The Hippo pathway and apico–basal cell polarity

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INTRODUCTION
Epithelia, cellular sheets that act as barriers between two environments, fulfill a variety of functions in animals and are the primary tissues of origin of cancer. To perform their function (barrier, absorption, secretion etc.), epithelial cells need to adhere cohesively to each other and to co-ordinate their behaviour. This can only be achieved through the establishment and maintenance of cell polarity, which is critical for generating robust cell–cell contacts.

The apico–basal polarity machinery in Drosophila
Apico–basal polarity divides the cell into two complementary membrane domains, an apical domain and a basolateral domain, which are separated from each other by cell–cell junctions [TJs (tight junctions) and AJs (adherens junctions) in mammals, adherens and septate junctions in flies]. The separation between these domains is achieved by the antagonistic action of the polarity complexes, groups of proteins that define the apical and basal regions and determine the position of the junctions (Figure 1). Many polarity proteins were initially identified through genetic screens in Caenorhabditis elegans early embryos or flies. The present review mainly focuses on Drosophila epithelia, but most aspects of apico–basal polarity are conserved across species, with some exceptions, such as the absence of TJs in insects [1–3]. The differences in polarity between different tissues and organisms are extensively reviewed in [4].

In Drosophila larval imaginal discs, sacks of epithelial tissues that are the precursors of most adult appendages, the AJs are linked with the underlying cortical cytoskeleton to form a belt known as the ZA (zonula adherens). Apical to the ZA lie the subapical region and the free apical surface, composed of densely packed microvilli. Just below the ZA sits the septate junction, the functional equivalent of the mammalian TJ, which provides a permeability barrier and constitutes the start of the basolateral domain. The apical domain is characterized by two main polarity complexes: the aPKC (atypical protein kinase C) complex, and the Crb (Crumbs) complex (see Figure 1 for details). The AJs are mainly composed of homophilic Drosophila E-cadherin transdimers, and of the adaptor proteins Arm (Armadillo; homologue of β-catenin) and α-catenin [1]. Two main groups of polarity proteins are present at the septate junctions and are required for their proper formation, although they appear to act independently of each other to maintain the basolateral domain: the Scribble module, which comprises Scrib, Dlg (Discs-large) and Lgl [lithal(2) giant larvae] [5], and the recently characterized Yurt/Coracle group [6,7]. Mutual antagonism between apical and basolateral complexes was found to be key to the establishment and maintenance of separate membrane domains [8,9]. For example, aPKC phosphorylates Lgl, causing its dissociation from the membrane, whereas Lgl promotes the disassembly of the aPKC complex [10–15]. The antagonism between the polarity complexes defines the position and assembly of the AJs and septate junctions, thus apico–basal polarity is crucial to the establishment of cell–cell contacts [1,4].

Loss of polarity and tumour formation
Loss of polarity is a hallmark of cancer and can participate in tumorigenesis through distinct routes [16,17]. First, epithelial

Abbreviations used: AJ, adherens junction; AMOT, angiomotin; aPKC, atypical protein kinase C; Arm, Armadillo; BMP, bone morphogenetic protein; Crb, Crumbs; Dlg, Discs-large; Dpp, decapentaplegic morphogen; Ds, Dachsous; dSTRIPAK, Drosophila Striatin-interacting phosphatase and kinase; Ex, Expanded; F-actin, filamentous actin; FERM, 4.1/ezrin/radixin/moesin; Fj, Four-jointed; Ft, Fat; Hpo, Hippo; Fz/Dsh, Frizzled/Dishevelled; ICM, inner cell mass; Jab, Jabba; LAT5, large tumour suppressor; Lgl, lethal(2) giant larvae; Mer, Merlin; MOBKL1A, Mps One Binder kinase activator-like 1A; PALS1, protein associated with Lin Seven 1; PATJ, PALS1-associated tight junction protein; PCP, planar cell polarity; PP2A, protein phosphatase 2A; RASSF, Ras-associated family; SARAH, Sav/RASSF/Hpo; Sav, Salvador; Scrib, Scribble; Ste20, Ste20; TGF-β, transforming growth factor β; TJ, tight junction; TSG, tumour suppressor gene; Wts, Warts; YAP, Yes-associated protein; Yki, Yorkie; ZA, zonula adherens; ZO, zonula occludens.

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Figure 1  Main polarity determinants in *Drosophila* imaginal discs and in vertebrates

Schematic diagram of the polarity complexes, as well as a table presenting the main polarity proteins, and their vertebrate orthologues. P, phosphorylation. For clarity, some components of the junctions were not mentioned or drawn, including components of the mammalian TJs, such as transmembrane claudins, or ZO proteins. **aPKC complex**: aPKC, Par-6, Cdc42 (small GTPase) and Baz (Bazooka; or *Drosophila* Par3). Rather than being a constitutive member of the aPKC complex, Baz is actually excluded from the apical domain by the combined activities of aPKC and Crb [141,142]. This results in Baz basal displacement, which defines the apical border of the ZA. **Crb complex**: Crb, Sdt (Stardust), PatJ, phospho-moesin (active form of the FERM protein moesin) and the spectrin cytoskeleton. **Scrib module**: Scrib, Dlg and Lgl. Although no direct physical interactions have been shown between Scrib/Dlg and Lgl, those three proteins interact genetically and at least partially co-localize [143]. **Yurt/Coracle group**: Yurt (Yrt) and Coracle (Cor) (FERM proteins), NrxIV (neurexin-IV; transmembrane protein), neuroglian and Na+/K+-ATPase.

In *Drosophila*, the aPKC complex is first recruited to the plasma membrane in a Cdc42-dependent manner [8,13]. Multiple protein–protein interactions occur between the aPKC and Crb complexes, as well as phosphorylation of Crb by aPKC, leading to correct localization of both complexes [93,144]. Both are involved in ZA formation, through the regulation of AJ endocytosis and via Baz/PAR3 [141,145–149]. On the basolateral side, Yurt negatively regulates Crb [150], whereas Scrib, Dlg and Lgl work as a genetic module whose role is to prevent spreading of apical determinants to the basolateral membrane [8,9,13,151]. Reciprocally, the Crb and aPKC complexes antagonize the Scrib group and prevent its accumulation at the apical membrane (see the text). DE-cad, *Drosophila* E-cadherin; E-CAD, E-cadherin; NrxIV, Neurexin IV.

In breast or colon cancer, loss of polarized architecture is usually the first sign of transformation. Can loss of polarity therefore lead to cancer? Several lines of evidence suggest that polarity determinants are inactivated during tumour formation (reviewed in [19–21]). For example, Scrib and Dlg are degraded upon targeting by the human papillomavirus E6 oncoprotein (transition) promotes a motile phenotype and therefore tumour invasion and metastasis [18].

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Hippo pathway and cell polarity

Figure 2 The Hpo pathway phosphorylation cascade in Drosophila

Schematic representation of the Hpo pathway in a fly epithelial cell. P, phosphorylation. A broken line represents a genetic interaction that is still molecularly unresolved. Main Hpo pathway components and their protein domains are represented. A list of the core Hpo pathway components and their mammalian orthologues is also provided (grey box). LIM, zinc-finger-containing LIM domain (protein–protein interaction); RA, Ras-association domain; NH, N-terminal homology region, mediates Scalloped (Sd)-binding to Yki; WW domain, protein–protein interaction domain that preferentially binds proline-rich regions, including PPXY sites.

[22,23]. The LKB1 gene, which encodes a kinase (PAR-4 in *C. elegans*) known to be involved in a nutritionally sensitive cell-polarity pathway [24,25], is mutated in an inherited cancer predisposition known as Peutz–Jeghers syndrome [26]. Furthermore, in *Drosophila*, *scrib*, *dlg* and *lgl* belong to the class of neoplastic TSGs (tumour suppressor genes), whose loss-of-function elicits massive overproliferation, as well as tissue architecture and differentiation defects in imaginal disc epithelia [27]. All of these results suggest that loss of polarity can promote or facilitate tissue growth, but relatively little is known about the mechanisms driving this growth. Below, we summarize recent work suggesting that cell polarity may limit growth through the Hpo (Hippo) tumour suppressor pathway.

**A NEW VIEW OF THE HPO PATHWAY**

From a ‘simple’ phosphorylation cascade...

Core Hpo pathway components and their adaptor proteins

Until recently, the Hpo pathway could be viewed as a straightforward phosphorylation cascade (Figure 2). The upstream kinase Hpo [mammalian Ste20 (Sterile 20)-like; MST1/2 in mammals], a member of the Ste20-like family of serine/threonine kinases, is thought to become active either through auto-phosphorylation or the action of an unknown kinase [28–30]. Active Hpo can then phosphorylate and activate the NDR (nuclear Dbf2-related) serine/threonine kinases, which...
in turn phosphorylates the transcription co-activator Yki [YAP (Yes-associated protein) in mammals]. This phosphorylation inactivates Yki by inducing its retention in the cytoplasm by 14-3-3 proteins and thereby preventing its association with its transcription factor partners, such as Scalloped (TEAD in mammals), Homothorax or Teashirt [33–42]. Yki inactivation leads to silencing of its target genes, which include pro-growth and anti-apoptotic factors such as cyclin E, DIA1 and the miRNA (microRNA) bantam. Thus the Hpo pathway restricts tissue size by antagonizing Yki’s function.

Two adaptor proteins, Sav and Mats, regulate the activity of the core kinases Hpo and Wts. Sav directly binds Hpo, via a C-terminal coiled-coiled region termed the SARAH domain [for Sav/RASSF (Ras-association family)/Hpo], and can also bind Wts via an interaction between its WW domains and PPXY motifs in Wts [43–48]. Sav, which is itself a Hpo phosphorylation target, potentiates Hpo-induced phosphorylation of Wts [43,45]. Similarly, Mats binds to Wts and potentiates its kinase activity [49]. Furthermore, Mats is also a Hpo substrate and Hpo-phosphorylated Mats displays a greater affinity for Wts, leading to an increase in Wts kinase activity [50,51]. Thus the core of the Hpo pathway is composed of a kinase cascade in which the upstream kinase Hpo, in co-operation with two scaffold proteins Sav and Mats, activates the downstream kinase Wts, leading to Yki inactivation.

Hunting for upstream regulators of the Hpo pathway

How Hpo activation is controlled to achieve the correct amount of growth inhibitory signal has been a subject of much interest in the field. By analogy to receptor tyrosine kinase pathways, the search has been on for a Hpo pathway receptor. The first upstream Hpo regulators to be identified were not transmembrane receptors, but two adaptor proteins containing a FERM (4.1/ezrin/radixin/moesin) protein–protein interaction domain, Ex and Mer [52,53]. FERM domain proteins are believed to act as membrane–cytoskeleton linkers by interacting both with the plasma membrane and F-actin (filamentous actin) [54]. Ex and Mer are two tumour suppressor proteins that co-operate to regulate organ growth. They co-localize apically in vivo, heterodimerize in vitro and are partially redundant [52,55]. Initial epistasis experiments placed Mer/Ex upstream of the core kinases and Mer/Ex overexpression was shown to promote Hpo/Wts activity in cell culture [52]. Reciprocally, Mer and Ex also are transcriptional targets of Yki, and are thus thought to be involved in a negative-feedback loop. However, until recently, no molecular relationship between those upstream regulators and the core components of the pathway (Hpo/Sav/Wts/Mats/Yki) had been established. More recently, Ft and Ds, two atypical cadherins, were shown to function as non-catalytic receptors for the pathway [56–59], and these proteins will be discussed in the planar polarity section at the end of the present review.

A change in perception of the Hpo pathway was initiated by the finding that the upstream protein Ex directly binds the downstream effector Yki [60,61], a result which blurred the distinction between upstream and downstream components. This interaction is mediated by PPXY motifs in Ex and the Yki WW domains, protein–protein interaction motifs that preferentially bind proline-rich regions (including PPXY sites) [62,63] and PPXY motifs in Ex. The Ex–Yki association participates in Yki inhibition by sequestering it out of the nucleus, independently of its phosphorylation status [60,61]. This mode of regulation was recently shown to be conserved in mammals: the junctional protein AMOT (angiomitin) and AMOT-like proteins sequester YAP/TAZ (the Yki orthologues) out of the nucleus, through an interaction between WW domains in YAP/TAZ and PPXY motifs in AMOT/AMOTL1/AMOTL2 [64–67]. Similarly to Ex, AMOT proteins can clearly antagonize YAP/TAZ through membrane tethering, independently of the core kinase cascade, since non-phosphorylatable forms of YAP are still inactivated upon AMOT overexpression [65–67]. In fact, the presence of a large number of PPXY motifs in pathway members (three in Ex, one in Hpo and five in Wts in the case of the Drosophila pathway) might act as a ‘magnet’, attracting Yki/YAP out of the nucleus [33,60,61].

AMOT appears to behave as Ex’s functional homologue, tethering Yki/YAP to the apical membrane, although both proteins share little sequence homology apart from their PPXY motifs. The FERM-domain-containing FRMD6 is the mammalian protein with most obvious sequence homology with Ex. Could Ex’s domains, as well as functions, have become evolutionarily split between AMOT and FRMD6? Is FRMD6 part of the mammalian Hpo pathway or has AMOT ‘taken over’ Ex’s Hpo regulation function? A detailed study of FRMD6 should answer these questions. Analogously to Ex and AMOT, the AJ component α-catenin has recently been reported to tether YAP to cell–cell contacts in keratinocytes [68]. In this case, rather than directly binding to YAP, α-catenin recruits 14-3-3, which in turn interacts with phospho-Ser127-YAP. It is not clear whether these tethering mechanisms operate in parallel or if they display tissue specificity.

Other scaffold proteins have been identified as regulators of the Hpo pathway. The WW-domain-containing protein Kibra was recently found to promote activation of the pathway and interacts with multiple components, ranging from upstream regulators Mer and Ex to the core kinases Hpo and Wts and their adaptor protein Sav, leading to pathway activation [69–71]. Drosophila RASSF, which binds Hpo, and the LIM-domain-containing protein Jub (Ajuba), which associates with Sav, Wts and possibly Yki, were also identified as inhibitors of the Hpo pathway [72,73]. Such a wide range of protein–protein interactions (Figure 3) complicates the view of the Hpo signalling network and prompts a careful investigation into the possible roles of these scaffolds.
has been described for α-catenin, which prevents association of YAP to PP2A, thereby preventing dephosphorylation on Ser127 and blocking YAP nuclear translocation [68].

Alternatively, interactions between different components of the pathway could directly influence protein conformation. Those conformational changes could for instance potentiate the activity of the kinases themselves, or reveal/conceal phosphorylation sites on their various substrates. For instance, binding of phosphorylated MOBKL1A (Mps One Binder kinase activator-like 1A; a Mats orthologue) to LATS1 (a Wts orthologue) promotes LATS1’s ability to autophosphorylate on Ser909, suggesting a change in conformation upon MOBKL1A binding [51]. It is possible that Sav and its orthologue SAV (or WW45) have a similar effect on Hpo/MST kinases. In addition, Mer exists either as an active unfolded form or an inactive folded form, whereby its C-terminal fragment folds on to its FERM domain, partially masking it [75]. Ex, as a FERM protein, might behave similarly, and reciprocal binding between Mer and Ex might trigger their unfolding into active forms able to bind Hpo pathway members with greater affinity [55]. Binding of Mer/Ex with Kibra might promote their active open conformation, potentially explaining why the Mer–Ex interaction is strengthened in the presence of Kibra [70].

Furthermore, protein–protein interactions can affect the stability of Hpo pathway components, as is the case for Sav and RASSF, both of which are stabilized after binding to Hpo [45,72]. Similarly, binding of LAT52 to mammalian KIBRA appears to protect LATS2 from ubiquitin-mediated proteasome degradation [76], which may be mediated by the E3 ligase Itch [77,78]. Interestingly, phosphorylation of YAP on Ser381 by LATS1/2 primes YAP for binding to the SCF (Skp1/cullin/F-box) ubiquitin ligase and subsequent ubiquitination and degradation [79], although this residue is not conserved in flies.

Finally, the Hpo pathway scaffolds could function as a platform to bring the core components to their site of activity at the plasma membrane, an issue that will be discussed in detail below.

In addition to the discovery of new adaptor proteins acting as bridges between different components of the Hpo pathway, a number of protein–protein interactions have recently been uncovered between established members of the Hpo network. Indeed, Mer and Ex were recently found to associate with Sav and Hpo, which constitutes the first biochemical link between these upstream regulators of the pathway and the core kinases [69]. Strikingly, Mer-bound Sav appears to be highly phosphorylated, suggesting it may be associated with Hpo [69]. Thus it is now clear that the interactions between Hpo pathway members are complex, involving WW domains, PPXY motifs, SARAH and FERM domains, as well as other protein–protein interaction motifs (Figure 3). The extent of these protein–protein interactions suggests the existence of a supra-molecular complex that controls Hpo pathway activity, although the existence of such a complex in vivo has not been proven. Indeed, most of the interactions between Hpo pathway components have been described by co-immunoprecipitation experiments of overexpressed proteins and should be interpreted with caution until they have been further validated. In particular, the multiplicity of possible WW/PPXY combinations between pathway members (Figure 2) makes it difficult to assess which are relevant in vivo. Nevertheless, the existence of a Hpo super-complex and its importance in pathway regulation are likely to be areas of intense future investigation.

The apical domain as the site of Hpo pathway activity

The adaptor proteins Mer, Ex and Kibra co-localize in vivo and are apically localized, similarly to Jub, which is located at the AJs. As these scaffolds interact with Hpo, Sav, Wts and Yki, the apical localization of the former would be expected to elicit an apical localization of the latter. Accordingly, overexpression of Ex, which directly binds Yki [60], leads to an increase in apical Yki [60,61], whereas AMOT family proteins and α-catenin promote YAP/TAZ junctional localization [64,65,68]. Under normal conditions, Hpo and Wts appear mostly cytoplasmic [56,80]. However, the existence of an apically localized pool of Hpo was recently reported in eye discs [81], whereas a significant portion of Mats is apically localized [82]. Finally, a fractionation assay in cell culture revealed that a membrane-bound fraction of Hpo exists, which is reduced upon combined

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**Figure 3** A new view of the Hpo pathway

Left-hand panel: the matrix of protein–protein interactions between components of the Hpo pathway, compiled from the literature. Right-hand panel: two views of the Hpo pathway: the successive phosphorylation cascade (above), and the scaffolding machinery complex (below). For the sake of clarity, not all protein–protein interactions could be represented in the scaffold model. GST, glutathione transferase; P, phosphorylation.
depletion of Kibra, Mer and Ex [69]. Is this membrane fraction the most biologically active? Indeed, forcing the apical membrane tethering of MST1 by adding a myristoylation signal leads to its activation [83]. Likewise, membrane tethering of Mats or its vertebrate orthologue leads to Wts/LATS activation [51,82,84]. Finally, similar to Ex [70], AMOT proteins are required for full YAP/TAZ phosphorylation since this decreases upon AMOT depletion [65], suggesting that tethering of Yki/YAP/TAZ to the junctions facilitates its phosphorylation. Thus multiple lines of evidence point at apical membrane recruitment being an important step in Yki inactivation by the Hpo pathway; but what is the link between the apical membrane and Hpo pathway components?

REGULATION OF HPO SIGNALLING BY POLARITY COMPLEXES

The Crb complex connects Hpo signalling by polarity

Several recent Drosophila studies suggest that the apical transmembrane protein Crb can influence Hpo signalling [81,85–87]. Crb was found to bind Ex via an interaction between Crb’s FBM (FERM-binding motif), situated in its short cytoplasmic tail, and Ex’s FERM domain [85]. This association is required for FB (FERM-binding motif), situated in its short cytoplasmic tail, and Ex’s FERM domain [85]. This association is required for Ex apical localization. Hence, in crb mutant cells, Ex is basally mislocalized, resulting in a down-regulation of the Hpo pathway and consequently in overgrowth [81,85–87]. Mer or Pt were not affected in this context, suggesting that Crb’s effect on Ex is specific. These results provide the first direct connection between a polarity protein and localization of a Hpo pathway member.

Interestingly, AMOT had previously been reported to associate with TJ components, and in particular with the mammalian orthologues of the Crb and aPKC polarity complexes such as PATJ [PALS1 (protein associated with Lin Seven 1)-associated TJ protein], PALS1 (the Stardust orthologue) and PAR3 [88]. Accordingly, the TJ fraction of YAP or TAZ was increased upon AMOT overexpression [65]. As AMOT is required for TJ maturation [88], its identification as a YAP/TAZ modulator suggests that mature junction formation in itself might regulate Hpo pathway signalling output. This notion is supported by Varelas et al. [64], who showed that disruption of TJs in a dense cell culture, through Ca2+ depletion or knockdown of TJ components CRB3 or PALS1, elicited a down-regulation of the Hpo pathway and YAP/TAZ nuclear accumulation. In keratinocytes, both Ca2+ depletion and α-catenin knockdown resulted in YAP nuclear accumulation, consistent with a role for α-catenin in YAP cytoplasmic retention in the skin [68]. Further connections between the Hpo pathway and AJs/TJs exist: mammalian MER [also called NF2 (Neurofibromin 2)] is required for TJ maturation and links α-catenin to PAR3 [89], whereas PATJ associates with KIBRA and TAZ through interactions between PATJ’s PDZ domains and KIBRA’s or TAZ’s PDZ-binding motifs [90,91]. In addition, YAP2 (one of the YAP isofoms) binds to the TJ component ZO-2 (zonula occludens 2) via a PDZ domain, although this interaction appears to favour nuclear translocation rather than membrane retention [92].

Intriguingly, in Drosophila, not only Crb depletion, but also its overexpression elicited a mislocalization of Ex and resulted in massive overproliferation due to Hpo pathway inactivation [81,85–87]. This phenotype might be due to a dominant-negative effect of Crb overexpression. However, an interesting possible explanation stems from the finding that Crb binding to Ex leads to Ex phosphorylation and degradation [85,86]. Rather than simply being a positive regulator of Hpo signalling, Crb may therefore fulfil a dual role: both localizing Ex to the membrane where it can sequester Yki and promote Hpo activity, and promoting Ex degradation once it has signalled to shut down excess Hpo signalling. Crb would then function as a buffer, allowing fine-tuning of Hpo signalling, but preventing excess activity. Several interesting questions remain open. For instance, what is the kinase responsible for Ex phosphorylation upon Crb binding? aPKC is a candidate, as it is known to phosphorylate Crb [93]. Could Ex phosphorylation (whether by aPKC or another kinase) prime it for removal from the membrane and/or degradation? More research is needed to answer these questions.

Other links between polarity complexes and Hpo signalling

In parallel, a study by Richardson and co-workers showed that the polarity determinants Lgl and aPKC influenced Hpo apical localization [81]. Overexpressing aPKC or loss of its antagonist Lgl induced cytoplasmic mislocalization of an apical pool of Hpo and increased co-localization between Hpo and its negative regulator RASSF. Consistently, a down-regulation of Hpo pathway signalling activity ensued. These results suggest that aPKC can reduce Hpo pathway activation, a function that is antagonized by Lgl, although it appears to be independent of Crb and the mechanism therefore remains to be elucidated. The newly discovered Hpo pathway regulator Kibra is an interesting candidate in this context. Human KIBRA was identified as an aPKC substrate [94], and the aPKC-binding region and one of the phosphorylation sites are conserved in Drosophila Kibra. aPKC could therefore reduce Hpo activation by phosphorylating pathway scaffolds, such as Kibra and Ex, modifying their binding affinities. Other basal polarity proteins such as Dlg or Scrib also appear to influence Hpo pathway targets in ovarian follicle cells [95].

Sensing social cues at the apical junctions

The emerging model is that the apical domain and cell–cell junctions play a key role in Hpo pathway regulation. However, what cues are being sensed through cell–cell contacts by the Hpo pathway remains the most important question. An attractive possibility is that the apical domain, and in particular the TJs/AJs, might function not only as a membrane anchor for pathway components but also as a signal-receiving platform for the Hpo pathway to sense ‘social’ signals from neighbouring cells (e.g. absence of neighbours or presence of an inappropriate neighbour). In mammals, the Hpo pathway is a crucial mediator of contact inhibition of proliferation [38,96,97] and is thus involved in ‘neighbour sensing’. At low cell density, YAP is mainly nuclear and drives proliferation with its partner TEAD [38,97]. In highly dense cell culture, YAP is phosphorylated on Ser127 and sequestered in the cytoplasm, thus eliciting proliferation arrest [97]. Furthermore, the timing of YAP translocation correlates with TJ assembly, and YAP becomes nuclear at high cell density upon depletion of CRB3, PAR3 or AMOT-like proteins [64,97]. Thus it appears that, through the CRB–AMOT complex, the Hpo pathway uses TJs to sense the presence of neighbours. Indeed, α-catenin, which tethers YAP at the AJs in skin cells [68], has also been implicated in contact inhibition and functions as a tumour suppressor in mouse skin [98,99]. Since loss of contact inhibition is a hallmark of cancer, this mechanism is likely to represent an important part of the Hpo pathway’s tumour suppressor function. Even in tumours where the Hpo pathway has not directly been affected by a genetic or epigenetic lesion, loss of tissue organization, which is a characteristic of many tumours, may cripple Hpo signalling through disruption of AJs/TJs and allow the tumour to co-opt YAP/TAZ pro-growth and anti-apoptosis activities.

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In *Drosophila*, wild-type cells neighbouring *crb* mutant tissue lose Crb expression along the border with the mutant cells, possibly as a result of loss of *trans*-homodimerization [87]. This loss of Crb then results in loss of Ex apical localization. Thus the level of Crb in a cell can transmit information to the Hpo pathway in a neighbour via Ex regulation. Crb could therefore conceivably be a mediator of contact inhibition of growth. However, it is not clear how the concept of contact inhibition in cell culture relates to development in an epithelium, where cells are in constant contact with their neighbours. Such ‘social’ sensing would nonetheless become particularly relevant in situations of tissue injury, when contact with a neighbour is lost or cell density dramatically drops, thus allowing the Hpo pathway to participate in tissue regeneration. Consistent with this idea, several studies recently have uncovered a role for the Hpo pathway in adult intestinal regeneration in *Drosophila* and mice [100–104], as well as in *Drosophila* wing imaginal disc regeneration [105,106]. Tissue injury elicited either through bacterial infection, toxic chemicals or genetic cell ablation leads to a disruption of Hpo signalling, Yki activation and proliferation of neighbouring cells to replace lost tissue. This suggests that the Hpo pathway might indeed act as a sensor of tissue damage, possibly through its ability to respond to disrupted cell–cell contacts.

The relationship between polarity and Hpo signalling activity is unlikely to be as simple as: polarized cell→Yki/YAP active, non-polarized cell→Yki/YAP active. This is exemplified by recent work in pre-implantation mouse embryos [40]. During the first stages of embryonic development, YAP is nuclear in all cells. However, at the blastocyst stage, when the mesenchymal ICM (inner cell mass) and the epithelial trophectoderm are specified (the former will form the embryo; the latter the extra-embryonic tissues), YAP localization becomes spatially regulated. Whereas polarized trophectodermal cells maintain nuclear YAP, non-polarized ICM cells activate the Hpo pathway, leading to translocation of YAP to the cytoplasm [40]. This Hpo signalling activation could be due to the high density of the ICM, which is enclosed inside the trophectoderm. It is, however, unclear how mesenchymal ICM cells, which do not maintain TJs [107], would be able to sense this high density and trigger the Hpo pathway. Alternatively, the ICM could be subjected to compression forces, which might activate Hpo signalling through mechanotransduction.

Mechanical stimuli have been proposed to play a crucial role in regulating growth arrest during development, in particular in the fly wing imaginal disc [108,109]. Could the Hpo pathway therefore respond to mechanical forces? The cell–cell junction and the cytoskeleton play an important role in transducing mechanical signals [110]. Interestingly, as well as being linked to junctions as described above, the Hpo pathway shares several connections with the actin cytoskeleton. First, Mer and Ex are FERM proteins, many of which associate with actin filaments [7]. Secondly, Jub, the recently discovered Hpo pathway inhibitor, belongs to an actin-associated family of LIM-domain-containing proteins [111]. Thirdly, AMOT has been shown to localize with F-actin and to regulate actin stress-fibre formation [112]. As YAP directly binds AMOT [65,67], a fraction of cytosolic YAP was found to co-localize with AMOT on cytoskeleton-like fibrous structures [65]. Finally, MST1/2 are associated with F-actin and mediate JNK (c-Jun N-terminal kinase) signalling in response to cytoskeletal disruption [113]. It is therefore plausible that the role of the apical scaffolding machinery might be to relay physical stimuli, such as tension or compression, to the Hpo pathway, although this possibility has not yet been addressed.

To summarize, given the multiple connections between the polarity complexes and Hpo pathway members (Figure 4), it is tempting to speculate that, rather than merely providing a scaffold for the assembly of the core Hpo kinase cascade, the polarity machinery plays an active role in signalling, relaying extrinsic (cell–cell contact, mechanical environment) or intrinsic (polarized state of the cell) information to the pathway core.

**REGULATION OF APICAL DOMAIN SIZE BY THE HPO PATHWAY**

As well as being regulated by apically localized proteins, the Hpo pathway appears in turn to influence apical determinant distribution. Apical ‘bulging’ of *wts* mutant cells was one of the first phenotypes observed by electron microscopy for a Hpo pathway core member and was later noted in *hpo* mutant tissue [43,114], suggesting a potential defect in apical domain
organization. Recent studies showed further that Hpo pathway loss-of-function elicited a hypertrophy of the apical domain and AJs correlated with an accumulation of apically localized proteins, such as the Notch receptor and apical polarity proteins (aPKC, Arm, etc.) [80,115]. Interestingly, in C. elegans, wts loss-of-function also induces mislocalization of apical components [116].

What causes this apical hypertrophy? Overexpression of some apical determinants, such as Crb, can lead to apical membrane accumulation [117], and such proteins could conceivably be Yki targets. Indeed, loss of crb is sufficient to rescue the apical protein accumulation [80,115], as well as the apical membrane hypertrophy [115] induced by Hpo pathway loss-of-function. However, as crb is not a clear direct transcriptional target of the pathway, other Yki targets might be responsible for the phenotype. Beside the excess transcription of apical determinants, an imbalance between exocytosis and endocytosis might account for the apical hypertrophy of hpo mutant cells. Interestingly, several pieces of evidence link the Hpo pathway to the regulation of endocytosis. hpo mutant clones in ovarian follicle cells showed increased bulk uptake of a lipophilic dye [118]. Combined loss of ex and mer leads to a defect in apical clearance of Notch and other surface receptors, suggesting a defect in endocytosis [119]. Future studies should reveal whether vesicle trafficking defects underlie the apical domain expansion upon Hpo pathway disruption.

What is the role of apical domain size regulation by the Hpo pathway? We have outlined the importance of the apical domain in regulating Hpo signalling activity. It is therefore tempting to speculate that apical hypertrophy upon Hpo signalling disruption might be part of a fail-safe negative-feedback loop in order to re-establish Hpo activity when it is compromised. Indeed, Kibra, Mer and Ex, three Hpo pathway apical scaffolds, are Yki targets and are therefore up-regulated upon Hpo inactivation [52,70], as was also recently shown for mammalian KIBRA [76]. Such a feedback loop would ensure that Yki activity is kept in check during development.

THE HPO PATHWAY AND OTHER FORMS OF CELL POLARITY

PCP (planar cell polarity)

Apico–basal polarity is not the only form of polarity in an epithelium. Frequent epithelial tissues also possess a form of PCP, which allows cells in an epithelium to sense their position in relation to the tissue axes (antero–posterior, dorso–ventral or proximo–distal) and orientate themselves accordingly. An example is the orientation of hair on the mammalian epidermis. Two signalling pathways ensure the robustness of PCP: the Fz/Dsh (Frizzled/Dishevelled) signalling pathway and the Ft pathway. Some studies suggest that the Ft pathway acts upstream of the Fz/Dsh pathway, whereas other evidence points to parallel roles ([112–122]; for a review, see [123]).

As mentioned above, the Ft signalling pathway is a major upstream branch that regulates Hpo signalling activity [56–59]. Ft forms trans heterodimers with Ds [124]. The Golgi kinase Fj (Four-jointed) modulates the Ds–Ft interaction by phosphorylating both the Ds and Ft extracellular domains [125–128]. Fj and Ds are expressed in opposite gradients along the proximo–distal axis of Drosophila limbs, thus resulting in a proximo–distal gradient of active (Ds-bound) Ft, such that each cell has more liganded Ft on its proximal than its distal side [120,121,129]. This small difference in Ft activity is thought to result in the planar polarization of the apical myosin Dachs, the membrane localization of which is antagonized by Ft, resulting in Dachs localization on the distal side of each cell [130]. Active Ft signalling in turn influences the Hpo pathway, partly by promoting Ex’s apical localization and partly by preventing Dachs-dependent Wts degradation [56–59]. In both cases, the molecular mechanisms underlying the Ft-mediated regulation of Hpo signalling are still unclear.

What is the signal relayed to the Hpo pathway by the Ft–Ds interaction? The ‘steepness hypothesis’ is an attractive model which suggests that the Ft–Ds module allows the Hpo pathway to ‘read’ the slope of the long-range morphogen gradients that are responsible for the growth and patterning of developing tissues [131]. It is based on the idea that: (i) each cell in the developing field of a tissue can sense the steepness of a linear morphogen gradient and divide accordingly until the gradient slope falls below a certain threshold; (ii) the steepness of the gradient decreases as the tissue grows; and (iii) Ft signalling senses morphogen gradient steepness and translates it into growth regulation (via the Hpo pathway) and PCP, thus co-ordinating three major morphogenetic processes: size, shape and patterning. Such a role for Ft–Ds signalling was suggested by studies [132,133] showing that: (i) disturbances in the gradient of the Dpp [decapentaplegic morphogen; the BMP (bone morphogenetic protein)/TGF-β (transforming growth factor β) orthologue] induced alterations in the graded expression of Fj and Ds, suggesting that Dpp signalling (directly or indirectly) controls Ds/Fj expression; (ii) that uniform expression of Fj and Ds led to an overall reduced tissue growth; (iii) that ectopic proliferation was induced in cells that were exposed to (and able to sense) a sharp gradient in Ft–Ds activity across their width; and (iv) that this ectopic proliferation was mediated by triggering of Hpo pathway targets. Such positional information sensing may explain the requirement for pathway components in mediating the limb regenerative response in cricket [134].

Although this model is extremely appealing, several important issues remain to be resolved [131]. For example, how would a cell be able to compare the difference of a few liganded Ft molecules on its proximal against its distal size, and compute this into Dachs planar polarization and a binary proliferation/arrest response remains unclear. Through its ability to differentially affect the Ds–Ft interactions (phosphorylation of Ds decreases binding to Ft, whereas phosphorylation of Ft increases binding to Ds) [127,128], Fj might play a crucial role in strengthening this weak initial asymmetry, but some form of positive-feedback regulation is also likely to be required. In addition, the relationship between Ft and Dpp signalling appears to be more complex than first envisaged. YAP and SMAD1, one of the transcription factors effectors of BMP signalling, as well as their orthologues Yki and Mad interact with each other, leading to transcriptional regulation of a subset of common target genes (e.g. bantam in flies) [135,136]. Furthermore, YAP/TAZ regulates SMAD2/3 nuclear localization in response to cell density [64]. This suggests that the Hpo and Dpp/BMP/TGF–β pathways are tightly intertwined at the level of their downstream transcription factors rather than Ft/Yki signalling simply being a growth control effector for Dpp/BMP/TGF–β. An alternative view to the gradient steepness hypothesis suggests that the Ft signalling and Dpp gradients act in a complementary, but independent, manner to regulate growth in the wing imaginal disc [137].

Polarity in cell migration

Migrating cells are also polarized, having a leading edge that faces the direction of migration. Even though this type of polarity is different from apico–basal polarity, it involves many of the same polarity determinants. However, their relationships might be different, as it appears that both the aPKC complex and the
Scrib module are required at the leading edge for cell migration [138]. Since the Hpo pathway influences and is influenced by apico–basal cell polarity, could it be implicated in migration? Interestingly, mammalian KIBRA has been reported to interact and co-localize with the polarity protein PATJ at the leading edge of migrating podocytes [90]. Furthermore, KIBRA is required for migration of NRK (normal rat kidney) cells, where it mediates delivery of PaPKC by the exocyt at the leading edge of migrating cells [139]. In addition, YAP promotes cell migration and invasion [39,41,140]. Thus, in mammals, the Hpo pathway might regulate cell migration, although this question has not been examined in detail in flies.

CONCLUDING REMARKS: THE HPO PATHWAY AS A SENSOR OF EPITHELIAL HEALTH

In summary, numerous studies have highlighted the interplay between the Hpo pathway and apico–basal cell polarity/cell–cell contacts. The idea of the Hpo pathway as a sensor of epithelial architecture/integrity rather than a stereotypical ligand–receptor signal transduction pathway is emerging. We can speculate that the Hpo pathway receives various inputs reflecting epithelial ‘health’. Such inputs would include polarity and positional information encoded by morphogens (are the cell’s neighbours correctly specified and polarized?), but also mechanical forces or stress signals. By computing these different inputs, ranging from information about cell-intrinsic (polarity) to ‘social’ or tissue properties (morphogen gradients, tension, adhesion), the Hpo pathway would ensure harmonious growth during development and participate in maintaining homeostasis in adult tissues through the control of regeneration. Just as the past decade has yielded a detailed understanding of the core Hpo pathway cascade, the next few years will hopefully see the elucidation of these regulatory inputs.

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