loss-of-function mutant flies, showing that JAK signaling is necessary and sufficient for *TotA* induction. Not only *TotA*, but also *TotM*, *TotC*, and probably the other *Turandot* genes as well, are regulated in the same way (Boutros et al., 2002; Agaisse et al., 2003). Although direct evidence is lacking, it is likely that this signal is mediated by the *Drosophila* STAT gene. It was previously shown that STAT becomes translocated to the nucleus in the fat body of mosquitoes after a bacterial challenge (Barillas-Mury et al., 1999), and now the same thing is demonstrated to happen in *Drosophila* (Agaisse et al., 2003).

Many mammalian cytokines act via a JAK/STAT signaling pathway. The results of Agaisse et al. (2003) provide strong evidence that a cytokine also initiates the JAK/STAT-mediated induction of *TotA* in the *Drosophila* fat body. So far, two cytokine receptor homologs have been identified in the *Drosophila* genome. At least one of them, Dome, plays a role during development, activating JAK/STAT signaling in response to a secreted ligand, Unpaired (Upd; Castelli-Gair Hombría and Brown, 2002). Agaisse et al. (2003) now overexpressed a dominant-negative version of Dome, and found that this blocks *TotA* induction in the fat body. However, in this case, the relevant ligand does not appear to be Upd itself but a related protein, Upd3, which is expressed in the hemocytes. Inactivation of the corresponding *upd3* gene by RNAi in the hemocytes, but not in the fat body, blocks *TotA* induction. This provides a long-sought link between signaling in the hemocytes and the fat body.

The Turandot peptides are not the only peptides produced by the fat body in response to a bacterial challenge. Several antibacterial and antifungal peptides are also secreted into the hemolymph. The complement-like TepIII protein is regulated by *hopscotch* (Lagueux et al., 2000), but the production of antimicrobial peptides does not generally depend on JAK/STAT signaling. Instead, they are mainly regulated by the NF-κB-like transcription factors Relish and Dif (Hetru et al., 2003; Hultmark, 2003). Surprisingly, Agaisse et al. find that the *Relish* gene is also required for *TotA* induction. This is in conflict with our finding that *TotA* can be fully induced in *Relish* and *imd* mutants (Ekengren et al., 2001), although we have noted that *TotA* expression can sometimes be reduced in these mutants (our unpublished data). At the time, we ascribed this observation to the inherently large variability in the *TotA* response (also noticed by Agaisse et al.). Apparently, *TotA* regulation is complex and it is possible that the *Relish* pathway may, under some conditions, contribute to *TotA* induction. However, the *Tot* genes are induced by a variety of different stress factors, most of which do not affect the expression of antibacterial peptides. This argues against a general requirement for *Relish* in *Tot* gene induction. An interesting question is whether Upd3 and the JAK/STAT pathway are involved in all cases. It is possible that other signaling pathways mediate the response to some stimuli.

The *upd3* gene forms a cluster together with the homologous *upd* and *upd2* genes (Castelli-Gair Hombría and Brown, 2002; Agaisse et al., 2003). None of them is obviously related to mammalian cytokines, but the results of Agaisse et al. demonstrate that at least Upd3 can act as a cytokine in the activation of Turandot peptide secretion. It mediates a signal from the hemocytes to a cytokine receptor homolog in the fat body, activating the JAK/STAT pathway in a way that is highly reminiscent of cytokine signaling in mammals. This makes *Drosophila* an interesting model for the cytokine field.

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Coupling Cell Growth, Proliferation, and Death: Hippo Weighs In

Hippo restricts cell growth and cell proliferation, promotes cell death, and interacts with the tumor suppressors Salvador and Warts. This, together with the ability of Mst2 to rescue *hippo* mutant phenotypes, argues that Mst/Hippo proteins are tumor suppressors.

Four recent papers describe the characterization in *Drosophila* of Hippo, a serine/threonine kinase of the Sterile 20 (STE20) group, resembling Mst1 and Mst2.

Tissue size is determined by the relationship between cell size, cell proliferation, and cell death. Normally, these processes are balanced so that tissue size in the

adult remains constant over time. Tumor suppressors are genes whose loss of function results in a proliferative advantage for the cell carrying the mutation. Many tumor suppressors have been identified in humans, and inactivation of tumor suppressors is likely to be obligatory for the development of cancer. Previous screens in *Drosophila* for loss-of-function mutations that induce tissue overgrowth—and thus constitute candidate tumor suppressors—have identified a number of interesting loci. Two of these are *warts* (*wts*; Justice et al., 1995), also known as *lats* (Xu et al., 1995) and *salvador* (*sav*; Tapon et al., 2002), also known as *shar-pei* (*shrp*; Kango-Singh et al., 2002). *wts* encodes a serine/threonine kinase and *sav* a multidomain protein with features of an adaptor. Loss of *wts* or *sav* results in increased proliferation and increased resistance to cell death. Wts and Sav negatively regulate proliferation and promote cell death, at least in part, by suppressing the transcription of Cyclin E, a positive regulator of cell cycle progression (Kango-Singh et al., 2002; Tapon et al., 2002), and by promoting the loss of DIAP1, a cell death inhibitor (Tapon et al., 2002; Wu et al., 2003). Importantly, the human homolog of *sav* is mutated in some cancer cell lines (Tapon et al., 2002), and loss of the mouse *wts* homolog, LATS1, results in tumor development and hypersensitivity to carcinogenic treatments (St. John et al., 1999), arguing that both genes are conserved tumor suppressors.

Work from four labs now adds a new player to this story, the gene *hippo* (*hpo*; Udan et al., 2003; Harvey et al., 2003; Wu et al., 2003; Pantalacci et al., 2003). Loss of *hpo* results in tissue overgrowth, and this is associated with increased cell proliferation and decreased cell death. *hpo* encodes a *Drosophila* member of the STE20 family Ser/Thr kinases, and is most closely related to human Mst2 and Mst1. The significance of this homology is suggested by the fact that expression of human Mst2 can rescue the tissue overgrowth phenotype of *hpo* mutants (Wu et al., 2003).

How does Hpo regulate tissue growth? Jun N-terminal kinase (JNK) activation, which occurs in response to expression of Mst (Graves et al., 1998) or Hpo (Pantalacci et al., 2003), appears not to be critical for Hpo's activity as a growth regulator (Harvey et al., 2003; Pantalacci et al., 2003). Instead, at least part of the story again involves negative regulation of Cyclin E and DIAP1. Hpo suppresses transcription of Cyclin E (Harvey et al., 2003; Pantalacci et al., 2003; Udan et al., 2003; Wu et al., 2003) and DIAP1 (Udan et al., 2003; Wu et al., 2003). Expression of Hpo also leads to phosphorylation of DIAP1. This is correlated with a decrease in DIAP1 stability, suggesting that Hpo-dependent phosphorylation may stimulate DIAP1 degradation (Harvey et al., 2003; Pantalacci et al., 2003). Finally, Hpo overexpression promotes transcriptional induction of the apoptosis inducer and DIAP1 binding protein *head involution defective* (*hid*; Udan et al., 2003). Hid inhibits DIAP1 function through several mechanisms. Therefore, it seems likely that Hpo can use multiple mechanisms to promote cell death.

In an effort to understand how Hpo exerts these effects, the various groups explored interactions with *sav* and *wts*, the two genes that give rise to similar loss-of-function phenotypes. All three genes show genetic interactions with each other (Harvey et al., 2003; Tapon et al., 2002; Wu et al., 2003; Udan et al., 2003), suggesting that Hpo, Sav, and Wts function together in a common pathway. How does this pathway work? Together, the four groups have made a number of intriguing observations.

Hpo phosphorylates both Sav (Harvey et al., 2003; Pantalacci et al., 2003; Wu et al., 2003) and Wts (Wu et al., 2003) in vitro; moreover, the in vivo phosphorylation of both Sav (Pantalacci et al., 2003; Wu et al., 2003) and Wts (Wu et al., 2003) requires Hpo, and is dependent on the ability of Hpo to bind Sav (Harvey et al., 2003; Pantalacci et al., 2003; Wu et al., 2003), which also binds Wts (Tapon et al., 2002). Expression of a Hpo kinase-dead mutant acts as a dominant negative, suggesting that Hpo's activity as a growth regulator requires it be active as a kinase (Wu et al., 2003; Udan et al., 2003). However, Hpo kinase activity is not required for Sav phosphorylation, suggesting that Hpo recruits other kinases to Sav, perhaps to promote Sav stabilization (Pantalacci et al., 2003), and that Hpo's most important substrate may be something else, for example, Wts. These observations (with the caveats that complexes containing all three proteins have not been identified, and that the significance of Wts phosphorylation by Hpo is unclear) are consistent with a model in which Sav functions to bring Hpo and Wts together, thus promoting phosphorylation of Wts by Hpo (Wu et al., 2003). As noted above, Hpo may also recruit other kinases to Sav, leaving open the possibility of a more indirect mechanism of action. This is an attractive model that may capture part of the story. *wts* does show the strongest overgrowth phenotype of the three mutants, consistent with the idea that Wts activity is a major output of this pathway. However, double mutants between *sav* and *wts* show stronger overgrowth phenotypes than either mutant alone (Tapon et al., 2002). Therefore, it seems likely that Sav and Wts, and perhaps Hpo as well, each have growth regulatory functions that are independent of the other proteins.

As with any exciting observations, these papers raise a number of questions. Hpo now has several targets, DIAP1, Cyclin E, Hid, and Sav, which are likely to be important in any conserved role of Mst proteins as tumor suppressors. It will be interesting to determine how Hpo regulates these genes and whether Mst proteins have similar activities. In addition, it is important to recognize that critical targets of Hpo/Sav/Wts remain to be identified. Cells mutant for *wts*, *sav*, or *hpo* are accelerated throughout every phase of the cell cycle, but yet the cells are of normal size. Therefore, loss of these genes must stimulate cell growth (cell mass accumulation) in addition to cell proliferation. Increased expression of Cyclin E is sufficient to stimulate entry into S phase, but it does not increase the rate of progression through other phases of the cell cycle (in fact there is a compensatory extension of S phase), nor does it promote cell growth. Therefore, other targets must exist. Finally, Hpo, Sav, and Wts are expressed ubiquitously. Presumably, their activity is regulated—but by whom, and in what contexts? Cells mutant for any of these three genes have a fascinating phenotype: they have not lost the ability to differentiate, but they seem to be very poor at recognizing or responding to signals that would normally promote differentiation and constrain growth within a

tissue to limit its overall size. Therefore, identification of the mechanisms that promote activity within the Hpo/Sav/Wts pathway(s) is likely to provide important insights into the question of how cells acquire, integrate, and utilize information on the status of their environment to make critical decisions about growth, survival, and differentiation.

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PtdIns(3,5)P_2 Finds a Partner

Phosphatidylinositol-3,5-bisphosphate (PtdIns(3,5)P_2) is required for the sorting of a subset of membrane proteins at the late endosome. Unlike other phosphoinositides, binding partners for PtdIns(3,5)P_2 and its mechanism of action have not been characterized. New work by Friant et al. (2003) in this issue of *Developmental Cell* describes the identification of a yeast epsin-like protein that binds PtdIns(3,5)P_2 and functions in the transport of proteins through late endosomes to the lysosome-like vacuole.

Phosphoinositides (PtdIns's), phosphorylated derivatives of the membrane lipid phosphatidylinositol, are crucial regulators of many basic cell biological processes. The importance of PtdIns's as signaling molecules is particularly evident in the regulation of membrane trafficking, where multiple kinases that synthesize distinct PtdIns's are required for efficient protein transport. Different versions of PtdIns's have specific cellular locations, where they recruit proteins carrying modular domains that interact with the phosphorylated inositol head group.

PtdIns(3,5)P_2 is thought to be concentrated at endosomes and the lysosome, and is important for the sorting of a subset of membrane proteins late in the endocytic pathway (Cooke, 2002; Katzmann et al., 2002). Membrane proteins traveling through the biosynthetic and endocytic pathways arrive at a late endosomal compartment known as the multivesicular endosome or body (MVE/MVB). The MVB forms when portions of the late endosome membrane invaginate and pinch off into the lumen to form intralumenal vesicles. Fusion of the MVB with the lysosome results in delivery of MVB vesicle lipids and proteins into the interior of the lysosome, where they are processed or degraded (Katzmann et al., 2002; Raiborg et al., 2003). Although not required for the formation of MVB vesicles per se, PtdIns(3,5)P_2 plays a key role in the sorting of some proteins into these vesicles. Yeast mutants defective in the Fab1 kinase, which converts phosphatidylinositol-3-phosphate (PtdIns(3)P) to PtdIns(3,5)P_2, fail to deliver MVB vesicle cargo proteins from the biosynthetic pathway into the vacuole lumen (Odorizzi et al., 1998).

Proteins that bind to PtdIns(3,5)P_2 and regulate MVB trafficking have been elusive, but now Friant et al. provide an exciting breakthrough (Friant et al., 2003). These authors identified a yeast epsin-like protein, Ent3, as a PtdIns(3,5)P_2 binding protein that is required for transport of cargo from the *trans*-Golgi network (TGN) to the vacuole. Ent3 and its close homolog, Ent5, are two of five yeast proteins that carry an ENTH (*e*psin *N*-*t*erminal *h*omology) domain (De Camilli et al., 2002). Earlier this year, Ent3 and Ent5 emerged in a yeast two-hybrid screen for proteins that bind to a clathrin adaptor complex (AP1) and to the GGA monomeric clathrin adaptors. Gene deletion experiments revealed that Ent3 and Ent5 have redundant functions in clathrin localization to intracellular membranes and for the transport of proteins from the Golgi to endosomes (Duncan et al., 2003).

Conventional epsins were originally identified as proteins that bind to Eps15, a component of the plasma membrane endocytic machinery. Epsins also bind clathrin and are required at the internalization step of endocytosis, where they bind phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) through their ENTH domain. They act as accessory proteins that function at the initial step of vesicle formation to recruit clathrin-based coats, and may link cargo proteins to the clathrin-based internalization machinery (Wendland, 2002). Friant and coworkers happened upon the epsin-like Ent3 in a screen for new mutants that missort newly synthesized