interact with and modulate the functions of LIM (Lin11, Isl1, Mec3) domain proteins that participate in spindle pole organization, actin filament assembly and cytokinesis (Abe et al., 2006; Hirota et al., 2000; Yang et al., 2004). More recent studies have identified that cell cycle-dependent changes occur in Mst activity and in the Mst-dependent phosphorylation of Mob (Praskova et al., 2008). Additionally, *Mats* mutations have recently been reported to result in aberrant chromosome segregation in the early *Drosophila* embryo (Shimizu et al., 2008), supporting both the conservation and the in vivo relevance of the association of Lats and Mob with mitotic chromosomes in cultured cells (Bothos et al., 2005; Nishiyama et al., 1999; Toji et al., 2004). Altogether, these observations imply that the Hpo kinase cassette acts at multiple steps to influence cell proliferation.

DNA-binding proteins for Warts signaling

Yki is a non-DNA-binding transcriptional co-activator, and since the discovery of its role in Warts signaling (Huang et al., 2005), a key issue has been the identity of its relevant DNA-binding partner(s). Recently, this has been at least partially answered by studies that have identified Scalloped (Sd) as being a partner protein for Yki, and mammalian homologues of Sd, the TEA domain/Transcription enhancer factor (Tead/Tef) proteins, as being partners for Yap (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008b; Zhao et al., 2008). Sd was first suggested as a candidate Yki-interacting protein through a genome-wide yeast two-hybrid screen (Giot et al., 2003). In addition, mammalian Tead/Tef proteins are among the several DNA-binding proteins that have been identified as partners for Yap in mammalian cells (Espanel and Sudol, 2001; Ferrigno et al., 2002; Komuro et al., 2003; Strano et al., 2001; Vassilev et al., 2001; Yagi et al., 1999; Zaidi et al., 2004). However, the functional significance of the Yki-Sd interaction was unclear, and, based on prior genetic studies, Sd was not an obvious candidate to be the DNA-binding partner of Yki, as *sd* is specifically required for wing and neuronal development (Campbell et al., 1992; Liu et al., 2000), whereas *yki* appears to be required for normal growth and survival in all imaginal cells (Huang et al., 2005). Indeed, in studies of *sd* mutant clones, *sd* was essential for cell proliferation only in the wing (Liu et al., 2000; Wu et al., 2008; Zhang et al., 2008b), where it functions as a DNA-binding partner for *vestigial* (*vg*) (Halder et al., 1998; Paumard-Rigal et al., 1998; Simmonds et al., 1998). However, genetic studies have clearly demonstrated that *sd* is required for the overgrowth phenotype that is associated with either the overexpression of Yki, or with the mutation of tumor suppressors in the Warts signaling pathway (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008b). In cultured mammalian cells, experiments using either RNA interference-mediated knockdown, or the expression of dominant-negative proteins, indicated that Tead proteins are similarly required for Yap-mediated gene expression and transformation (Zhao et al., 2008). The linkage of Sd to Warts signaling in *Drosophila* was further supported by the identification of an enhancer within the downstream transcriptional target gene *Diap1* (*thread*). This enhancer mediates Sd:Yki-dependent transcription in vivo and in cultured cells, and is bound by Sd in vivo and in vitro (Wu et al., 2008; Zhang et al., 2008b).

Although these recent studies have provided convincing evidence that Sd is a Yki partner, they left unanswered the question of why *sd* and *yki* mutant phenotypes differ. Indeed it is striking that *sd* is required for the effects of the overexpression of Yki on *Diap1* expression, but outside of the *Drosophila* wing, *sd* is not required for the endogenous expression of *Diap1* (Wu et al., 2008; Zhang et al., 2008b). One possibility is that other DNA-binding transcription factors that partner with Yki might contribute to Warts signaling (Fig. 3B). Another possibility, however, is that Sd might function as a transcriptional activator in the presence of Yki, but as a transcriptional repressor in the absence of Yki (Fig. 3A). Such repression of normal Warts pathway targets might explain the observation that overexpression of *sd* actually inhibits growth and promotes apoptosis (Liu et al., 2000). Switching from repressor to activator isoforms is typical of the DNA-binding transcription factors at the end of many signaling pathways (Barolo and

Posakony, 2002). In this case, the absence of *sd* would differ from the absence of *yki* because target genes would be derepressed without Sd, but repressed without Yki (Fig. 3).

Sd was previously identified as the DNA-binding partner protein of Vg (Halder et al., 1998; Paumard-Rigal et al., 1998; Simmonds et al., 1998), with which it functions to promote wing development. Both loss-of-function and gain-of-function experiments argue that Vg and Yki have different functions and thus must have at least some distinct targets. Studies of Vg have determined that, in addition to providing a transcriptional activation domain to Sd, it also influences Sd DNA-binding specificity (Halder and Carroll, 2001). If this is also the case for Yki, it would support a simple explanation for how they execute different functions, despite complexing with the same DNA-binding protein. Although both *yki* and *vg* influence growth and some of the same target genes in the wing, expression of *yki* in *vg* mutant clones, or of *vg* in *yki* mutant clones confirmed that *yki* and *vg* can function independently (Wu et al., 2008). The issue of how different co-activator proteins regulate different sets of downstream genes using the same DNA-binding transcription factor is even more complex in mammals, as there are four Tef/Tead proteins, and multiple Vg-related proteins, one of which (Tondu) has also been shown to interact with Tef/Tead proteins (Vaudin et al., 1999), as does the Yap-related protein Taz (Mahoney et al., 2005).

Phosphorylation of Yki/Yap regulates its subcellular localization

Several recent studies have also increased our understanding of the molecular and cellular basis for the regulation of Yki/Yap by Warts/Lats. One crucial phosphorylation site is Ser168 of Yki (Ser127 of Yap) (Dong et al., 2007; Oh and Irvine, 2008; Zhang et al., 2008b; Zhao et al., 2007). Phosphorylation of this Ser creates a binding site for 14-3-3 proteins (Basu et al., 2003; Dong et al., 2007; Oh and Irvine, 2008; Zhao et al., 2007), a class of proteins that act as cytoplasmic anchors for several phosphorylated transcription factors (Mackintosh, 2004). Indeed, experiments have shown that the phosphorylation of Yki by Wts/Lats influences its subcellular localization: when Warts/Lats is active, Yki/Yap is phosphorylated and is retained in the cytoplasm, but when Warts/Lats are mutant or inactive, active Yki/Yap can enter the nucleus (Dong et al., 2007; Hao et al., 2008; Oh and Irvine, 2008; Zhang et al., 2008b; Zhao et al., 2007). This provides a simple mechanism for the regulation of Yki/Yap by Warts signaling. However, complicating the story is the fact that both in vivo and cell culture experiments indicate that Yki and Yap have multiple Wts/Lats sites (Hao et al., 2008; Oh and Irvine, 2008; Zhao et al., 2007). Moreover, even though the Yki-S168A/Yap-S127A mutation hyperactivates Yki/Yap, the mutant protein still exhibits some sensitivity to Wts/Lats (Oh and Irvine, 2008; Zhao et al., 2007). The other sites have not yet been as well characterized, but appear to fall within a HXRXXS consensus motif (Hao et al., 2008; Zhao et al., 2007). As the site at 127/168 is the only 14-3-3 consensus binding site within Yki/Yap, and mutation of 127/168 alone appears to eliminate 14-3-3 binding (Basu et al., 2003; Dong et al., 2007; Oh and Irvine, 2008; Zhao et al., 2007), the mechanism by which these other sites influence Yki remains to be determined.

Downstream targets of Warts signaling

Warts signaling regulates gene expression, and studies in *Drosophila* over the years have led to the identification of several downstream genes that could contribute to the growth phenotypes associated with pathway mutants. One important target is *bantam* (Nolo et al., 2006; Thompson and Cohen, 2006), a gene that encodes a microRNA that is not obviously conserved in vertebrates. The genes encoding other key growth regulators that are downstream of Warts signaling in *Drosophila* include *cyclin A*, *cyclin B*, *cyclin E*, *E2F1* and *Diap1* (Goulev et al., 2008; Shimizu et al., 2008; Silva et al., 2006; Tapon et al., 2002; Wu et al., 2003). Microarray studies in cultured mammalian cells have recently added substantially to the list of potential targets (Dong et al., 2007; Hao et al., 2008; Zhang et al., 2008a; Zhao et al., 2007), although many of these may be indirect. There are many differences between the lists of downstream genes identified in these different microarray studies, and more needs to be done to define crucial downstream targets for growth control in both flies and mammals.

Another important class of target genes in *Drosophila* imaginal discs are upstream components of signaling pathways that influence Warts. *fj*, a regulator of Fat signaling, and *expanded* (*ex*), a regulator of Hippo signaling (Table 1, Fig. 1), are also both downstream targets of Yki (Cho et al., 2006; Hamaratoglu et al., 2006; Yang et al., 2002). The mammalian homologue of *fj*, *four-jointed box 1* (*Fjx1*), is a Fat target gene in the mammalian kidney (Saburi et al., 2008). Thus, as in most signaling pathways, feedback regulation occurs in Fat and Warts pathways.

A third class of targets are those involved in local cell fate and patterning decisions. Activation of Yki in the proximal wing of *Drosophila* induces expression of the Wingless (Wg) signaling molecule (Cho et al., 2006; Cho and Irvine, 2004), which contributes to the overgrowth phenotypes associated with Fat signaling in this

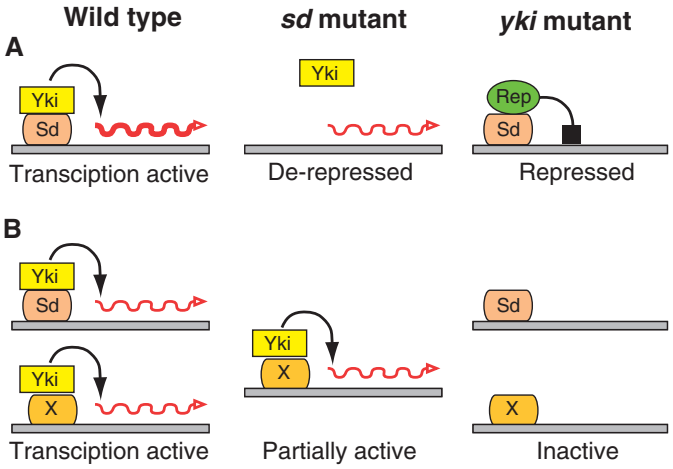

Fig. 3. Transcriptional regulation by Yki and Sd. In *Drosophila*, Yorkie (Yki) and Scalloped (Sd) form a heterodimeric transcription factor that regulates downstream targets of Warts signaling. Their mammalian homologues Yap and Tead/Tef1-Tef4 (not shown) perform a similar function in mammalian cells. Genetic studies in *Drosophila* indicate that *yki* mutation reduces organ growth, whereas *sd* mutation has little effect outside of the wing. Two possible explanations (which are not mutually exclusive) for this are proposed. (**A**) In the absence of Yki, target genes might be actively repressed by Sd (right image), presumably in concert with, as yet, unidentified repressors (Rep). Target genes would be expressed at modest levels (thin red line) in the absence of Sd (owing to derepression), but would not to be expressed at all in the absence of Yki. (**B**) Alternatively, Yki might complex with other DNA-binding proteins (X). These other complexes could then act independently of Sd to promote the expression of the same downstream target genes. In this case, partial expression of targets would occur in the absence of Sd, but not in the absence of Yki.

region (Cho and Irvine, 2004), but Wg is not induced by Yki in other regions of the wing disc. The Notch ligand Serrate (Ser) is induced by Yki within the leg disc (Cho et al., 2006; Mao et al., 2006), but Ser is not a Yki target in the *Drosophila* wing or eye. Components of Warts signaling have also been implicated in a variety of other processes in *Drosophila*, including regulating neural fate during early eye development (Feng and Irvine, 2007; Maitra et al., 2006; Pellock et al., 2007), photoreceptor cell type during later eye development (Mikeladze-Dvali et al., 2005), posterior follicle cell fate in the ovary (MacDougall et al., 2001; Meignin et al., 2007; Polesello and Tapon, 2007; Yu et al., 2008) and dendritic maintenance (Emoto et al., 2006). Considering the variety of tissue-specific functions for components of Warts signaling in *Drosophila*, one reason for some of the differences in gene expression detected by microarray experiments on cultured mammalian cells may be that they employed different cell types (Dong et al., 2007; Hao et al., 2008; Zhang et al., 2008a; Zhao et al., 2007). Microarray targets identified in mammalian cells include not only genes implicated in the regulation of cell proliferation and cell death but also genes implicated in processes like epithelial-mesenchyme transition, cytoskeletal organization, cell adhesion and cell migration, which also supports the conclusion that Warts signaling has functions beyond growth control, presumably involving the regulation of a variety of cell-type specific targets.

Regulation by Merlin and Expanded

In *Drosophila*, two related genes, *ex* and *Merlin* (*Mer*), have been identified as being upstream regulators of Hippo signaling (Hamaratoglu et al., 2006) (Fig. 1). *ex* and *Mer* both encode members of the Band 4.1 super family, a group of cytoplasmic proteins characterized by the inclusion of a FERM (Four-point one, Ezrin, Radixin, Moesin) domain, which mediates membrane association; many family members are also associated with cytoskeletal regulation (Mangeat et al., 1999). *ex* was first identified as a *Drosophila* tumor suppressor (Boedigheimer and Laughon, 1993). *Mer* was identified as the *Drosophila* homologue of a human tumor suppressor responsible for a congenital syndrome (neurofibromatosis type 2, NF2) that is associated with a high frequency of tumors in nervous tissue (LaJeunesse et al., 1998; McClatchey and Giovannini, 2005). Mutation of *Drosophila Mer* on its own has only minor effects on growth, but characterization of *Mer*; *ex* double mutants suggests that they are partially redundant (McCartney et al., 2000). Each gene also has unique functions (McCartney et al., 2000; Pellock et al., 2007; Silva et al., 2006; Willecke et al., 2006), but it is not yet clear whether these reflect unique functions of each protein or simply differences in expression.

Mer and *ex* have been linked to Hpo signaling in *Drosophila* by several observations. Mutation of these genes not only influences growth and cell survival, resulting in phenotypes similar to the effects of mutation of Hippo kinase cassette genes, they also influence the same downstream target genes (Cho et al., 2006; Hamaratoglu et al., 2006). Genetic epistasis experiments have suggested that *ex* and *Mer* act upstream of *hpo* (Hamaratoglu et al., 2006), and, consistent with this, they can influence Hpo and Wts phosphorylation in cultured cells (Hamaratoglu et al., 2006; Silva et al., 2006), and can influence Yki phosphorylation and Yki subcellular localization in vivo (Oh and Irvine, 2008).

The precise mechanisms by which these proteins influence Hpo signaling has not yet been determined. One study identified an accumulation of several different transmembrane receptors, including Fat, on the cell surface in *Mer; ex* double mutant clones, and raised the possibility that *Mer* and *ex* might exert a general influence on receptor endocytosis (Maitra et al., 2006). However, *ex* null mutant animals can be largely rescued by overexpression of Wts (Feng and Irvine, 2007), which suggests that the effects *ex* has on the levels of cell surface receptors are a consequence, rather than a cause, of its influence on the Hippo kinase cassette.

In *Drosophila*, Ex is the more crucial regulator of Hippo signaling in most contexts, but it is not yet clear whether this is also the case in mammals. Two mammalian genes with some sequence similarity to Ex have been identified (Hamaratoglu et al., 2006), but there are some differences in their domain structure when compared with *Drosophila* Ex, and mutants have not been described. Nonetheless, one Ex-related protein, Ex1/Frmd6, influenced Yap activity in a cultured cell assay (Zhao et al., 2007).

Regulation by contact inhibition

Mammalian Merlin has been extensively studied for its tumor suppressor function (reviewed by McClatchey and Giovannini, 2005). These studies have identified several proteins that can interact with Merlin, and have tied Merlin to the activity of cytoskeletal regulators, but the mechanisms by which the loss of Merlin leads to tumor formation had remained unclear. However, one important clue comes from experiments that implicate Merlin in the contact-dependent inhibition of cell proliferation. Normal cells will proliferate in culture at low density, but stop proliferating when they become confluent. Loss of contact information is a hallmark of oncogenic transformation, and is not specific to Merlin. However, Merlin has been tightly linked to contact inhibition by the observation that it is subject to cell density-dependent phosphorylation in culture (Morrison et al., 2001), as many FERM-domain proteins are regulated by phosphorylation (Mangeat et al., 1999). This study also implicated CD44 in this regulation of Merlin; CD44 is a transmembrane protein, the extracellular domain of which can interact with the extracellular matrix, while its intracellular domain can interact with Merlin.

More recent studies have now clearly implicated the Warts pathway in contact inhibition (Zhao et al., 2007). The phosphorylation status and subcellular localization of Yap in cultured cells depends on cell density, and correlates with the proliferative status of these cells. Thus, at low cell density, Yap is predominantly unphosphorylated and nuclear, but when cells become confluent and stop proliferating, Yap is predominantly phosphorylated and cytoplasmic (Fig. 4). Moreover, the expression of the Yap^{S127A} mutant overcomes contact inhibition. This regulation of Yap was tied to Hippo signaling by the observations that Lats2 kinase activity is also influenced by cell density, and that Yap remains nuclear even at high cell density in a *Merlin* mutant cell line.

Warts signaling and cancer

Several studies have linked the Hippo kinase cassette to cancer in mammals. *Lats1* mutant mice are sensitive to carcinogen treatment, and develop soft tissue sarcomas and ovarian tumors (St John et al., 1999). A gene targeted mutation of *Lats2* causes embryonic lethality, but mutant embryos show overgrowth in mesodermal lineages, and *Lats2* embryonic fibroblasts are refractory to contact inhibition (McPherson et al., 2004). More recently, studies in *Ww45* mutant mice have uncovered a requirement for *Ww45* during cell cycle exit in epithelial tissues (Lee et al., 2008). Consequently, these tissues display hyperproliferation and are defective in terminal differentiation. *Ww45* mutations have also been identified in some human renal cancer cell lines (Tapon et al., 2002), and mutations in *Mats* were identified in a human skin melanoma and a mouse mammary gland carcinoma (Lai et al., 2005). Promoter

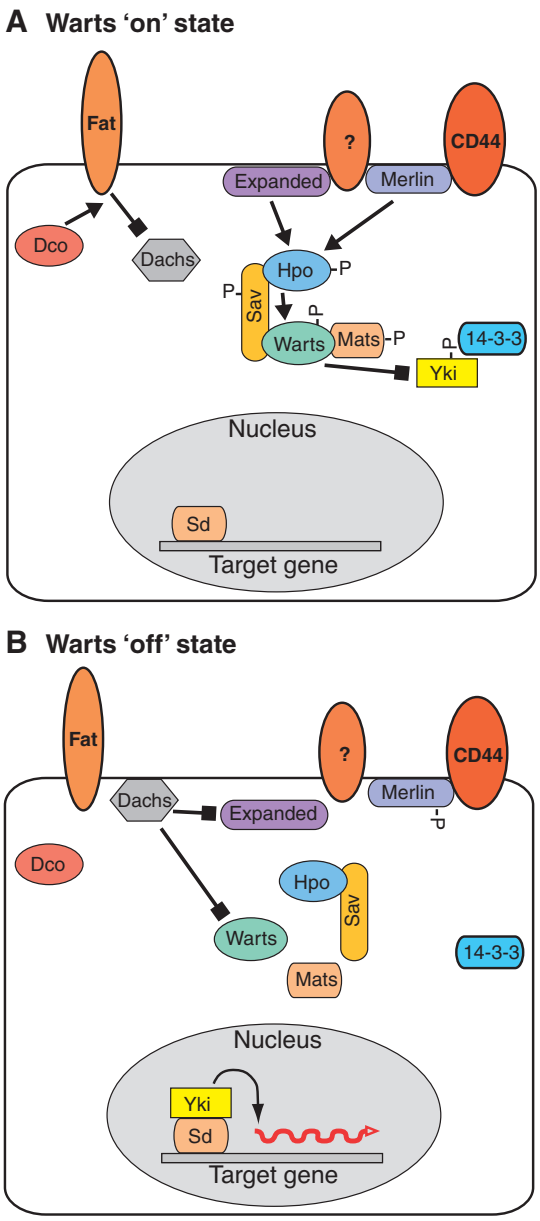

Fig. 4. Warts signaling pathways. A cellular perspective of Warts signaling pathways. (**A**) In the Warts 'on' (phosphorylated) state, Dachs is inhibited by Fat and not detected at the plasma membrane, and does not decrease Warts levels. Discs overgrown (Dco) promotes Fat signaling upstream of Dachs, through an undetermined mechanism. Expanded accumulates at the membrane, and Expanded and Merlin are activated by unknown regulators, and, in mammalian cells, by CD44. Expanded and Merlin promote Hpo phosphorylation (P), which in turn promotes phosphorylation of Salvador (Sav), Warts and Mob-as-tumor suppressor (Mats), contributing to the assembly of these proteins into complexes. Active Warts phosphorylates Yorkie (Yki), which inhibits Yki by promoting its association with 14-3-3 proteins in the cytoplasm, thereby excluding it from the nucleus. (**B**) In the Warts 'off' (unphosphorylated) state, Dachs accumulates at the membrane, reduces levels of Warts protein and reduces levels of Ex protein at the membrane. Merlin is in its inactive, phosphorylated, state. Components of the Hippo (Hpo) kinase cassette are unphosphorylated, and interactions between them are reduced. Yki is not phosphorylated, and enters the nucleus where it complexes with Scalloped (Sd) to promote the transcription of downstream target genes.

hypermethylation and decreased expression of *MST1* and *MST2* in soft tissue sarcomas, and of *LATS1* and *LATS2* in aggressive breast cancers, have also been reported (Seidel et al., 2007; Takahashi et al., 2005).

In addition to this evidence implicating the four core components of the Hippo kinase cascade as tumor suppressors, several studies have identified Yap as an oncogene. For example, Yap overexpression transformed human MCF10A mammary epithelial cells (Overholtzer et al., 2006). Moreover, the amplification of the chromosomal region that harbors Yap has been observed in several animal tumor models, including mouse liver and mammary tumors (Zender et al., 2006). Elevated Yap protein and nuclear localization was also observed in human liver and prostrate cancers, and expression of Yap^{S127A} in mice can cause overgrowth of the liver and other organs (Camargo et al., 2007; Dong et al., 2007).

Linkage of Fat to Warts signaling

fat was identified as a *Drosophila* tumor suppressor 20 years ago (Bryant et al., 1988), but the basis for its tumor suppressor activity was unknown. Within the past few years, however, it has become clear that the influence of *fat* on growth reflects its role as a receptor for an intercellular signaling pathway that influences gene expression (Fig. 4). The first gene identified as a downstream effector of Fat signaling was *dachs*. The mutation of *dachs* in *Drosophila* reduces growth, especially in the wing and leg, and reduces the expression of Fat target genes (Cho and Irvine, 2004; Mao et al., 2006). These phenotypes are opposite to those of *fat* mutants. Moreover, *dachs* mutations completely suppresses the effects of *fat* mutations on growth and gene expression. This epistasis of *dachs* to *fat* suggested that *dachs* might act downstream of Fat, which was confirmed by the observation that Fat regulates the subcellular localization of Dachs protein (Mao et al., 2006).

More recently, Fat and Warts signaling have been linked by the observation that they regulate a common set of downstream target genes (Bennett and Harvey, 2006; Cho et al., 2006; Silva et al., 2006; Tyler and Baker, 2007; Willecke et al., 2006). Thus, *fat* regulates the expression of genes that were first identified as Warts pathway targets, such as *Diap1*, *Cyclin E* and *ex*, whereas components of Warts signaling regulate the expression of genes that were first identified as being Fat pathway targets, such as *wg*, *Ser* and *ft*. The inference that Fat signaling mediates its effects on gene expression through the regulation of Yki is also supported by the observations that heterozygosity for *yki* partially suppresses *fat* phenotypes (Bennett and Harvey, 2006; Silva et al., 2006; Willecke et al., 2006), and that loss of *fat* influences Yki phosphorylation and its subcellular localization in vivo (Oh and Irvine, 2008). Moreover, *fat* tumor suppressor phenotypes can be partially rescued by the overexpression of Wts (Feng and Irvine, 2007). One additional *Drosophila* tumor suppressor, *discs overgrown* (*dco*), which encodes a Casein kinase Iε homologue (Zilian et al., 1999), has also been linked to Fat-Warts signaling by its regulation of common downstream target genes and by genetic epistasis experiments that position the action of *dco* as being upstream of *dachs* (Cho et al., 2006).

Two distinct mechanisms by which Fat could intersect with Warts signaling have been described. One involves an influence that Fat has on the levels of Warts protein (Cho et al., 2006). The mutation of *fat* or *dco* is associated with a post-transcriptional reduction in Warts protein levels. *dachs* is required for this influence on Warts, and Dachs can associate with Warts in cultured cells, which suggests that Dachs might be involved in a turnover of Warts protein. This effect on Warts levels is specific to Fat signaling, as opposed to

Hippo signaling, because it was not observed with mutations in *ex*, *sav* or *mats*. A second proposed mechanism involves an influence of Fat on the levels of Ex protein at the subapical membrane, which are reduced in *fat* mutants (Bennett and Harvey, 2006; Silva et al., 2006; Willecke et al., 2006); this effect of *fat* also depends on *dachs* (Feng and Irvine, 2007). This reduction in Ex levels was hypothesized to influence Hippo signaling, which was supported by the observation that the overexpression of the Fat intracellular domain in cultured S2 cells could influence the expression of a Yki-dependent reporter. Two observations indicate that this effect of *fat* on Ex levels does not suffice to explain Fat signaling. First, *ex fat* double mutants have more severe phenotypes (Feng and Irvine, 2007; Willecke et al., 2006), and stronger effects on Yki phosphorylation and localization (Oh and Irvine, 2008), than either single mutant, consistent with the inference that they act in parallel to influence Warts. Second, the reduction in Ex levels can be reversed by the overexpression of Ex, yet Fat still affects tissue growth and gene expression in these cells (Feng and Irvine, 2007). These observations indicate that Fat can signal independently of Ex, but they do not exclude the possibility that Fat could also signal through Ex, and hence influence Warts through two parallel pathways, one affecting Warts levels and the other affecting Warts activation (Figs 1, 4). Distinguishing the respective contributions of these two mechanisms in different tissues in vivo will require the development of reagents that can reliably detect the levels, localization and phosphorylation status of components of the Hippo kinase cassette in situ.

Fat PCP signaling

In addition to its affects on Warts signaling, Fat also affects planar cell polarity (PCP). PCP is the polarization of cells within the plane of a tissue, perpendicular to the apical-basal polarity of epithelial cells. Most studies of PCP have focused on Frizzled-dependent PCP signaling, which involves a set of core PCP proteins, including Frizzled, Dishevelled, Starry night and Prickle (Klein and Mlodzik, 2005). Several years ago, however, PCP phenotypes were reported for *Drosophila fj*, *ds* and *fat* mutants (Adler et al., 1998; Casal et al., 2002; Rawls et al., 2002; Strutt and Strutt, 2002; Yang et al., 2002; Zeidler et al., 1999; Zeidler et al., 2000); a weak PCP phenotype can also be seen in *dachs* mutants (Held et al., 1986; Mao et al., 2006). The relationship between Fat PCP signaling and Frizzled PCP signaling remains unclear. Some studies in the *Drosophila* eye and wing suggested that Fat PCP signaling acts upstream of Frizzled PCP signaling (Adler et al., 1998; Ma et al., 2003; Matakatsu and Blair, 2004; Yang et al., 2002). More recently, a detailed examination of the relationship between Fat and Frizzled PCP signaling in the abdomen has indicated that these pathways can act in parallel to influence PCP (Casal et al., 2006). There is also at least one PCP phenotype that depends only on Fat PCP signaling: the elongated shape of the wild-type *Drosophila* wing depends in part on cell divisions that are oriented along the proximodistal axis (Baena-Lopez et al., 2005). The normal polarization of these cell divisions is lost in *ds* mutants, and this correlates with the rounder shape of the wing (Baena-Lopez et al., 2005), whereas genes involved in Frizzled PCP signaling do not affect wing shape.

Although events downstream of Fat in PCP signaling remain poorly understood, two genes have been implicated in this process (Fig. 1). Atrophin has been linked to Fat PCP signaling by the observations that *Atrophin* (*grunge*) mutant clones have PCP phenotypes similar to *fat* mutant clones, and that Atrophin can bind to the Fat cytoplasmic domain (Fanto et al., 2003). Atrophin is a transcriptional co-repressor, and influences the expression of *fj* (Fanto et al., 2003), but has not been reported to influence growth or

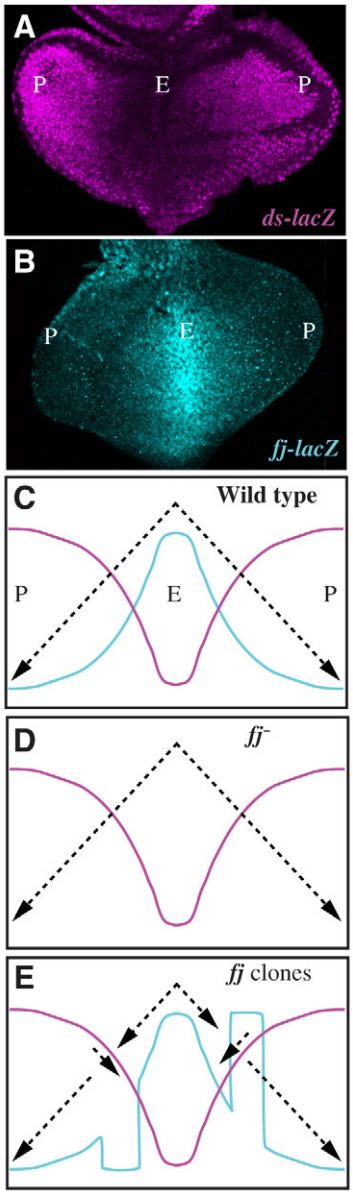

Fig. 5. Fj and Ds expression gradients and the regulation of PCP. (**A**) *dachsous* (*ds*) expression, revealed by a *ds-lacZ* enhancer trap, is graded in the *Drosophila* eye, with higher levels at the poles (P) and lower levels at the equator (E). (**B**) *four-jointed* (*fj*) expression, revealed by a *fj-lacZ* enhancer trap, is in a complementary pattern, with levels high at the equator and low at the poles. (C-E) Schematic perspectives of polarity in the eye in different genotypes. Broken lines with arrows indicate vectors of planar cell polarity, which in the eye is manifest in the orientation of ommatidia. Magenta and blue lines represent the Ds and Fj expression gradients, respectively. (**C**) In wild-type flies, the arrangement of ommatidia is symmetrical with respect to the equator of the eye, represented here by arrows pointing out towards the poles. The vector of polarity can thus be thought of as ascending the Ds slope and descending the Fj slope. (**D**) In an *fj*$^{-}$ mutant, the vector of polarity continues to ascend the Ds slope and PCP is essentially normal. (**E**) In an eye with *fj* mutant clones (left side) or *fj* overexpressing clones (right side), reversals of polarity occur where the change in *fj* expression causes a local reversal of the gradient (Zeidler et al., 1999).

the expression of other Fat-Warts target genes (Cho and Irvine, 2004; Fanto et al., 2003). Dachs has been linked to Fat PCP signaling by the observations that *dachs* mutants partially suppress *fat* PCP phenotypes, and that the subcellular localization of Dachs itself is polarized (Mao et al., 2006).

Regulation of Fat activity

PCP can be represented as a vector of polarity within a tissue. A particularly striking aspect of Fat PCP signaling, then, is that *fj* and *ds* are expressed in gradients in developing tissues, and these vectors parallel their influence on PCP (Fig. 5A-E). The instructive nature of these gradients has been established by both loss- and gain-of-function genetic mosaic experiments (Adler et al., 1998; Casal et al., 2006; Casal et al., 2002; Matakatsu and Blair, 2004; Simon, 2004; Strutt and Strutt, 2002; Yang et al., 2002; Zeidler et al., 1999). These experiments also indicate that *fj* and *ds* have opposite effects on PCP, which is consistent with the observation that they are normally expressed in opposing gradients. Intriguingly, the PCP information in these opposing gradients is partially redundant (Simon, 2004; Zeidler et al., 1999). Thus, in the *Drosophila* eye, as long as *ds* expression is normally graded, the loss of *fj* or the uniform expression of *fj* has only minor effects on PCP, and strong PCP phenotypes are only observed when there is a sharp difference in *fj* expression levels created by a genetic mosaic (Fig. 5D,E). Conversely, *ds* mutants have strong effects on PCP, but its expression does not need to be graded as long as *fj* expression is graded. The contributions of these gradients to PCP in different tissues can vary, however, as neither the *ds* nor *fj* gradient is required for normal PCP in much of the *Drosophila* wing (Matakatsu and Blair, 2004; Simon, 2004). Genetic epistasis experiments suggest that *fj* and *ds* act upstream of *fat* in regulating PCP, consistent with the conclusion that they act as *fat* regulators (Yang et al., 2002).

The hypothesis that *fj* and *ds* act as regulators of Fat is also supported by their influence on gene expression. Wg is expressed in a ring of cells in the proximal *Drosophila* wing. In the absence of *fat*, Wg expression within the proximal wing is elevated and broadened, and this effect of *fat* on Wg is cell autonomous (Cho and Irvine, 2004). Manipulations of *fj* and *ds* expression also influence Wg expression, but their effects are non-autonomous (Cho and Irvine, 2004). Similarly, various studies have reported non-autonomous effects of *fj* and *ds* on *fj*, *Ser* and *Diap1* expression (Buckles et al., 2001; Cho et al., 2006; Rogulja et al., 2008; Zeidler et al., 1999), whereas the expression of these genes is upregulated cell autonomously within *fat* mutant clones (Cho et al., 2006; Mao et al., 2006; Yang et al., 2002). The effects of *fj* and *ds* on *Diap1* expression and cell proliferation are suppressed in *dachs* mutants (Rogulja et al., 2008). Together, these observations indicate that Fj and Ds act on the signaling side, and Fat on the receiving side, of a pathway that influences gene expression. The hypothesis that Fat acts as a receptor is also consistent with the observation that the expression of a truncated Fat protein that is missing almost its entire extracellular domain can partially rescue *fat* mutant phenotypes (Matakatsu and Blair, 2006).

Direct support for Ds binding to Fat and has come from cell aggregation and protein localization experiments. Cultured *Drosophila* S2 cells do not normally aggregate, but can be induced to aggregate when they express interacting proteins. Fat- and Ds-expressing cells specifically bind to each other in this assay (Matakatsu and Blair, 2004). Studies of Fat and Ds protein localization in vivo also suggest that they engage in heterophilic binding (Cho and Irvine, 2004; Ma et al., 2003; Mao et al., 2006; Strutt and Strutt, 2002). When expression of Ds is manipulated in a mosaic fashion, such that a Fat-expressing cell is confronted with neighbors that differ in the amount of Ds expressed, Fat protein concentrates at the interface with neighbors that express higher levels of Ds, and is lost from interfaces with neighbors that express lower levels of Ds. Ds localization can be similarly affected by the manipulation of Fat expression, and the localization of both proteins can be affected by Fj. These observations imply that Fat and Ds bind to each other, acting as a ligand-receptor pair, and further suggest

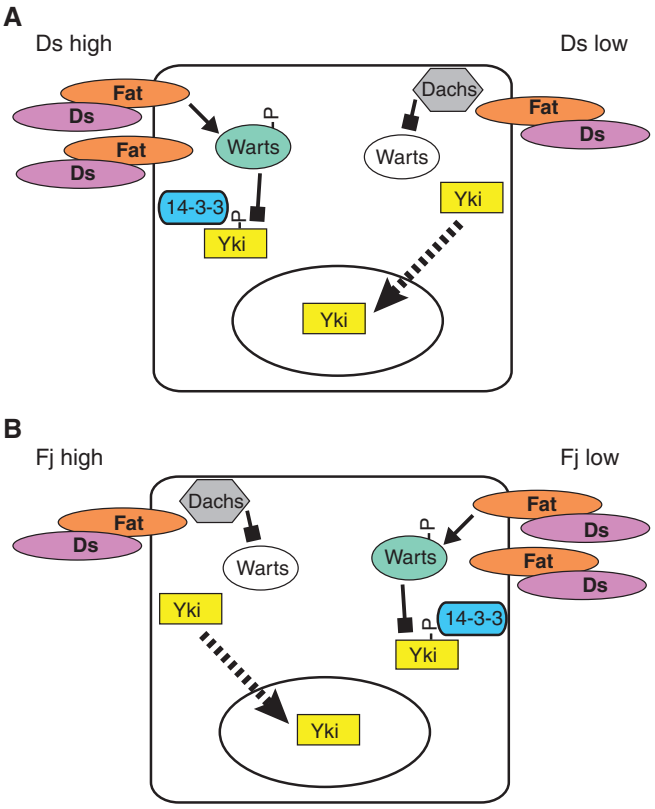

Fig. 6. Model for how polarization of Fat activity might influence Warts signaling. A proposed model for how differences in Dachsous (Ds) or Four-jointed (Fj) expression might affect both planar cell polarity (PCP) and Warts signaling pathways (Rogulja et al., 2008). (**A**) A cell that encounters higher levels of Ds on the cell to its left and lower levels of Ds on the cell to its right. Ds gradients are associated with the polarization of Dachs localization, which is mediated by Fat (Mao et al., 2006). The establishment of polarized protein localizations, including that of Dachs, but presumably also of other proteins, may initiate the cellular polarization associated with PCP. Dachs also inhibits Warts. In the model, this occurs locally, such that when Dachs is polarized, Warts could be degraded and rendered inactive (colorless oval) on one side of a cell (right, in this case), but abundant and active (colored oval) on the other side. Where Warts is present and active, it would phosphorylate and inhibit Yorkie (Yki), but where Warts is missing or inactive, Yki would not be phosphorylated and hence could enter the nucleus. (**B**) A cell that encounters higher levels of Fj expressed in the cell to its left and lower levels of Fj expressed in the cell to its right. The opposing influences of Fj and Ds on PCP and Dachs localization suggest that this is functionally equivalent to a situation in which Ds levels are higher in the cell to the right and lower in the cell to the left. This polarizes the cell in the opposite direction, such that Dachs now accumulates on the membrane on the left side of the cell, rather than on the right side. Even though the cell is polarized in the opposite direction, the transcriptional response associated with failure to locally phosphorylate Yki could be the same for A and B.

that Fj influences this binding. Although a fraction of Fj is secreted from cells (Buckles et al., 2001), it also localizes to the Golgi, and experiments with chimeric proteins have indicated that the Golgi localization is relevant (Strutt et al., 2004). A biochemical explanation for the influence of Fj on Fat signaling has recently been provided by the discovery that it is a protein kinase that can phosphorylate some of the cadherin domains of Fat and Ds (Ishikawa et al., 2008).

The mechanism by which Ds regulates Fat has not yet been determined. However, as Fat antagonizes the localization of Dachs to the membrane, the polarized localization of Dachs implies that Fat activity is normally polarized within cells. This polarization parallels the *fj* and *ds* expression gradients (Mao et al., 2006; Rogulja et al., 2008), which suggests that the polarization reflects an ability to compare the relative levels of Ds presented on one side of a cell versus the other, and, consistent with this, genetic experiments have confirmed that Dachs localization can be altered by manipulating *fj* or *ds* expression (Mao et al., 2006). As Fat signaling can polarize Dachs localization, it could influence PCP through a similar mechanism, but how might this be related to effects on Warts signaling? A recent model proposes that polarization of Dachs could also influence Warts signaling if the influences of Dachs on Warts levels and activity, and if the influence of Warts on Yki phosphorylation, occur locally at the membrane (Rogulja et al., 2008) (Fig. 6). This model provides an explanation for how Ds can act as a ligand that activates Fat, yet inhibit Fat-Warts signaling when cells with different levels of Ds expression are juxtaposed (Rogulja et al., 2008). Additionally, because in this model the influence of Fj and Ds on PCP depends on the vector of their expression gradients, but their influence on Fat-Warts signaling depends on the slope, it provides an explanation for why Fj and Ds have opposite effects on Fat PCP signaling, but similar effects on Fat-Warts signaling.

A variety of observations implicate Ds as a Fat ligand, but *ds* mutants have weaker effects on growth than do *fat* mutants. Thus, some degree of Fat activity might be ligand independent. Alternatively, there might be other Fat ligands, although there are no obvious *ds* homologues encoded by the *Drosophila* genome. There is another *fat*-related gene in *Drosophila*, *fat2*, but its cytoplasmic domain appears structurally distinct, and it has been implicated in the morphogenesis and maintenance of tubular organs rather than in PCP or growth control (Castillejo-Lopez et al., 2004). In mammals, there are two *ds*-related genes, *Dchs1* and *Dchs2* (Rock et al., 2005), and four *fat*-related genes, *Fat1-Fat4* (Katoh and Katoh, 2006). Sequence analysis suggests that *Fat4* is the closest homologue of *Drosophila fat*, and this is supported by the recent discovery that *Fat4* mutant mice have PCP phenotypes and have elevated mammalian *fj* (*Fjx1*) expression in the kidney (Saburi et al., 2008).

Conclusion

The last few years have given us the basic outline of a novel set of interconnected signaling pathways, the Fat-Warts signaling network (Figs 1, 4). This network has multiple inputs and outputs. On the input side, Fat is the only transmembrane receptor protein identified in *Drosophila* thus far. However, as double mutants of *fat* with both *Mer* and *ex* have additive phenotypes (Bennett and Harvey, 2006; Feng and Irvine, 2007; Silva et al., 2006; Willecke et al., 2006), we expect that there will be other transmembrane receptors that regulate this pathway. CD44 may be one such protein in mammals (Morrison et al., 2001), but as CD44 is not obviously conserved in *Drosophila*, there must be others, and their identification will be crucial for understanding the regulation of Warts signaling.

Outputs of this network can be broadly classified as Warts dependent (Warts signaling) or Warts independent (Fat PCP signaling). Although the influence on PCP is largely Warts independent (Fanto et al., 2003; Feng and Irvine, 2007; Mao et al., 2006), feedback regulation, such as the regulation of *fj* expression, is a complicating factor. Warts signaling incorporates both effects on Warts levels (The Fat-Warts pathway), and effects on Warts phosphorylation and activity (The Hippo pathway). The principal substrate of Warts signaling in terms of effects on growth and gene expression is Yki/Yap. The identification of a DNA-binding partner for Yki/Yap is an important advance, but the divergence between *sd* and *yki* mutant phenotypes indicates that there must be other proteins that participate in the transcriptional regulation mediated by this pathway. Additionally, the cell cycle-dependent localization and mitotic phenotypes of Warts/Lats and Mats/Mob suggest that other Warts substrates that are not transcription factors will be important for cell division.

In between these inputs and outputs, there is a series of identified biochemical interactions, and many unanswered questions, such as how does Dachs influence Warts levels and how does Ex influences Hpo activity? Indeed, current pathway models are best considered as frameworks, the details of which will continue to be added to over the coming years. A better understanding of the cell biology of Fat-Warts signaling, including the localization and dynamics of proteins and protein complexes, would be especially valuable. Nonetheless, tremendous progress has been made in just the past few years, and enough has been learnt to establish Fat-Warts signaling as one of the core conserved signaling pathways that acts throughout the metazoans to direct their growth and patterning.

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