establishment proteins. However, these proteins cannot recognize Gβγ on their own. Instead, the polarity-establishment proteins Cdc24 and Bem1 bind to Far1, which in turn binds to Gβγ[4,5]. In support of a function for Far1 in polarity establishment, alleles of both *FAR1* and *CDC24* have been identified[6,7], termed *far1-s* and *cdc24-m*, respectively, that can polarize in response to the internal cell-cycle signal generated during vegetative growth, but fail to polarize properly in response to a gradient of pheromone.

Shimada *et al.*'s studies[3] now reveal that Far1 is more than just a linker for assembly of a polarity-establishment complex — it also plays a crucial part in the temporal regulation of polarity. Far1 controls polarity temporally by regulating the access of Cdc24 to sites of polarity establishment, so modulating Cdc24's activity. The localization of Cdc24 within the cell changes as a function of cell-cycle position — it is localized to the nucleus in early G1 and to the bud tip from the G1-to-S-phase boundary until mitosis, at which time it becomes localized to the bud neck[3,5,8]. It is Far1 that brings Cdc24 into the nucleus during G1, sequestering it there until a polarity signal is received either from the cell cycle or as a result of activation of the pheromone-response pathway.

Regulation of Far1 by a cell-cycle kinase provides the internal cue that triggers movement of Cdc24 to the cytoplasm during vegetative growth, leading to its activation and polarization of the actin cytoskeleton. At the boundary between the G1 and S phases of the cell cycle, Far1 is phosphorylated by the G1 cyclin–cyclin-dependent kinase (CDK) complex (Cln–Cdc28), inducing degradation of Far1 by a ubiquitin-dependent pathway[9]. Shimada *et al.*[3] show that when Far1 is degraded, Cdc24 is released and exported to the cytoplasm, where it can associate with the nascent bud site through interactions with Bud1. Thus, Far1 anchors Cdc24 in the nucleus during early G1, preventing it from functioning in the cytoplasm until the internal cue derived from the cell cycle is received, signified by activation of Cln–Cdc28.

Regulation of Far1 activity in response to pheromones leads to Cdc24 activation by a mechanism that is completely different from that used to respond to the internal, cell-cycle-derived cue[3]. When yeast cells sense a gradient of pheromone, the pheromone-response pathway is activated, leading to phosphorylation of Far1 by the MAPK Fus3. The phosphorylated form of Far1 is then able to associate with the transport receptor Msn5, which escorts Far1 out of the nucleus[10]. Intriguingly, Cdc24 comes along for the ride and thus is exported to the cytoplasm in association with Far1, where it is then targeted to the pheromone-induced site of polarization by the interaction of Far1 with Gβγ. Export of the Cdc24–Far1 complex thus has two consequences: it promotes its association with the polarization site directed by the external pheromone signal, and also prevents Cdc24 from

Wingless and Naked

Patterning of the embryo — the process by which asymmetrically organized cell types and organs are generated from a single cell to make up an individual — is controlled by a surprisingly limited number of signalling pathways (for example, the Hedgehog, Wingless, FGF, TGF-β and EGFR pathways). What's amazing is that patterning of structures as different as the limb or appendage and hair or bristles (for vertebrates or invertebrates, respectively), in species as different as the fruitfly *Drosophila melanogaster* and mammals, is controlled by the same conserved pathways. Specificity is achieved by the fine cross-regulation of these pathways by one another. Negative feedback loops have been described whereby activation of these signalling pathways leads to expression of negative regulators. These inhibitors may directly antagonize the signal, possibly by interfering with ligand binding to the receptor at the start of the signal cascade in the case of soluble inhibitors or membrane inhibitors, or by interfering with the downstream signalling cascade in the case of intracellular inhibitors. Alternatively, inhibitors may generate an antagonizing signal. But, until now, a negative feedback loop had not been described for the Wingless pathway.

Because the phenotype of *Drosophila* with a loss-of-function mutation in the *naked cuticle* (*nkd*) gene resembles that of *wingless* (*wg*) gain-of function mutants and *wg* transgenic animals, Nkd has been proposed to be an antagonist of Wg signalling. Genetic evidence also indicated that *nkd* might be a direct transcriptional repressor of Engrailed (a target of Wg), or might somehow influence Wg transport. Matthew Scott and colleagues (*Nature* **403,** 789–795; 2000) now show that Nkd indeed opposes the effect of a Wg signal. The *nkd* mutation, which is lethal in the embryo and causes multiple segmental defects, has been described previously and named after the absence of denticles in the mutant *Drosophila* embryos. Scott and colleagues have now cloned the *nkd* gene, and show that its expression pattern parallels that of *wg* in the *Drosophila* embryo and larva, indicating that *nkd* may be a target for Wg. They confirm this by monitoring the expression of *nkd* in gain-of-function and loss-of-function *wg* mutants. Overexpression of *nkd* (right-hand panel in figure below) and decreased *wg* activity (middle panel) in the *Drosophila* larva produce similar adult phenotypes (such as the absence of wings in the middle and right-hand panels). Scott *et al.*'s results indicate that Nkd interferes with Wg signalling, which they also show to be the case for Wnt signalling in the frog *Xenopus laevis*. So, as is the case for Hedgehog signalling with Patched, EGFR signalling with Argos, Kekkon and Sprouty, and TGF-β signalling with Dad, Wg signalling now has its own inhibitor and its own negative feedback loop.

The question now will be how Nkd inhibits Wg signalling. The evidence so far is that it does not, at least initially, interfere with the expression or transport of Wg, and so it most likely interferes with the signalling cascade downstream of the Wg receptors, Frizzled and Dishevelled. But where in the cascade, and how, does it act? Nkd bears a region of homology to calcium-binding EF-hand domains, which might provide a hint to its mode of action. However, Nkd seems to lack other structural features of EF-hand-containing proteins such as EF-hand repeats or myristoylation sequences, so this clue might be misleading.

VALERIE DEPRAETERE