becomes either cytosolic or is seen in patches around the cortex instead of as a basal crescent. Importantly, the apical Inscuteable crescent remains intact so myosin VI is only required downstream of, or in parallel with, the apical complex to localize proteins to the basal cortex.

What is not clear is the mechanism of action of myosin VI in neuroblasts. Previous studies of myosin VI in mammalian cells, flies, and worms have implicated it in the trafficking of vesicles and organelles. For instance, in mammalian cell lines, it is involved in endocytosis (Buss et al., 2001); in *Drosophila* syncytial blastoderm embryos, it plays a role in the delivery of vesicles to ingressing membranes (Mermall and Miller, 1995); during *C. elegans* sperm development, it is required for the partitioning of organelles in or out of spermatids (Kelleher et al., 2000). However, an alternative function in the stabilization of adherens junctions has been demonstrated in *Drosophila* oocytes (Geisbrecht and Montell, 2002). Since neuroblasts lose adherens junctions when they delaminate from the neuroectoderm, a trafficking role seems more likely, and the immunofluorescence staining in this study points in that direction. The authors do not see myosin VI enriched in the cortex, as one might predict if it played a role in maintenance of crescents. Rather, they see a punctate pattern reminiscent of the myosin VI distributions seen previously. Class VI myosins are unusual in that they move toward the minus, or pointed, ends of actin filaments in contrast to most other members of the superfamily. Unfortunately, there is no information available about the orientation of actin filaments in neuroblasts so the significance of this "backward" movement in basal targeting is unclear. Nevertheless, the simplest model would be that Jaguar is involved in transporting cargo that includes a complex of molecules nucleated by Miranda to the basal cortex.

The authors noted that unconventional myosin II (encoded by *zipper*) also co-IPs with Miranda but that myosin II and myosin VI could not be found in the same complex, implying that they might compete for Miranda binding. Certainly, this scenario helps to explain a genetic and molecular interaction previously found between myosin II and a cortically localized tumor suppressor, Lethal Giant Larvae (Lgl). Lgl is required for basal protein targeting and acts downstream of, or in parallel with, the apical complex. Genetically, myosin II antagonizes and myosin VI promotes Lgl function but *zipper* mutants themselves show no targeting defects. Since myosin II is a barbed end-directed myosin, it is possible that Zipper and Jaguar have antagonistic effects on protein targeting, although myosin II is largely cortically localized in neuroblasts.

One other important finding of this paper is that the mitotic spindle becomes misoriented when myosin VI function is attenuated. In neuroblast asymmetric divisions, the mitotic spindle undergoes a 90° reorientation to align along the apical-basal axis. It is known that an intact apical complex is required for spindle rotation and orientation, and now it seems that myosin VI may also play a role, possibly by delivering regulatory molecules to the spindle or indirectly by targeting a regulatory molecule to the basal cortex.

Myosins have been prime candidates to help target proteins during asymmetric division, but until now the evidence for their involvement in neuroblasts has been circumstantial. That myosin VI, implicated in trafficking events in other systems, should be the first shown to have a direct role is satisfying, although it would not be surprising to find that other members of the family are also involved.

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Selected Reading

Buss, F., Arden, S.D., Lindsay, M., Luzio, J.P., and Kendrick-Jones, J. (2001). EMBO J. *20*, 3676–3684.

Doe, C.Q., and Bowerman, B. (2001). Curr. Opin. Cell Biol. *13*, 68–75.

Geisbrecht, E.R., and Montell, D.J. (2002). Nat. Cell Biol. *4*, 616–620.

Jan, Y.N., and Jan, L.Y. (2001). Nat. Rev. Neurosci. *2*, 772–779.

Kelleher, J.F., Mandell, M.A., Moulder, G., Hill, K.L., L'Hernault, S.W., Barstead, R., and Titus, M.A. (2000). Curr. Biol. *10*, 1489–1496.

Knoblich, J.A. (2001). Nat. Rev. Mol. Cell Biol. *2*, 11–20.

Matsuzaki, F. (2000). Curr. Opin. Neurobiol. *10*, 38–44.

Mermall, V., and Miller, K.G. (1995). J. Cell Biol. *129*, 1575–1588.

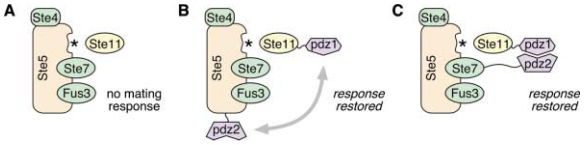
Petritsch, C., Tavosanis, G., Turck, C.W., Jan, L.Y., and Jan, Y.N. (2003). Dev. Cell *4*, 273–281.

Wodarz, A. (2002). Nat. Cell Biol. *4*, E39–44.

Enforced Proximity in the Function of a Famous Scaffold

Recent studies by Park, Zarrinipar, and Lim with reengineered Ste5 scaffold proteins underscore the fundamental importance of proximity in enzyme regulation and of keeping a proper distance for maintaining signaling specificity.

The scaffold protein Ste5 is critical for mating pheromone responses in budding yeast. When Ste5 is present, pheromone causes Fus3 to be activated and mating occurs; when Ste5 is absent, there is no Fus3 activation and no mating response. Ste5 binds all three members of the Ste11-Ste7-Fus3 MAP kinase cascade, thereby allowing them to be activated by their upstream regulators (Ste4-Ste18, the β- and γ-subunits of a trimeric G protein, and Ste20, a PAK-family protein kinase) and to relay signals to their downstream effectors. Ste5 is a complicated protein that does many things: it undergoes regulated dimerization, shuttles between the nucleus and the plasma membrane, and localizes to the region

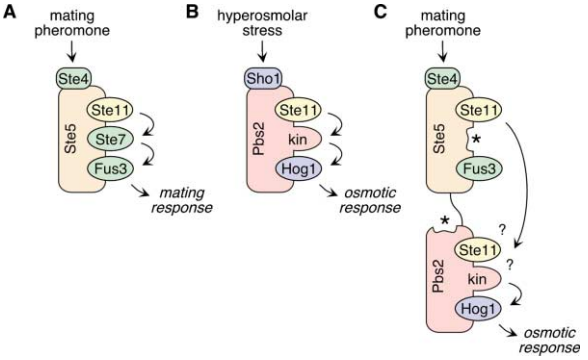


Restoring Scaffold Function through Enforced Proximity

(A) A Ste5 mutation that blocks Ste11 docking does not support mating responses.

(B) Adding dimerizing pdz domains to Ste11 and Ste5 restores mating responses.

(C) Adding dimerizing pdf domains to Ste11 and Ste7 restores mating responses.



Rerouting Cascade Signaling through Enforced Proximity

(A and B) Schematic of the normal mating pheromone pathway (A) and the normal hyperosmolar response pathway (B).

(C) Rerouting a mating pheromone input to a hyperosmolar response output by tethering the two pathways' scaffolds together.

of the cell closest to the source of pheromone (Dohlman and Thorner, 2001; Elion, 2001). But the most central aspect of its function is, arguably, the facilitation of signal transmission between Ste11, Ste7, and Fus3.

One way Ste5 might facilitate signaling is by acting as a "meta-enzyme": just as enzymes bind, orient, align, and push and pull on their substrates, Ste5 might bind, orient, align, and allosterically regulate its kinases, allowing them to efficiently relay signals. It is also possible that Ste5 might bind and orient, but not allosterically regulate its kinases. Or even more simply, Ste5 might bring the kinases into proximity without orienting or allosterically regulating them. This last possiblity, that Ste5 facilitates signal transmission mainly through enforced proximity, is the focus of a new paper by Park and coworkers (Park et al., 2003).

A priori it is clear that enforced proximity has the potential to play a powerful role in Ste5 function. Since Ste5 acts upon relatively scarce signaling proteins, the simple act of dangling two kinases near each other could increase their encounter rate enormously. For example, Ste7 is estimated to be present at a concentration of less than 2000 molecules per 100 fl yeast cell (Bardwell et al., 1996) or 20 molecules per fl. If Ste5 constrains Ste11 and Ste7 to within, say, 10 nm of each other, then the effective concentration of the scaffold-bound Ste7 in the eyes of the scaffold-bound Ste11 will be 250,000 molecules per fl, an increase of more than 10^4-fold. A scaffold could also convert weak binding interactions between the unscaffolded kinases into strong ones. By either mechanism, the result could be a substantial increase in the rate of Ste11-to-Ste7 signaling. If the scaffold also helps orient or allosterically activate the proteins, so much the better.

Park and coworkers have now tested these ideas experimentally by determining whether Ste5 can still function as an effective scaffold if it is stripped of any ability to align or allosterically regulate the kinases it coordinates. They began with a mutation in Ste5 known to disrupt the mating response and to prevent Ste5 from binding Ste11 (see first Figure, [A]). They attached a prosthetic protein-protein interaction domain—a PDZ domain derived from nitric oxide synthetase—to Ste11, and a second PDZ domain to which the first could bind with reasonable (~600 nM) affinity to the mutated Ste5 protein (see first Figure, [B]). They then asked whether mating pheromone could now induce mating. The answer was a clear yes—mating pheromone induced Fus3 activation, albeit more slowly and to a 5- to 10-fold lesser extent than it could with wild-type Ste5. In addition, pheromone induced mating, albeit ~100-fold less efficiently than it could with wild-type Ste5. The discrepancy between the biochemical (Fus3) and cell biological (mating) readouts of Ste5-mediated signaling is noteworthy; it suggests that there is some sort of noise filtration downstream of Fus3 or some way of authenticating a mating pheromone signal that the bogus scaffold does not get quite right. However, the most remarkable aspect of this result is the fact that the reengineered scaffold worked at all—that simply dangling Ste11 in the vicinity of Ste4-Ste18, Ste20, Ste5, Ste7, and Fus3 was sufficient to restore function to the system.

Of course, this conclusion assumes that the prosthetic protein-protein interaction domains did not inadvertently reproduce Ste5's ability to orient and/or allosterically regulate Ste11. So as an additional control, Park et al. tried tethering Ste11 to Ste7 rather than to Ste5 (see first Figure, [C]). Success again. And they examined a different Ste5 mutation, one that was defective for Ste7 binding rather than Ste11 binding. Again, restoring proximity—here by tethering Ste7 to either Ste5 or Ste11—restored signaling. In all cases, the prosthetic protein binding domains worked and always to about the same extent. It is exceedingly unlikely that all of these arrangements had accidentally restored an enzyme-like, precise alignment of the cascade protein kinases. Evidently enforced proximity is enough to restore a substantial degree of function to the mating pheromone cascade.

In a final, dramatic feat of scaffold reengineering, Park et al. built an entirely novel signaling pathway by tying two defective scaffolds together and thereby tested one simple and appealing model for signaling specificity. This experiment relies upon the curious fact that four distinct MAP kinase cascades in *S. cerevisiae* use the same MAPKKK, Ste11, and yet each cascade receives signals from distinct upstream inputs and relays them to specific downstream effectors, with little interfering crosstalk. Probably the two best-characterized of these are the Ste11-Ste7-Fus3 mating pheromone cascade discussed above and the Ste11-Pbs2-Hog1 osmosensing cascade, the latter of which is organized by a scaf-

fold-like sequence in the MAPKK Pbs2 (see second Figure, [A] and [B]).

The prevailing idea has been that scaffolds keep these distinct cascades distinct. One way a scaffold might accomplish this is by acting as an insulator—a Ste11 molecule on a Ste5 scaffold might be sterically prevented from interacting with Pbs2, and a Ste11 bound to Pbs2 might be prevented from interacting with Ste5-bound Ste7. In support of this idea, Harris and coworkers have shown that a constitutively active form of Ste11 can turn on both Hog1 and Fus3, but becomes less able to turn on Hog1 when fused to Ste5, and less able to turn on Fus3 when fused to Pbs2 (Harris et al., 2001).

An additional way a scaffold could contribute to pathway specificity is by simply failing to concentrate the "wrong" downstream effectors the way it concentrates the right ones—specificity by omission. To test this idea, Park et al. linked a Ste5 protein that could receive signals from Ste4, but relay them only as far as Ste11, to a Pbs2 protein defective for receiving osmotic signals from the upstream Sho1 protein (see second Figure, [C]). Expression of this diverter scaffold caused mating pheromone to trigger typical hyperosmolar responses (Hog1 phosphorylation, survival in high salt, and a characteristic gene expression profile) rather than mating responses. Exactly where the crossover occurred between the two cascades is not completely clear; it is possible that a Ste11 bound to Ste5 autophosphorylated a Ste11 bound to Pbs2 in *trans*, or perhaps the Ste5-bound Ste11 diffused off Ste5 and onto Pbs2, or maybe the Ste5-bound Ste11 directly phosphorylated Pbs2. In any case, something remarkable was achieved—the meaning of mating pheromone receptor activation was completely changed—simply by inviting a new guest to the Ste5 party.

One of the long-recognized appeals of enforced proximity as a mechanism for protein regulation is the inherent evolvability of the mechanism (Austin et al., 1994; Ptashne and Gann, 1998). By combining simple, modular binding interactions in a combinatorial fashion, scaffolds could generate molecular complexity and impose high specificity without requiring evolution of the signaling enzymes themselves. The Park et al. work underscores this idea in dramatic fashion: Park et al. apparently had little trouble creating a novel signaling pathway by recombining scaffold proteins whose main function seems to be simply dangling proteins near each other. Surely nature carries out the same sort of scaffold shuffling, allowing new signaling pathways to be generated and tested for usefulness.

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Selected Reading

Austin, D.J., Crabtree, G.R., and Schreiber, S.L. (1994). Chem. Biol. *1*, 131–136.

Bardwell, L., Cook, J.G., Chang, E.C., Cairns, B.R., and Thorner, J. (1996). Mol. Cell. Biol. *16*, 3637–3650.

Dohlman, H.G., and Thorner, J.W. (2001). Annu. Rev. Biochem. *70*, 703–754.

Elion, E.A. (2001). J. Cell Sci. *114*, 3967–3978.

Harris, K., Lamson, R.E., Nelson, B., Hughes, T.R., Marton, M.J., Roberts, C.J., Boone, C., and Pryciak, P.M. (2001). Curr. Biol. *11*, 1815–1824.

Park, S.H., Zarrinpar, A., and Lim, W.A. (2003). Science, in press.

Ptashne, M., and Gann, A. (1998). Curr. Biol. *8*, R812–R822.