CELL SIGNALING

A Sophisticated Scaffold Wields a New Trick

Many cellular processes are governed by proteins that are docked onto a scaffold protein. In addition to providing binding sites, scaffolds influence the signaling output of these multiprotein complexes.

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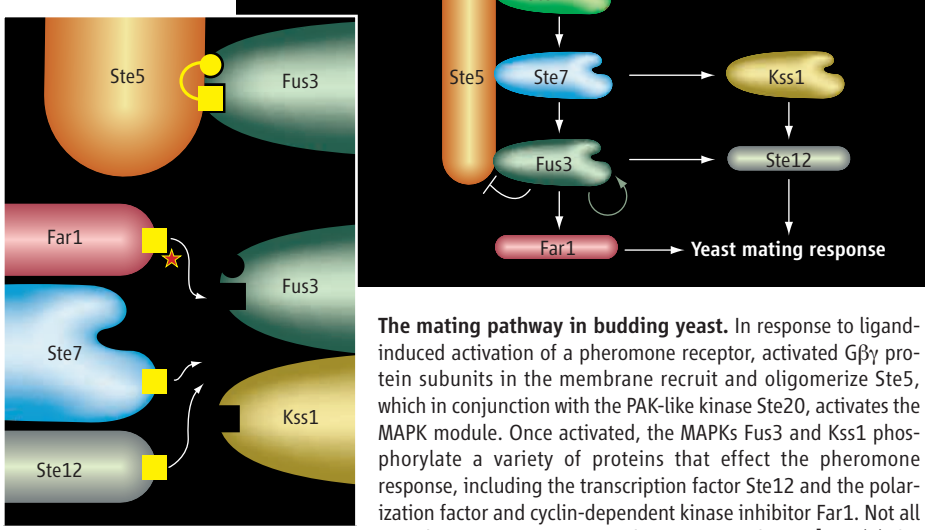
A complex and dynamic network of signaling proteins enables eukaryotic cells to respond to different stimuli, including changes in their physical environment, contacts with other cells, and growth and differentiation factors. Mitogen-activated protein kinase (MAPK) modules often help accomplish this task. (*1*). The archetypal MAPK signaling cascade mediates the mating response of budding yeast (see the figure). In response to pheromone, upstream events activate the MAPK kinase kinase Ste11, which then phosphorylates and activates the MAPK kinase Ste7, which in turn phosphorylates and activates two partially redundant MAPKs, Fus3 and Kss1 (*2*). MAPK activation requires dual phosphorylation on a threonine (T) and a tyrosine (Y) in a conserved T-X-Y (X, any amino acid) motif in the kinase activation loop. In addition to the tiered activation steps in the MAPK cascade, the pheromone pathway kinases are functionally connected by the scaffold protein Ste5 (*3*). Metazoan MAPK scaffold proteins, such as KSR (kinase suppressor of Ras) and JIP (c-Jun N-terminal kinase inhibitory protein), have also been discovered (*1*). As befits their name, scaffolds were originally thought to act as passive docking sites, functioning to localize and concentrate the appropriate components for signal transmission (*3*, *4*). However, at least two lines of evidence hint that Ste5 might play a more active role in signal transmission. First, in vitro studies that reconstituted the yeast pheromone–responsive MAPK cascade suggest that Fus3 is activated in a distinctly stepwise fashion, with highest activity achieved in the presence of Ste5 (*5*). Second, synthetically tethering the kinases to Ste5 with ectopic protein interaction domains only partially rescues the mating defect of *ste5* mutants (*6*). On page 822 of this issue, Bhattacharyya *et al*. (*7*) demonstrate that Ste5 indeed plays an active role in signaling, and a surprisingly dynamic one at that.

In the first glimpse of a scaffold bound to a MAPK, Bhattacharyya *et al*. crystallized a 29-residue polypeptide of Ste5 in complex with Fus3. The Ste5 peptide extends over the back side of Fus3 and engages one site on the N-terminal lobe of the kinase (site A) and another on the C-terminal lobe (site B). An intervening disordered region of eight residues links the two MAPK-binding regions of the Ste5 peptide. At the A site, Ste5 interdigitates into the normal five-stranded β-sheet structure of the N-terminal lobe to create a seven-stranded β-sandwich architecture. The B site precisely overlaps with a previously described MAPK surface groove that binds to docking peptides present in a variety of substrates, as well as in the upstream MAPK kinase (*8*, *9*). Unexpectedly, the Ste5 peptide allosterically induces autophosphorylation of the tyrosine in the T-X-Y activation loop of Fus3, which leads to a substantial degree of kinase activation (*7*). This autoactivation event is intramolecular in nature and requires an optimal length linker between the two MAPK-binding regions in the Ste5 peptide. Other peptides known to interact with Fus3 did not have this effect. Ste5 is also phosphorylated on a threonine residue by autoactivated Fus3. Mutation of this Ste5 modification site increases the expression of a mating-responsive gene, implying that the Ste5-MAPK module attenuates its own activity (*7*). All told, these findings dramatically elaborate the repertoire of Ste5 functions.



The mating pathway in budding yeast. In response to ligand-induced activation of a pheromone receptor, activated Gβγ protein subunits in the membrane recruit and oligomerize Ste5, which in conjunction with the PAK-like kinase Ste20, activates the MAPK module. Once activated, the MAPKs Fus3 and Kss1 phosphorylate a variety of proteins that effect the pheromone response, including the transcription factor Ste12 and the polarization factor and cyclin-dependent kinase inhibitor Far1. Not all signaling components or substrates are shown [see (*2*) for details]. **(Inset)** Circle indicates peptide motif from Ste5 that docks specifically into the A site of Fus3. Squares indicate the peptide motifs from Ste5, the MAPK kinase Ste7, and various substrates that dock into the B sites of both Fus3 and Kss1; the star on Far1 indicates that it is a Fus3-specific substrate (*9*).

As with any breakthrough, a host of new questions arise. How the presumed tension exerted by the Ste5 linker on the two lobes of Fus3 facilitates autophosphorylation is not clear. However, this type of mechanism is not entirely without precedent. For example, modulation of the N-terminal lobe through peptide interactions is used to activate other kinases, such as protein kinase A or Akt/protein kinase B (*10*, *11*). In another sense, Ste5 is loosely analogous to the protein cyclin, which binds and activates the cyclin-dependent kinases that control the cell division cycle. (*12*). And, as Bhattacharyya *et al*. note, the activation of Fus3 by Ste5 is reminiscent of the autoactivation of the mammalian MAPK p38α by its binding partner, TAB1 (*13*). Finally, intramolecular autophosphorylation has also been docu-

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mented as an intrinsic maturation step for the dual-specificity tyrosine phosphorylation–regulated protein kinases that are distantly related to MAPKs (*14*). The fact that Fus3 phosphorylates itself on tyrosine belies its categorization as a serine-threonine kinase, and begs the question as to whether Fus3 may phosphorylate other substrates on tyrosine residues.

Given the overlapping binding sites on Fus3, competition for binding partners both within the Ste5-MAPK complex and for other Fus3 substrates must be rife, especially because many of the peptide interactions occur with moderate to weak affinity (*7*, *9*). Fus3 does not detectably interact with either the A- or the B-site fragment of Ste5 alone, in agreement with the finding that the Ste5-Fus3 and Ste7-Fus3 interactions are competitive (*15*). This competition implies that a dynamic series of events occurs during signal transmission. Then there is the issue of Kss1, which is also activated in the pheromone response (*5*, *16*). Kss1 does not, however, interact with the Ste5 fragment that binds Fus3, and instead requires Ste7 for recruitment to Ste5 (*5*). Because Kss1 is still activated by pheromone in the presence of a catalytically inactive form of Fus3 (*5*, *17*), it seems plausible that both Fus3 and Kss1 are found together in oligomerized Ste5 complexes, which are necessary for signaling (*18*).

From the signal transmission perspective, yet other issues come to the fore. If Ste5 triggers partial activation of Fus3 by autocatalytic tyrosine phosphorylation, what then is the role of Ste7? Through mutational analysis, Bhattacharyya *et al*. clearly demonstrate that the pheromone response requires Ste7-mediated dual phosphorylation of Fus3 and not Ste5-mediated monophosphorylation (*7*). Indeed, it is unknown whether Fus3 is ever monophosphorylated on tyrosine in vivo. If this form of Fus3 does exist, what role might it play in modulating the signaling response? Another canonical MAPK module, the p42/44 Erk1/2 pathway that triggers maturation of frog (*Xenopus laevis*) oocytes, displays a profound all-or-none or "switchlike" response to stimulus (*19*). In yeast, however, careful single-cell analysis has recently demonstrated that yeast respond gradually to increasing doses of pheromone in a graded rather than switchlike response (*20*). By potentially eliminating the distributive phosphorylation of Fus3, Ste5 might flatten the dose-response curve of Fus3 activation, as would the observed feedback inhibition of Ste5.

Unlike the conserved enzymatic components of signaling networks, scaffolds are not easily recognized by sequence similarity alone and so have undoubtedly been understudied to date. The rather dull moniker "scaffold" has also perhaps not helped the profile of these interesting proteins. Scaffolds have been discovered not only as components of MAPK modules, but also in many other signaling pathways, such as the ubiquitous A-kinase anchoring proteins (AKAPs) that confer spatial specificity on protein kinase A activation (*21*). Spurred on by the ground-breaking studies of Bhattacharyya *et al.*, odds are that more scaffold magic will soon be discovered.

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CHEMISTRY

Building Molecules with Carbon Monoxide Reductive Coupling

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A new reaction can generate complex organic molecules from carbon monoxide. This method for creating organic materials could provide fuel and chemicals that do not depend on our dwindling petroleum supply.

Uncertainty in petroleum cost and supply demands that alternative sources of primary organic materials be developed (*1*). Carbon monoxide as a component of synthesis gas (nH_2 + CO) from coal and biomass will have a leading role in meeting these needs (*2*). One prominent objective of energy-related research is to develop strategies that use CO as a two-carbon or larger building block to construct the organic materials needed for fuels and chemical manufacturing. Transition metal catalysts are highly effective in directing reactions of carbon monoxide as a one-carbon building block, but catalysts that promote C–C coupling of CO have not yet been developed. Determining the scope and nature of carbon monoxide reactions with metal complexes that result in C–C bond formation provides guidelines for the design of catalysts for CO coupling reactions. An important new type of CO coupling is reported on page 829 of this issue by Summerscales *et al*. (*3*) in which a set of three CO molecular units combine to form an oxocarbon dianion ring, $C_3O_3^{2-}$, by means of an organouranium complex.

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Observation of the actinide complex containing the elusive $C_3O_3^{2-}$ unit both encourages a renewed exploration for additional classes of metal-induced CO reductive coupling reactions and advances the historic search for carbon monoxide reactions that directly produce the three-carbon member of the oxocarbon dianion series ($C_nO_n^{2-}$; $n = 3$ to 6). This quest began in the very earliest chapter of organic chemistry when in 1834 Liebig (*4*) reported that CO reacts with molten potassium to produce salts of the croconate ($C_5O_5^{2-}$) and rhodizonate ($C_6O_6^{2-}$) dianions. These five- and six-membered oxocarbon ring compounds had been reported to be formed from high-temperature reactions of KOH and carbon by Gmelin (*5*) in 1825. Croconic and rhodizonic acids are now known to be products of biological oxidations, and thus in hindsight Gmelin could be credited with the earliest organic synthesis from nonorganic substances. More than a century passed before the

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