this is indeed the case[1,2]. We now know that Jeb is secreted by somatic mesoderm cells and binds to Alk on the surface of adjacent mesoderm cells that are due to become visceral muscle (Fig. 1). This leads to Alk activating the MAP kinase pathway, via the Ras protein; this pathway in turn triggers the expression of the gene-transcription factors Duf and Org-1, which 'tell' the mesoderm cells to become visceral founder cells.

There are some further insights. The Frasch and Weiss groups[1] discovered that Jeb–Alk signalling simultaneously inhibits a transcription factor that is required for somatic muscle 'determination'. In this way, Jeb–Alk signalling definitively determines that cells become visceral, not somatic, muscles. Palmer's group[2] showed that internalization of the Jeb–Alk complex depends on the receptor's kinase activity, suggesting that the internalization and subsequent degradation of the signal might itself be regulated by signalling, possibly in some kind of feedback loop. Both papers agree that when cells are prevented from becoming visceral founders as usual, they abnormally fuse with somatic muscles.

These papers extend our understanding of how cell-to-cell signalling controls cell-fate decisions. They also marry two signalling proteins in search of partners. It will, of course, be a high priority to discover whether mammalian Jeb-like proteins also bind to Alk, and whether their role in muscle development is conserved. The fruitfly is a powerful model for mammalian biology, but it cannot be assumed that any given system is identical. Indeed, two other proteins — pleiotrophin and midkine — have been shown to activate mammalian Alk[9,10], so the relative roles of these different potential binding partners must be assessed. But genetics has now definitively established that, in fruitflies at least, Jeb is a physiologically significant partner for Alk.

Finally, although one of the obvious attractions of these papers is the discovery of a normal function for a protein that is implicated in cancer, it is important to remember that the relationship between the developmental and tumour-causing functions of signalling proteins can be quite indirect. The cancer-inducing form of Alk is misexpressed and does not require a partner to activate it, and has therefore escaped from normal physiological control. It has run amok, and the damage it causes may be unrelated to its normal function. ■

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Cell biology

The hippo hypothesis

Michael E. Rothenberg and Yuh-Nung Jan

The perfection of a fly's eye and the chaotic nature of tumours provide eloquent examples of the need to coordinate cell death and proliferation. The intricacies of the underlying mechanism are now being uncovered.

Just as the number of working parts in a machine is crucial, so too is the number of cells in a tissue. This means that the creation of new cells (by proliferation) and the elimination of excess ones (by programmed cell death, or 'apoptosis') must be tightly coordinated. Although many genes have been shown to regulate either proliferation or apoptosis, little is known about how the two are coupled. Recently, however, geneticists have begun to uncover some of the genes involved in this coordination, and, writing in *Cell*, Wu *et al.*[1] and Harvey *et al.*[2] describe another such gene, *hippo*. Their findings significantly advance our understanding of this fundamental problem in organ development and cancer biology.

Both groups[1,2] looked at the imaginal discs of fruitflies. Imaginal discs are packets of cellular monolayers (hence the name disc) that are set aside during larval development, and differentiate during metamorphosis (the pupal stage) to give rise to most of the tissues of the adult fly. In the case of the eye, on which the two groups focused, undifferentiated retinal imaginal disc cells proliferate rapidly throughout much of development. Later, waves of differentiation sweep across the eye disc to pattern the tissue (that is, to determine the fates and arrangements of the cells) and to halt cell division. This leads to the formation of a highly organized retina consisting of about 750 regularly spaced 'seeing units', called ommatidia (Fig. 1a, overleaf). During this process, the imaginal disc has an excess of cells between the ommatidia. Most of these leftover

100 YEARS AGO

Die Schule der Chemie. **Prof. Ostwald is an ingenious man; in his own language, the attribute might be expressed by the adjective "schlau." Having, as he tells us in his preface, published volumes of the greatest importance, and of the widest range, on physical chemistry for the use of investigators in the domains of chemistry and physics… he now makes an attempt in this very elementary work to reach a larger public, and has written this most amusing book for the use of youngsters about ten to thirteen years of age. The plan adopted is to introduce by means of dialogue some chemical facts… Talking of the combustion of a candle and its disappearance, the pupil says, "But it really vanishes before my eyes." "Yes," says the teacher, "it becomes invisible. But can't it change into something invisible?" "There are no invisible things," says the pupil. "Oho!" replies the teacher. "No," says the pupil, "ghosts and goblins don't exist." "Even they are said to be sometimes visible," answers the teacher. "But can you see the air?" "Hum — no," says the pupil. "But the air is changed by burning. I don't see how." And so the formation of an invisible gas is brought out, and the method of determining its weight.**

From *Nature* 1 October 1903.

50 YEARS AGO

The loofah of commerce, also known as the '*Luffa* sponge', 'dishcloth' or 'dishrag gourd', 'snake gourd' and 'vegetable sponge', is the cleaned, dried inner fibrous tissue of the fruit or gourd of *Luffa* spp., which grow readily in most warm countries. It is a climbing annual plant, and has been grown in Japan from olden times. The plant and fruits resemble cucumbers; but the fruits are fatter and hang heavily on the vines, while the stems are more woody and stronger. In Japan, the plant blooms during June–July, and the flowers last well into the autumn... The best type of loofah for the commercial market is the one produced in Japan, where the soil and climate have proved well suited to their cultivation. An account of the loofah industry in Japan has been given by J. S. Ingram in *Colonial Plant and Animal Products*… The cultivation and possibilities of the plant in Colonial territories is also described.

From *Nature* 3 October 1953.

'interommatidial' cells (some 2,000) are later eliminated by a strictly controlled wave of apoptosis, leaving a thin layer. The end result is a retina so perfectly organized that it has been called a "neurocrystalline lattice"[3]. Its perfection provides a sensitive assay for probing the link between proliferation and apoptosis.

Previous work had already indicated that cell proliferation and death in the developing retina are tightly coupled. First, inducing apoptosis early on in a group of retinal precursors — a manipulation that should dramatically reduce the size of the eye if cell death and proliferation were completely independent and inflexible — does not result in a much smaller eye[4]. Second, driving extra proliferation causes the imaginal disc to grow too large only if cell death is simultaneously inhibited[5]. Third, several genes, including *salvador* (also known as *shar-pei*)[6], have been shown to coordinately regulate both proliferation and apoptosis[7,8].

This coupling of proliferation and apoptosis seems to be a general rule in multicellular organisms, and the dysregulation of these processes can lead to cancer. Indeed, tumour development is thought to require both an increase in proliferation and a decrease in cell death[9]. Thus, a gene that promotes both apoptosis and exit from the cell-division cycle should be a tumour-suppressor gene. In fact, *salvador* is such a gene, and its human relative *hWW45* has been implicated in several cancers[8].

Quite how *salvador* and other such genes couple cell proliferation and death was unknown. But our understanding of this is now advanced by the characterization of *hippo*[1,2], a gene that functions with *salvador* and another tumour suppressor called *warts* (also known as *lats*)[10]. Wu *et al.*[1] and Harvey *et al.*[2] embarked on genetic screens to identify genes that negatively regulate tissue growth. They found *hippo*, a gene that belongs to a family of kinases — enzymes that add phosphate groups to other proteins. The Hippo protein is 60% identical to the human kinase Mst2, a close relative of which, Mst1, has been implicated in apoptosis[11].

The authors then found[1,2] that tissues lacking *hippo* contain too many cells. Within those mutant tissues, cell size and many aspects of patterning and cell-fate determination are remarkably normal — but the number of cells is dramatically increased. For instance, in the mutant eye, early patterning is not affected and photoreceptor cells differentiate and assemble into normal ommatidia. Yet there are many more interommatidial cells than normal, resulting in overgrown and folded eyes (Fig. 1b).

What causes the increase in cell number? First, there is increased proliferation. After the wave of differentiation has swept across the imaginal disc, normal cells stop dividing (or divide only once more). But cells lacking *hippo* continue to cycle for some time. At least one reason is that these cells upregulate Cyclin E, a limiting factor for entry into the DNA-replication phase of proliferation in imaginal disc cells[5,12]. Both groups agree that transcription of the *Cyclin E* gene is increased, although Harvey *et al.* suggest that a post-transcriptional mechanism might also contribute. So why are these extra cells not simply pruned away by apoptosis? The reason is that *hippo* is also required for apoptosis. Cells lacking *hippo* have increased levels of a key inhibitor of apoptosis, DIAP1, and are strongly resistant to treatments that should induce apoptosis.

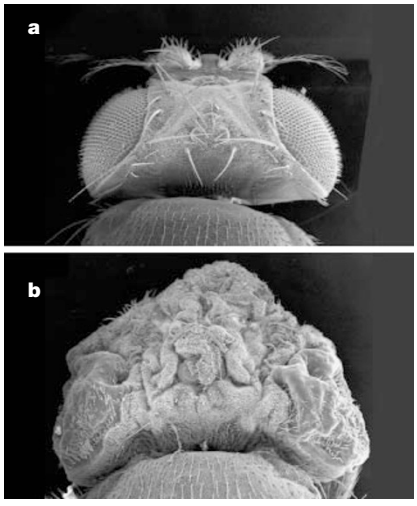

Figure 1 **Eyes right, and wrong.** a, **The head of a normal fruitfly, showing its perfectly organized eyes (left and right).** b, **The new papers[1,2] show that mutations in the *hippo* gene increase cell proliferation and decrease cell death, resulting in vastly overgrown tissues, including eyes. (Reproduced with permission from ref. 1.)**

The defects produced by a lack of *hippo* are remarkably similar to those produced when *warts* or *salvador* is missing, and indeed Wu *et al.* observed strong, specific genetic interactions between the three genes. These experiments, as well as other studies described in the papers, show that Hippo can associate with and phosphorylate Salvador, and that this interaction helps Hippo to phosphorylate Warts, another kinase. This suggests a model in which Salvador functions as an adaptor protein that brings Hippo and Warts together in a ternary complex. Presumably, phosphorylated Warts — as the major (but not the only) output of this complex — then goes on to regulate Cyclin E, DIAP1 and other downstream effectors. This work has thus begun to define a genetic pathway for the coordinated regulation of cell-cycle exit and apoptosis.

So what remains to be discovered? First, there is the question of mechanism. What is the exact nature of the physical, genetic and biochemical interactions between Hippo, Salvador and Warts? What is the significance of the phosphorylation of Warts by Hippo? And when, where and how does phosphorylation occur in this pathway *in vivo*? Also, given that any pathway that includes kinases also has regulatory phosphatases (proteins that remove phosphate groups), are phosphatases also involved in coordinating proliferation and death?

Second, there is the question of downstream targets. Tissues lacking *salvador*, *hippo* or *warts* show reduced cell death and more proliferation — but the cells also divide faster, yet remain of normal size. So this pathway also regulates cell growth (mass accumulation), and downstream targets in addition to DIAP1 and Cyclin E must exist. Might Hippo, as a member of the Ste20 family of kinases[13], be connected to signalling modules that contain mitogen-activated protein kinases — modules that regulate cell proliferation, death and growth elsewhere?

Third, there is the question of upstream control. How do cells receive and process the signals that work through the Hippo–Salvador–Warts pathway to control proliferation, death and growth? As all three proteins are ubiquitously expressed, it is likely that their activity (or the ability of cells to respond to them) is regulated, rather than their expression. Perhaps, as in other pathways involving Ste20-family kinases, regulatory enzymes such as members of the Ras family will be found upstream.

Finally, as a first step towards testing whether Hippo's role in coupling proliferation to apoptosis is evolutionarily conserved, Wu *et al.*[1] investigated whether the human Mst2 protein could restore the status quo to fruitflies that lack Hippo. They found that it could, raising the exciting possibility that Mst1 and Mst2 are tumour suppressors. This question could be investigated in several ways: by knocking out the genes in mice; by analysing them in tumour cell lines; and by searching for human families with heritable cancer syndromes that are caused by mutations in the genes for Mst1 or Mst2. ■

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