In the work of Ezhkova et al., the conditional knockout of *Ezh2* occurs in the basal keratinocytes, cells that have already entered the epidermal pathway. In these cells, the genes controlling the pluripotent state as well as other differentiation pathways have been silenced by PcG-independent processes. Perhaps a model for this can be found in the regulation of the *Oct4* gene: when ES cells are induced to differentiate, the *Oct4* gene promoter is bound by transcriptional repressors, which recruit the H3K9 methyltransferase G9a, in turn leading to de novo DNA methylation and permanent shutdown of its expression (Feldman et al., 2006; Epsztejn-Litman et al., 2008). In this view, PcG-based mechanisms emerge as the flexible dynamic field artillery of genomic repression as opposed to the heavy artillery represented by DNA methylation.

Is the process of differentiation always as simple as the winding down of repression and consequent activation of terminal differentiation genes? In vivo there are clearly multiple inputs. Lessons from the fruit fly *Drosophila* indicate that correct morphogenesis results from new signals derepressing key genes modulating epigenetic patterns of expression and repression that are pre-established at earlier developmental stages and maintained by PcG-based mechanisms. This is the case for the *Hox* genes and for key morphogens such as Hedgehog or Wingless/Wnt.

How are the genes that initiate epidermal commitment protected from PcG repression so that they can activate the pathway? The classical model derived from *Drosophila* suggests that PcG target genes are also targets for Trithorax (TRX) or its mammalian counterpart MLL. However, how TRX/MLL antagonizes PcG repression is poorly understood.

Little is known about the way PcG genes themselves are regulated to permit the unfolding of developmental programs. In later postnatal development, there appears to be little or no Ezh2 detectable in basal cells, yet they must continue to act as epidermal stem cells to maintain the epidermal layers. How do these cells continue to divide if *Ink4* genes are now derepressed in the absence of Ezh2? Perhaps, as Ezhkova et al. suggest, Ezh1 takes over at later stages. Untangling the multiple regulatory networks involved in differentiation is likely to keep researchers busy for years to come.

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A MAPK Scaffold Lends a Helping Hand

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The scaffold proteins of signaling pathways are thought to act as passive tethering devices bringing together catalytic components of signaling cascades. Good et al. (2009) now reveal that in the budding yeast the scaffold protein Ste5 acts as an allosteric activator of the mitogen-activated protein kinase Fus3, rendering it competent to be a kinase substrate for signal transmission.

Many signaling pathways depend critically on scaffold proteins, which provide docking sites for various signaling components, thus organizing them for efficient communication. In insulin signaling, for example, the insulin receptor substrate 1 (IRS-1) scaffold protein provides multiple docking sites for SH2 proteins that can activate the phosphoinositide 3-kinase (p85) and stimulate the insulin response (Backer et al., 1992; Pawson and Scott, 1997). Perhaps the most famous of the scaffold proteins, however, is Ste5 in the budding yeast *Saccharomyces cerevisiae*. Ste5 serves as a hub that brings the protein kinases of the mitogen-activated protein kinase (MAPK) pathway into close

proximity, thereby enabling signal transduction and activation of the yeast mating response. Despite their central role in coordinating specific interactions between signaling pathway components, scaffold proteins have long been considered the passive wall flowers at the signaling dance, in contrast to kinases and other catalytic molecules. Reporting in this issue of *Cell*, Good and colleagues (Good et al., 2009) now turn this notion on its head by showing that the scaffold protein Ste5 takes a much more active role in determining the outcome of MAPK signaling in the yeast mating response than previously appreciated.

Vertebrates have multiple distinct MAPK pathways that signal to the MAPKs p38, JNK, and ERK, among others, and require different types of scaffold proteins (Morrison and Davis, 2003). In the budding yeast, the archetypical MAPK signaling pathway requires the successive activation of three protein kinases, MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK), and MAPK (Figure 1). The MAPK cascade responsible for activating the budding yeast mating response is organized around an essential scaffold protein Ste5, which has separate binding sites for MAPKKK, MAPKK, and MAPK. In the mating response signaling cascade, a MAPKKK called Ste11 is activated first by upstream signals; it then activates the MAPKK Ste7, which in turn activates the MAPK Fus3 (Chen and Thorner, 2007). The central question addressed by Good and coworkers concerns how the scaffold protein Ste5 helps the MAPKK Ste7 distinguish between two possible MAPK substrates—Fus3 or the very similar Kss1—in the last step of the MAPK cascade. Activation of Fus3 results in correct induction of the mating response, whereas activation of Kss1 triggers filamentous growth. Although Fus3 and Kss1 share a high degree of sequence identity (55%), Fus3 is an extremely poor substrate for the MAPKK Ste7 in the absence of a scaffold, whereas Kss1 is a good substrate for Ste7 without requiring a scaffold.

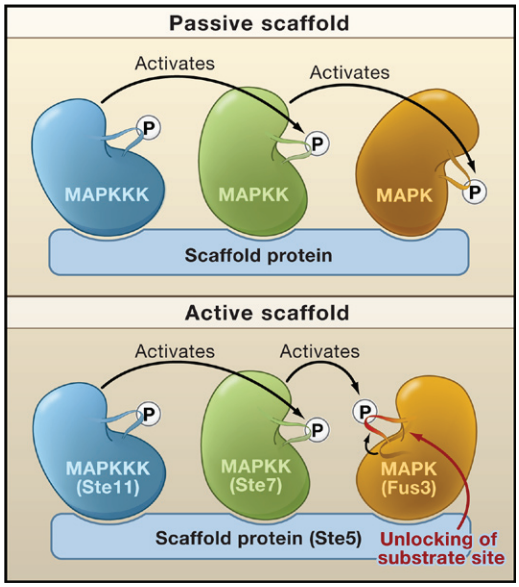

Figure 1. Scaffold Proteins and Their Clients
(Top) Passive tethering of kinases of the mitogen-activated protein kinase (MAPK) signaling pathway by a scaffold protein brings the kinases into proximity, enabling signal transduction. The MAP kinase kinase kinase (MAPKKK) that is activated by phosphorylation itself phosphorylates and activates MAP kinase kinase (MAPKK), which in turn phosphorylates and activates MAP kinase (MAPK). (Bottom) In the budding yeast, the MAPKKK Ste11 activates the MAPKK Ste7, which in turn activates the MAPK Fus3. Beyond tethering all three kinases together, the scaffold protein Ste5 also "unlocks" the Fus3 MAPK, enabling it to be phosphorylated and activated by the MAPKK Ste7.

In their new study, Good et al. (2009) now report the unexpected finding that a newly identified domain in the scaffold protein Ste5 (Ste5ms) allows the scaffold to change the activity of the MAPKK Ste7 toward Fus3 and Kss1. The authors find that the Ste5ms domain boosts the activity of Ste7 for Fus3 by ~5000-fold without affecting the activity of Ste7 when Kss1 is the substrate. What is particularly intriguing is that the Ste5ms domain does not achieve this differential activation of Ste7 by preferentially tethering one of the two possible substrates to the kinase, as would be predicted on the basis of the classical notion of how a scaffold protein works. Indeed, Good et al. observe that the MAPKK Ste7 actually binds very tightly to Fus3 without requiring the scaffold protein at all. Amazingly, Fus3 has no detectable affinity for Ste5ms, the minimal activating domain of the scaffold protein. So, if tethering is not involved, how does the Ste5ms domain transform Fus3 from a poor substrate into a more palatable one for the MAPKK Ste7?

Good and coworkers determined the crystal structure of the Ste5ms domain and show that there are two interfaces necessary for stimulating Fus3 activation. One interface mediates binding to Ste7, and the other is required for specific coactivation of Fus3. On the basis of this structure and an impressively detailed biochemical dissection of the activation mechanism, the authors propose a model in which the Fus3 MAPK exists by default in a "locked" conformation that makes it a poor substrate for the upstream MAPKK Ste7. Thus, even though Fus3 and Ste7 are capable of binding tightly to one another, the phosphorylation site on Fus3 remains inaccessible to Ste7 until the coactivator-like Ste5ms domain joins the two kinases and "unlocks" Fus3 (Figure 1). In the unlocked conformation, the phosphorylation site of Fus3 is now accessible, making this MAPK a good substrate for phosphorylation by Ste7. How does the Ste5ms domain "unlock" the Ste7 substrate at the molecular level? It is tempting to speculate that the Ste5ms domain achieves this by acting like a chaperone and locally changing the MAPK's fold to reveal its phosphorylation site to the MAPKK.

The model introduced by Good and coworkers illustrates a characteristic advantage of protein colocalization: the high local concentrations of Fus3 and Ste7 on the Ste5ms scaffold domain provide a thermodynamic boost that can be used to pay the energetic penalty of "unlocking" Fus3. It is not surprising that scaffold proteins are turning out to be allosteric regulators of the signaling molecules that use them as docking sites. The docking function of scaffold proteins may in fact predestine them to evolve additional roles as allosteric regulators. Colocalization of proteins on scaffolds or within cell membranes increases their local concentration so dramatically that protein-protein interactions can occur frequently even if the intrinsic binding affinities of the proteins are low (Kuriyan and Eisenberg, 2007). Under these circumstances, even single mutations can drastically change the propensity for a

protein pair to interact, thereby allowing specific interactions and allosteric regulatory mechanisms to evolve relatively quickly. Scaffold proteins can therefore act as "catalysts" for the evolution of specific interactions between the proteins that are bound to them. They can also acquire the ability to directly control the activities of the docked proteins, as illustrated here by the action of the scaffold protein Ste5 on its clients Ste7 and Fus3. This incisive mechanistic analysis of MAPK signaling by Good and coworkers may well change our view of scaffold proteins as the boring partners of catalytically active kinases. These results also show us that unexpected relationships can develop when evolution tinkers with molecules that are tethered together.

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A Gate Keeper for Axonal Transport

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The axon and dendritic arbor of neurons require different sets of membrane proteins to carry out their functions. In this issue, Song et al. (2009) describe how a cytoplasmic diffusion barrier in the axon initial segment of rat hippocampal neurons ensures that only axonal (and not dendritic) membrane proteins enter the axon.

Neurons are polarized cells harboring two distinct subcellular domains: the axon and the somatodendritic region including the dendritic arbor. The dendritic arbor receives signals from neighboring neurons, whereas the axon sends signals to neighboring neurons, providing the basic building blocks of the neuronal circuitry. Consistent with their specialized functions, the axon and dendrites have different protein and lipid compositions, including distinct sets of membrane proteins. The localization of membrane proteins to their sites of action in the specialized subdomains of the neuron is crucial for the maintenance of proper neuronal polarity and function. In this issue of *Cell*, Song et al. (2009) report the discovery of a cytoplasmic barrier in the axon initial segment of the neuron that prevents the free diffusion of macromolecules between the dendritic arbor and axon subdomains. This cytoplasmic selectivity filter allows entry into the axon of only axonal proteins (Figure 1).

Mechanisms for specific targeting of membrane proteins in neurons include selective sorting and transport along the secretory and endosomal pathways, selective retention of proteins at the membrane, and barriers in the membrane that prevent unwanted mixing of proteins (Horton and Ehlers, 2003) (Figure 1). Vesicular transport of membrane proteins to axons and dendrites is generally carried out by molecular motors of the kinesin and dynein superfamilies, which move along microtubules to transport vesicles toward the plus and minus ends of microtubules, respectively (Hirokawa and Takemura, 2005). Different molecular motors of the kinesin superfamily (KIFs) recognize specific transmembrane cargo proteins and direct them from the cell body to the axon or dendrites. Interestingly, the motor protein KIF5 is responsible for both the dendritic targeting of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors and the axonal transport of certain membrane proteins including amyloid precursor protein and VAMP2, a synaptic vesicle protein. This raises the question of how a motor protein determines whether its cargo is destined for the axon or for dendrites.

Maintaining the asymmetric distribution of membrane proteins in the different neuronal subdomains is crucial for proper neuronal function. A membrane diffusion barrier that restricts the lateral mobility of membrane proteins and lipids has been identified in the axon initial segment of neurons (Nakada et al., 2003; Winckler et al., 1999). The axon initial segment harbors a high density of ankyrin G, adaptor proteins that connect the spectrin-actin cytoskeleton with integral membrane proteins. In their new study, Song et al. (2009) now find that in