Minireview

Sequence patches on MAPK surfaces define protein-protein interactions
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Abstract

Recent studies on the modularity of mitogen-activated protein kinases show how redesigning 'surface patches' on a protein can change the topology of a signaling network.

In cells, protein-protein interaction domains control the organization of multiprotein complexes in signal transduction networks, thereby determining the responses of cells to many different stimuli [1]. Such domains are generally defined as independently folded structural modules that can bind a protein ligand or a peptide motif. There are at least 81 defined protein-interaction domains in eukaryotic cells that control the organization and responses of signaling networks [2]. Even a given domain can have significant complexity and be used repeatedly in different contexts. For example, more than 120 Src-homology 2 (SH2) domains - which recognize phospho-tyrosines - are encoded in the human genome. Each SH2 domain has amino acid variations that alter the sequence context within which it recognizes a phospho-tyrosine residue. In higher eukaryotes especially, a single protein is typically composed of multiple domains, and so the ability to reconfigure the repertoire of domain composition and position within a protein provides a powerful mechanism for reconfiguring the architecture of signaling networks both in evolution and by design engineering [3-5].

Although domain-wiring models, defined by domain-dependent protein metrications, have proved to be particularly valuable in predicting protein interactions within complex networks, they best describe how the primary backbone of the network is laid out. The high-fidelity choice of interaction partner can only be partly explained by domain-wiring. For instance, a degree of interaction specificity can be controlled by variation within the domain itself, as evidenced by the 120 or so different members of the SH2-domain family. However, it is clear that in many cases the specificity of a protein interaction cannot lie entirely with the interacting amino acids in the binding site, and a degree of 'fine-tuning' of specificity occurs elsewhere in the protein. The recent work of Mody *et al.* [6] published in *Nature Cell Biology* helps shed light on how the modularity of two yeast mitogen-activated protein kinases (MAPKs) establishes a capability for altering the specificity of interaction and, therefore, for changing the topology of a signaling network.

The modular nature of MAPKs

MAPKs are relatively small proteins with an average mass of around 40 kDa. The three-dimensional structures of several MAPKs are known and show them to be compact globular proteins [7,8]. MAPKs are serine-threonine kinases that phosphorylate diverse transcription factors, intracellular enzymes and cytoskeletal proteins to control gene expression and the physiological program of the cell. They are activated by MAPK kinases (MKKs) via the phosphorylation of a threonine and a tyrosine in a conserved Thr-X-Tyr motif on the 'phosphorylation lip' of the kinase domain, and are inactivated by specific phosphatases that remove these phosphate groups. In addition, MAPKs often bind specific scaffold proteins such as Ste5 in yeast and KSR in mammalian cells [9,10]. In response to a particular signal (such as pheromone signaling), scaffold proteins such as Ste5 bind

and organize specific components of a 'MAPK cascade' - MAPK kinase kinases (MKKKs), MKKs and MAPKs - in such a way that they interact effectively with each other [9,10]. Each different MAPK must therefore interact with high specificity with multiple proteins so that MAPK signaling networks responding to different stimuli can be formed and regulated.

The specific interactions MAPKs make with their cognate MKKs, substrates, scaffolds and phosphatases contribute significantly to pathway specificity, and involve a docking groove found in all MAPKs that contains a basic region and a hydrophobic region and binds the hydrophobic docking-peptide motif ϕ_A-X-ϕ_B (where ϕ_A and ϕ_B are hydrophobic residues - Leu, Ile or Val) [11-13]. However, given the relative conservation of docking-groove amino acid sequence among MAPKs, it is unlikely that the docking groove and the cognate binding motifs are the only mechanism for controlling the specific interaction of MAPKs with their many ligands.

The recent work of Mody *et al.* [6] provides a significant advance beyond the docking groove in our understanding of MAPK modularity and the determinants of its interaction with other proteins. These investigators examined the sequence alignments of multiple yeast, human and plant orthologs of *Saccharomyces cerevisiae* MAPKs. Focusing on Fus3 and Hog1, *S. cerevisiae* orthologs of the mammalian MAPKs ERK1/2 and p38, respectively, they hypothesized that variable residues in particular surface regions or 'patches' in the two proteins could contribute to the different activation and substrate specificities of Fus3 and Hog1. Fus3 is activated by the MKK Ste7 and phosphorylates substrates such as the cell-cycle arrest mediator Far1 in response to mating pheromone. In contrast, Hog1 is activated by the MKK Pbs2 in response to hyperosmolar shock and phosphorylates several transcription factors, including Hot1 and Sko1, thus initiating a response to osmolyte imbalance. Mody *et al.* investigated the significance of the sequence patch in controlling the specificities of Fus3 and Hog1 for their upstream MKKs and downstream transcription-factor substrates by constructing kinases containing different combinations of amino acids from the Fus3 and Hog1 patches (Figure 1). The chimeric proteins were expressed in appropriate genetic backgrounds in *S. cerevisiae* and tested for their ability to signal pheromone- or hyperosmolar-stimulated responses.

Mody *et al.* [6] used six different segments of Fus3 and Hog1 in the combinations shown in Figure 1. The segments 'B' (or 'b') and 'F' (or 'f') contain the docking-groove sequences. The BF segments from Fus3 were effective in maintaining the mating-pheromone response mediated by the upstream MKK Ste7, and the bf segments from Hog1 were similarly effective in maintaining the sorbitol hyperosmolar response (Figure 1a and 1b, respectively). These effects were most
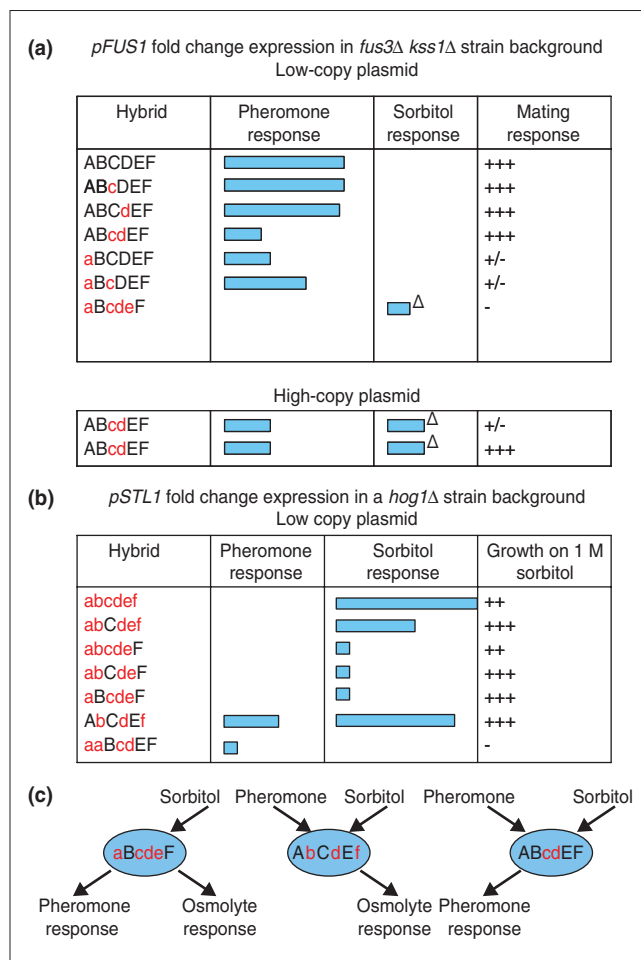

Figure 1

The responses to pheromone and sorbitol in the presence of different Fus3 and Hog1 hybrid proteins. **(a)** The hybrids of Fus3 and Hog1 are shown on the left. Capital letters ABCDEF (black) each represent a segment of Fus3, while the lower-case letters abcdef (red) each represent a segment of Hog1. The relative responses to pheromone and sorbitol were measured using a *FUS1* promoter-driven reporter gene to detect Fus3 activity (horizontal blue bars). Plasmids bearing the hybrid genes were introduced into cells deleted for endogenous Fus3 (*fus3Δ*) and the MAPK Kss1 (*kss1Δ*), an alternative target for Ste5 activation. Mating activity is scored from +++ to - (none). The lower panel in (a) shows the crossover response in which sorbitol activates the *FUS1*-driven reporter gene when there is high-copy expression of the ABcdEF hybrid. The Δ symbol indicates that the response was maintained in a Ste7-deleted background. **(b)** The relative responses to pheromone and sorbitol were measured using a *STL1* promoter-driven reporter gene to detect Hog1 activity (represented by blue bars). Relative efficiency of growth on sorbitol is scored from +++ to -. Data in (a) and (b) are from [6]. **(c)** Model modified from [6] depicting the ability of different sequence patches in Fus3/Hog1 hybrids to regulate the pheromone and osmolyte activation of hybrid MAPKs.

clear-cut when the chimeric MAPKs were expressed from low-copy-number plasmids, which is more representative of their physiological levels. When high-copy plasmids for high protein expression were used, there is significant crossover, and responses to both pheromone and sorbitol were seen with each MAPK. This is due to the high protein expression

enabling lower-affinity interactions to occur to a much greater extent.

Notably, cells expressing ABcdEF or ABcdeF had constitutive Fus3 activity. This is particularly interesting because replacing Thr and Tyr in the Thr-X-Tyr activation-loop motif with phosphomimetics does not activate Fus3 or Hog1. These results suggest that the CD segments have a role in controlling the inactive state of Fus3 and that substitution with the cd region of Hog1 relieves this inhibition. The three hybrids ABcdEF, ABcdeF and aBcdeF showed Fus3 activity in response to sorbitol, even when Ste7 (the Fus3 MKK) and Hog1 were deleted. That result indicated a direct activation of the chimeric protein by Pbs2, the MKK for Hog1, which was now able to recognize Fus3. This is particularly telling, because these three hybrids encode the docking-groove BF segments of Fus3, and it implies that segments A/a, C/c, D/d and E/e in Fus3 and Hog1 make significant contributions to recognition by their cognate MKKs.

Figure 1c summarizes some of the salient findings from the hybrid analysis. These indicate that an aBCDEF hybrid produces only a low-level mating response, thus implicating segment A in the interaction of Fus3 with Far1. This is not too surprising, as segment A/a includes the ATP-binding pocket and includes residues involved in substrate recognition. Segment 'd' is important for transducing a hyperosmolar response to either pheromone or sorbitol. It is required, although not sufficient, for activation of hybrid MAPKs by sorbitol at low-copy expression. Segment d has a significant deletion relative to D, with a somewhat neutral drift in the amino acid differences in D, suggesting that the insert might be significant for the selectivity of Fus3 for Ste7 and Far1 in the mating response. Overall, the hybrid analysis shows that the different sequence patches in A/a, C/c, D/d and E/e play significant roles in specificity in addition to the roles played by the docking groove and activation loop (comprising segments B/b and F/f).

Engineering MAPK signaling dynamics

Switching or modification of specific sequences on the MAPK surface enables the generation of promiscuous enzymes that respond to multiple activators and act on multiple substrates, the evolution of new specificities within signaling networks, and the engineering of MAPK interactions to rewire network behavior. The identification by Mody *et al.* [6] of regions outside the docking groove that support interaction specificity expands the ability to engineer MAPKs to have new functions. Thus, engineering these sequence patches as well as the docking groove will enable the development of MAPKs with unique connections for upstream activators, downstream substrates, inactivating phosphatases and the scaffolds that organize the MAPK signaling complexes. Such specificity modifications could be engineered in combination with scaffold modifications to allow assembly of MAPK cascades that

modulate positive- and negative-feedback loops controlling duration and magnitude of activation, sensitivity of the system to specific stimuli, and the ability to tune the system [14,15].

The modular nature of MAPKs and their scaffolds allows rational design principles to be used to build synthetic responses for therapeutic uses. For example, one can imagine a surface receptor expressed in vascular sentinel cells that binds a specific disease-related biomarker released into the bloodstream that, in turn, activates a synthetic MAPK system and sounds the alarm for early diagnosis and therapeutic intervention. The extensive and growing knowledge base for designing synthetic MAPKs and scaffolds suggests that such ideas are probably already in the making.

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