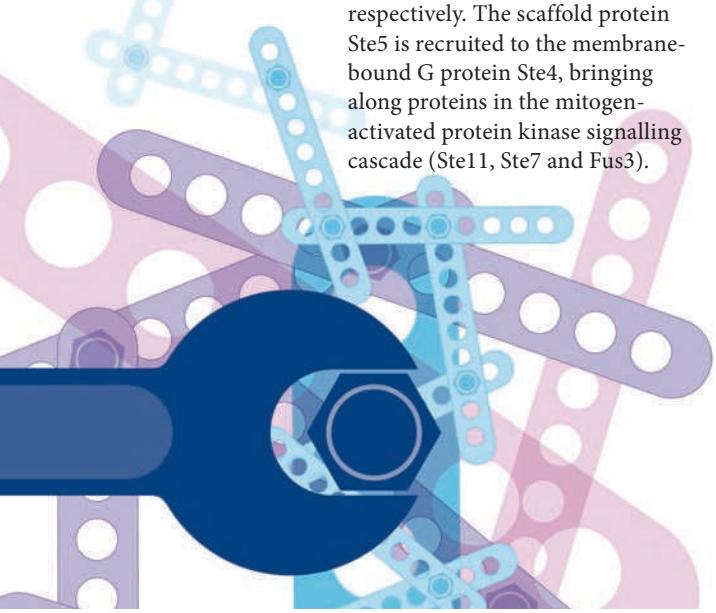
Nature Reviews Molecular Cell Biology | AOP, published online 12 May 2010; doi:10.1038/nrm2909

CELL SIGNALLING

Making new connections

> recombining domains from proteins in the budding yeast mating pathway leads to diversity in pathway response dynamics



In signalling networks, protein domains usually have a catalytic function or play a part in the regulation or localization of a protein and it has been suggested that the reorganization of domains during evolution leads to new signalling activities. Peisajovich *et al.* present data in support of this, showing that recombining domains from proteins in the budding yeast mating pathway leads to diversity in pathway response dynamics and changes in mating phenotype.

The yeast mating pathway is activated when the mating pheromone (from an 'a' or 'α' cell type) binds to the G protein-coupled receptor in an 'α' or 'a' cell type, respectively. The scaffold protein Ste5 is recruited to the membrane-bound G protein Ste4, bringing along proteins in the mitogen-activated protein kinase signalling cascade (Ste11, Ste7 and Fus3). Activated Ste11 phosphorylates Ste7, which phosphorylates Fus3. Phosphorylated Fus3 then translocates to the nucleus and regulates gene expression to result in fusion between the 'a' and 'α' cells.

The authors used the domains of the 11 proteins in the mating pathway to construct a library of 66 recombinant proteins. Chimeric proteins representing all possible recombinations of domain-containing amino-terminal and carboxy-terminal portions were made, and each was transformed into yeast expressing endogenous mating pathway genes so that the recombinant protein was added to the existing signalling network. Mating pathway activation (as judged by the expression level of a green fluorescent protein (GFP) driven by a mating-responsive promoter) revealed that, although expressing duplicate domains has little effect on pathway activation, domain recombination results in a wide range of dynamic responses, with variants that prevent or strengthen pathway activation. As co-expression of analogous, unlinked C-terminal and N-terminal portions has limited effect on the pathway, these variations must depend on domain recombination.

To determine whether the changes in GFP expression translate to changes in pathway output, the efficiency of mating between 'a' cells expressing domain-recombinant proteins and wild-type 'α' cells was measured. Yeast strains expressing domain-recombination variants that strengthen mating pathway activation mated more efficiently than wild-type yeast, whereas those expressing recombinants that weaken pathway activation mated poorly. Thus, domain recombination produces yeast strains with altered mating efficiency.

How do recombination variants alter the mating response? Analysis of the ten recombination variants that most markedly change yeast behaviour revealed that seven created new links between the different signalling complexes, whereas only three created links in an individual functional complex. Thus, new behaviours might arise when key signalling domains change their complex formation or their localization. Indeed, the fusion of the Ste4-binding domain of Ste5 to the kinase domain of Ste11 strengthens pathway activation as Ste11 is efficiently localized to the membrane, enabling an extra pool of Ste11 to increase signalling.

Thus, recombining catalytic domains with different regulatory domains results in the novel regulation or localization of the catalytic domain and distinct changes in signalling behaviour and phenotype. This might play a part in the evolution of signalling networks.

Katharine H. Wrighton

ORIGINAL RESEARCH PAPER Peisajovich, S. G. *et al.* Rapid diversification of cell signaling phenotypes by modular domain recombination. *Science* **328**, 368–372 (2010)
FURTHER READING Lim, W. A. Designing customized cell signalling circuits. *Nature Rev. Mol. Cell Biol.* **11**, 393–403 (2010)