

Science

 AAAS

Rewiring MAP Kinase Pathways Using Alternative Scaffold Assembly Mechanisms

Sang-Hyun Park, *et al.*
Science **299**, 1061 (2003);
DOI: 10.1126/science.1076979

The following resources related to this article are available online at www.sciencemag.org (this information is current as of January 10, 2008):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/299/5609/1061>

Supporting Online Material can be found at:

<http://www.sciencemag.org/cgi/content/full/1076979/DC1>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/cgi/content/full/299/5609/1061#related-content>

This article **cites 23 articles**, 10 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/299/5609/1061#otherarticles>

This article has been **cited by** 98 article(s) on the ISI Web of Science.

This article has been **cited by** 32 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/cgi/content/full/299/5609/1061#otherarticles>

This article appears in the following **subject collections**:

Cell Biology

http://www.sciencemag.org/cgi/collection/cell_biol

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/about/permissions.dtl>

References and Notes

1. L. Van Parijs, A. K. Abbas, *Science* **280**, 243 (1998).
2. S. Sakaguchi, *Cell* **101**, 455 (2000).
3. K. J. Maloy, F. Powrie, *Nature Immunol.* **2**, 816 (2001).
4. A. Coutinho, S. Hori, T. Carvalho, I. Caramalho, J. Demengeot, *Immunol. Rev.* **182**, 89 (2001).
5. E. M. Shevach, *Nature Rev. Immunol.* **2**, 389 (2002).
6. S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, M. Toda, *J. Immunol.* **155**, 1151 (1995).
7. M. Itoh et al., *J. Immunol.* **162**, 5317 (1999).
8. M. Asano, M. Toda, N. Sakaguchi, S. Sakaguchi, *J. Exp. Med.* **184**, 387 (1996).
9. E. Suri-Payer, A. Z. Amar, A. M. Thornton, E. M. Shevach, *J. Immunol.* **160**, 1212 (1998).
10. S. Read, V. Malmstrom, F. Powrie, *J. Exp. Med.* **192**, 295 (2000).
11. M. A. Currotto de Lafaille et al., *J. Exp. Med.* **194**, 1349 (2001).
12. R. S. Wildin, S. Smyk-Pearson, A. H. Filipovich, *J. Med. Genet.* **39**, 537 (2002).
13. V. L. Godfrey, J. E. Wilkinson, L. B. Russell, *Am. J. Pathol.* **138**, 1379 (1991).
14. P. J. Blair et al., *J. Immunol.* **153**, 3764 (1994).
15. L. B. Clark et al., *J. Immunol.* **162**, 2546 (1999).
16. J. L. Zahorsky-Reeves, J. E. Wilkinson, *Eur. J. Immunol.* **31**, 196 (2001).
17. M. E. Brunkow et al., *Nature Genet.* **27**, 68 (2001).
18. T. A. Chatila et al., *J. Clin. Investig.* **106**, R75 (2000).
19. R. S. Wildin et al., *Nature Genet.* **27**, 18 (2001).
20. C. L. Bennett et al., *Nature Genet.* **27**, 20 (2001).
21. Materials and methods are available as supporting material on Science Online.
22. L. A. Stephens, D. Mason, *J. Immunol.* **165**, 3105 (2000).
23. O. Annacker et al., *J. Immunol.* **166**, 3008 (2001).
24. T. Takahashi et al., *J. Exp. Med.* **192**, 303 (2000).
25. B. Salomon et al., *Immunity* **12**, 431 (2000).
26. J. Shimizu, S. Yamazaki, T. Takahashi, Y. Ishida, S. Sakaguchi, *Nature Immunol.* **3**, 135 (2002).
27. R. S. McHugh et al., *Immunity* **16**, 311 (2002).
28. J. Lehmann et al., *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13031 (2002).
29. T. Takahashi et al., *Int. Immunol.* **10**, 1969 (1998).
30. A. M. Thornton, E. M. Shevach, *J. Exp. Med.* **188**, 287 (1998).
31. E. Suri-Payer, H. Cantor, *J. Autoimmun.* **16**, 115 (2001).
32. S. Hori, T. Nomura, S. Sakaguchi, unpublished data.
33. We thank K. J. Wood, Z. Fehervari, and T. Takahashi for critically reading the manuscript; W. S. Pear and T. Kitamura for reagents; and T. Matsushita for histology. Supported by grants-in-aid from the Ministry of Education, Sports and Culture, the Ministry of Human Welfare, and the Organization for Pharmaceutical Safety and Research of Japan.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1079490/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Table S1
References

17 October 2002; accepted 23 December 2002
Published online 9 January 2003;
10.1126/science.1079490
Include this information when citing this paper.

Rewiring MAP Kinase Pathways Using Alternative Scaffold Assembly Mechanisms

Sang-Hyun Park, Ali Zarrinpar, Wendell A. Lim*

How scaffold proteins control information flow in signaling pathways is poorly understood: Do they simply tether components, or do they precisely orient and activate them? We found that the yeast mitogen-activated protein (MAP) kinase scaffold Ste5 is tolerant to major stereochemical perturbations; heterologous protein interactions could functionally replace native kinase recruitment interactions, indicating that simple tethering is largely sufficient for scaffold-mediated signaling. Moreover, by engineering a scaffold that tethers a unique kinase set, we could create a synthetic MAP kinase pathway with non-natural input-output properties. These findings demonstrate that scaffolds are highly flexible organizing factors that can facilitate pathway evolution and engineering.

Scaffold proteins are known to play a critical role in a growing number of signaling pathways, including several mitogen-activated protein kinase (MAPK) cascades (1–4). In the budding yeast *Saccharomyces cerevisiae*, the scaffold proteins Ste5 and Pbs2 are essential for the mating and high-osmolarity response MAPK pathways, respectively (5–8). These scaffold proteins contain binding sites for each of the pathway kinases, as well as for upstream signaling input proteins (Fig. 1).

Despite their importance, little is known about the mechanism by which scaffold proteins such as Ste5 contribute to efficient and specific signaling (9). One model is that scaffold proteins simply tether pathway components, increasing their likelihood of acting on one another. However, one might expect a simple tethering scaffold to enhance but not

be required for signaling. Thus, because Ste5 is essential for signaling, and because of evidence supporting conformational changes induced by scaffold-kinase association (10, 11), an alternative model is that Ste5 plays a more complex catalytic role, precisely orienting and/or allosterically regulating pathway kinases (4). One way to distinguish between these models would be to probe pathway sensitivity to perturbations in assembly mechanisms. If pathway function depended on precise catalytic participation of the scaffold, then strict stereochemical requirements for kinase recruitment would be expected.

We therefore tested whether non-native protein-protein interactions could be used to build a scaffolded assembly capable of mediating proper mating pathway connectivity and function. We took advantage of several known mutations in Ste5 that selectively destroy recruitment of the MAPK kinase kinase (MAPKKK) Ste11 and the MAPK kinase (MAPKK) Ste7. These mutations, respectively termed Ste5* and Ste5**, each resulted in a nonfunctional mating pathway (12). Defective recruitment interactions were then re-

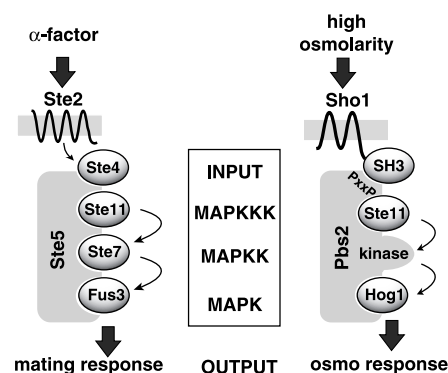


Fig. 1. Yeast mating and high-osmolarity MAPK pathways require scaffold proteins Ste5 and Pbs2. Both pathways require the shared MAPKKK Ste11 but exhibit no cross-signaling under normal conditions. Ste5 has distinct docking sites for Ste11, the MAPKK Ste7, and the MAPK Fus3 (or the partially redundant MAPK Kss1, not shown for simplicity) (5–7). Input to Ste5 occurs through a docking site for Ste4, the G_{β} subunit of the heterotrimeric guanine nucleotide-binding protein activated upon pheromone binding to the α -factor receptor, Ste2. Pbs2 functions as both the scaffold and MAPKK of the osmolarity pathway: It has a MAPKK domain, and it has been shown to bind Ste11 and the MAPK Hog1 (precise binding sites have not been identified) (8). Pbs2 also binds the Src homology 3 (SH3) domain from the upstream osmosensor Sho1 through a proline-rich docking site (residues 94 to 100), indicated by PxxP (26). A second branch of the osmolarity pathway involving the two-component sensor protein Sln1 has been omitted for simplicity (27). This branch of the pathway does not require Sho1 or Ste11. All of the studies described here were performed with strains lacking this pathway branch (*ssk2 Δ* and *ssk22 Δ*).

Department of Cellular and Molecular Pharmacology and Department of Biochemistry and Biophysics, University of California, 513 Parnassus Avenue, San Francisco, CA 94143, USA.

*To whom correspondence should be addressed. E-mail: wlim@itsa.ucsf.edu

REPORTS

16), but they are absent in yeast (17). This interaction has a modest affinity (dissociation constant $K_d = 0.6 \mu\text{M}$) (14).

Each missing kinase (Ste11 or Ste7) was re-recruited to the mutant Ste5 (Ste5* or Ste5**) complex via two possible topologies. A scaffold-kinase recruitment topology (analogous to the wild-type recruitment topology) was achieved by fusing the partner PDZ domains to the C-termini of the mutant Ste5 scaffold and the missing kinase (Fig. 2C). An alternative kinase-kinase recruitment topology was achieved by fusing the partner PDZ domains to the C-termini of the missing kinase and a kinase that was still recruited via native scaffold interactions (Fig. 2D). The ability of the PDZ pairs to restore physical recruitment in vivo was confirmed by coimmunoprecipitation (Fig. 2B). All of these diverse alternative recruitment strategies rescued the mating response (Fig. 2, C and D). Signaling specificity is also well maintained by these alternative recruitment complexes: No cross-signaling to the osmolarity pathway was observed, as assayed by phosphorylation of the osmolarity MAPK, Hog1 (18). Thus, signaling function was maintained even when individual components were recruited by interactions that differ radically in their stereochemical properties from the native recruitment interactions. The pathway components are known to interact with one another with low affinity, but they require reinforcement by either the native or heterologous scaffolding interactions to mediate signaling (19). Similar studies show that heterologous interactions can also restore signaling in the yeast Hog1 pathway (20). Signaling efficiency, however, was attenuated in all cases. In quantitative mating assays, these rebuilt scaffolds increased mating efficiency by three to four orders of magnitude over the defective scaffold background. Nonetheless, these efficiencies were still two orders of magnitude below that observed with wild-type Ste5 (Fig. 2E). Similarly, an assay of direct pathway output—phosphorylation of the MAPK Fus3—revealed a decrease in amplitude (by factors of 5 to 10) and slower kinetics (Fig. 2F). Thus, it is likely that the precise stereochemical properties of native Ste5 play an important secondary role in optimizing the mating response.

Overall, these alternative recruitment studies show that there are diverse stereochemical solutions for assembly of a functional mating pathway complex. Simple recruitment—even by interactions completely unrelated to the native recruitment interactions in their stereochemical properties—is sufficient to specify the basic functional connections for the kinase network examined here.

The robustness of the mating MAPK pathway to perturbations in the recruitment mechanism has important implications for the role of scaffolds in facilitating pathway evolution:

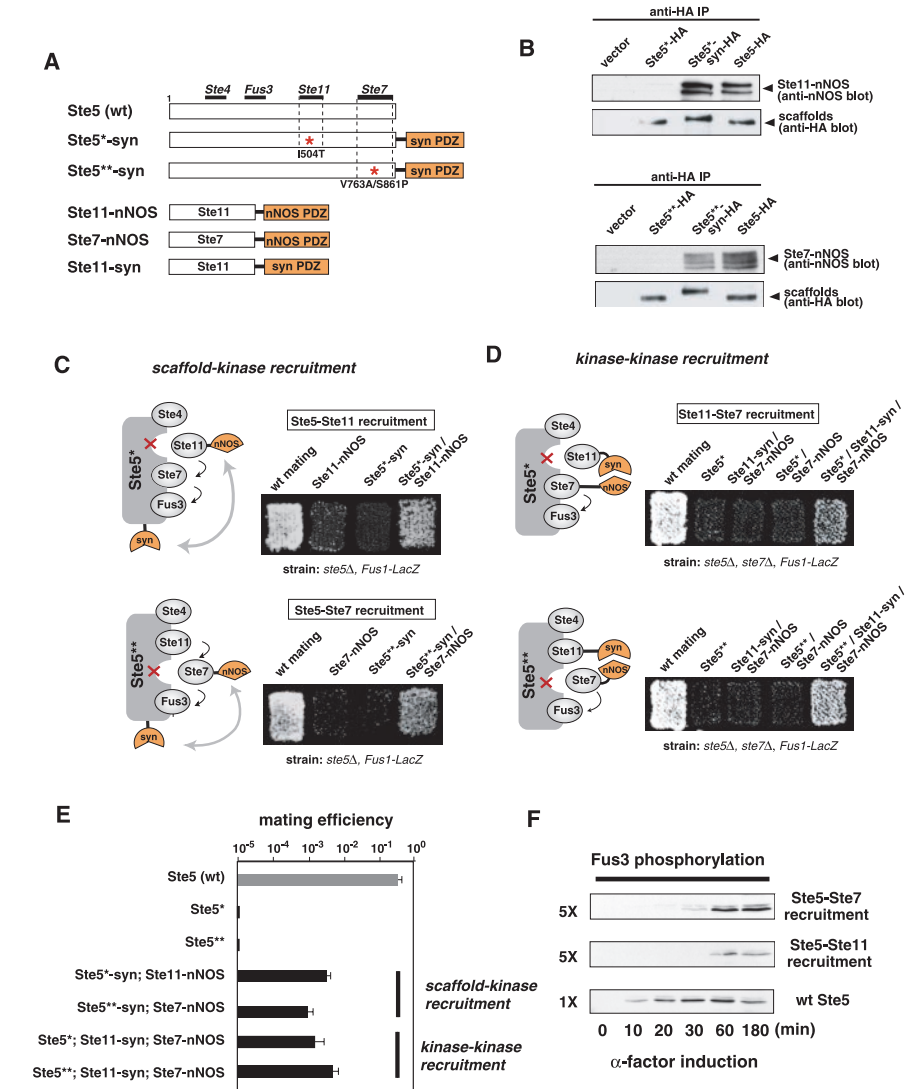
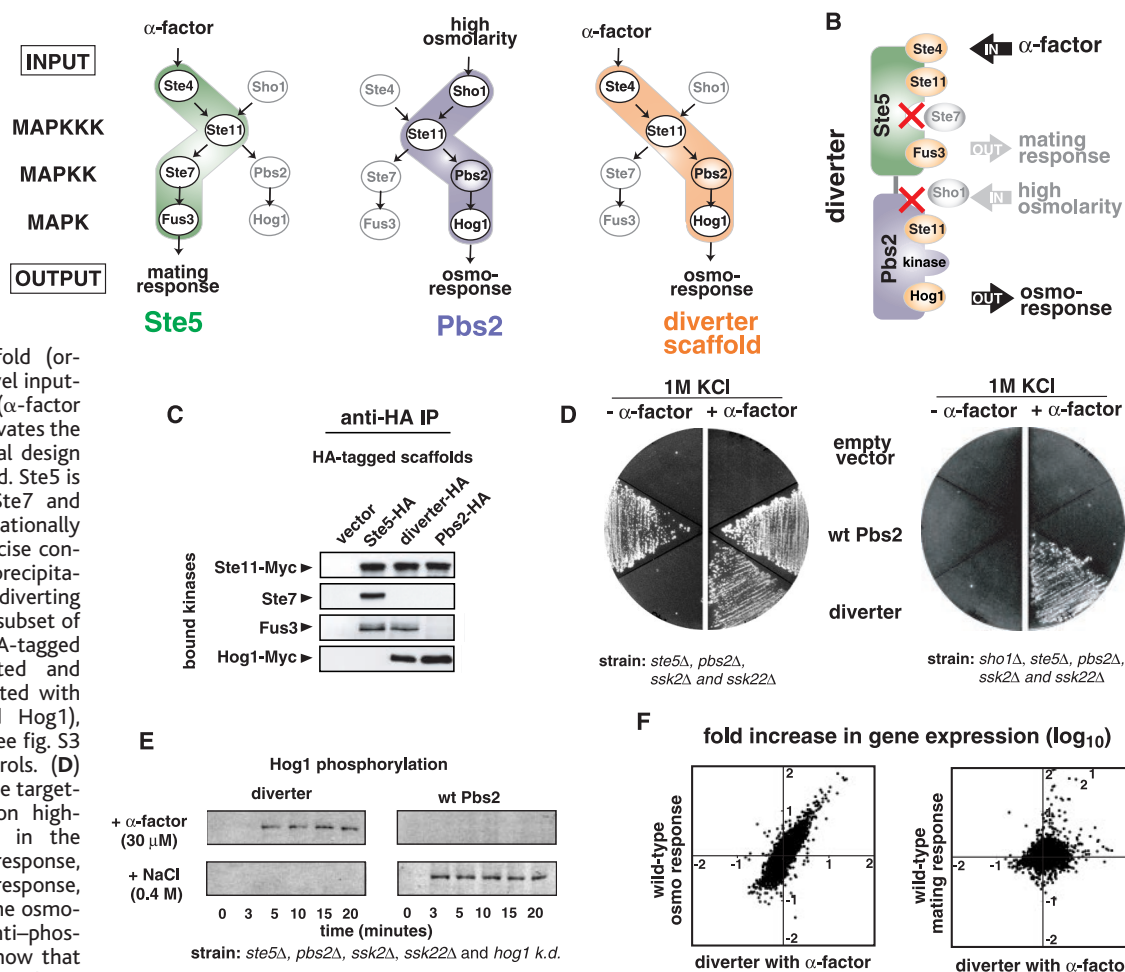


Fig. 2. Scaffold recruitment interactions can be replaced by heterologous PDZ domain-mediated interactions. (A) Constructs used for the recruitment studies. Ste5*-syn bears a mutation (I504T) that selectively disrupts Ste11 docking (12) as well as a C-terminal fusion of the syntrophin PDZ domain; Ste5**-syn bears a double mutation (V763A/S861P) that disrupts Ste7 docking (12) as well as a C-terminal fusion of the syntrophin PDZ domain (I, Ile; T, Thr; V, Val; A, Ala; S, Ser; P, Pro). Ste11-nNOS and Ste7-nNOS are fusions of the kinases to the nNOS PDZ domain; Ste11-syn is a fusion of the kinase to the syntrophin PDZ domain. The nNOS and syntrophin PDZ domains specifically heterodimerize with an affinity of $K_d = 0.61 \mu\text{M}$. (B) Immunoprecipitation assays show that heterologous PDZ-PDZ interactions restore recruitment of missing kinases. Hemagglutinin (HA)-tagged Ste5 variants were precipitated with agarose beads coated with antibody to HA (anti-HA) and the recruited kinase-nNOS fusions were detected with anti-nNOS. (C) Complementation of mating deficiency in *ste5Δ* cells when native kinase recruitment interactions are replaced by PDZ-mediated scaffold-kinase interactions. Growth on the media selective for diploid cells indicates mating. (D) Complementation of mating deficiency in *ste5Δ, ste7Δ* cells when native kinase recruitment interactions are replaced by PDZ-mediated kinase-kinase interactions. (E) Quantitative mating assays for rescued scaffold signaling pairs. Negative control experiments in (C) to (E) were performed on strains in which only one partner protein is PDZ-tagged. Although omitted in labels for simplicity, untagged partner proteins were also coexpressed to control for any variation in mating due to expression of fusions. Background mating efficiency of the negative controls is $\leq 10^{-5}$. (F) Flux through mating MAPK cascade directly assayed by immunoblot detection of phosphorylation of endogenous Fus3. For experiments with replacement scaffolds, lysate concentration was increased fivefold. The antibody (anti-phospho p44/42) also detects phosphorylation of the semi-redundant MAPK Kss1 (faint band just above major Fus3 band).

It implies that primitive tethering scaffolds generated by recombination or fusion events could in principle be sufficient to generate new pathways (and hence phenotypes) from

combinations of preexisting kinases. To experimentally test this hypothesis, we attempted to engineer a synthetic “diverter” scaffold that assembles a non-native complex of ki-

Fig. 3. A synthetic diverter scaffold mediates an artificial MAPK pathway. (A) Concept of diverter scaffold. MAPKKK Ste11 is a node participating in multiple pathways. The wild-type mating pathway is mediated by Ste5 (green); the high-osmolarity pathway is mediated by Pbs2 (purple). A diverter scaffold (orange) would mediate a novel input-output linkage via Ste11 (α -factor stimulation selectively activates the osmoresponse). (B) Physical design of chimeric diverter scaffold. Ste5 is fused to Pbs2 and the Ste7 and Sho1 binding sites are mutationally disrupted [see (28) for precise construction]. (C) Coimmunoprecipitation assays show that the diverting scaffold binds to a unique subset of component kinases. HA-tagged scaffolds were precipitated and bound kinases were detected with anti-Myc (for Ste11 and Hog1), anti-Ste7, and anti-Fus3. See fig. S3 for HA blot of bait controls. (D) Diverter scaffold confers the targeted behavior of survival in high-osmolarity medium only in the presence of α -factor. This response, unlike the wild-type osmoresponse, occurs in a strain lacking the osmosensor Sho1 (right). (E) Anti-phospho Hog1 immunoblots show that diverter scaffold selectively induces Hog1 phosphorylation upon stimulation by α -factor, but not by 0.4 M NaCl (left). Reverse behavior is observed with the wild-type osmolarity scaffold Pbs2 (right). (F) Microarray analysis shows a linear correlation between the gene expression program triggered by stimulation



nases and consequently might yield a new, nonphysiological response pathway.

We targeted the design of a non-natural pathway in which mating pheromone α -factor selectively triggers the osmolarity response (Fig. 3A). This input-output relationship would be generated if input from the α -factor receptor could be directed to Ste11, as normally occurs, but then shunted to the osmolarity pathway components (Pbs2 and Hog1) instead of the corresponding mating pathway components (Ste7 and Fus3). Thus, we constructed a diverter scaffold by fusing the scaffolds Ste5 and Pbs2 and mutagenically destroying interactions with the downstream mating output (Ste7) and the upstream osmolarity input (the osmosensor Sho1) (Fig. 3B) (fig. S1). This strategy takes advantage of the fact that Ste11 is a node common to both natural pathways. Coimmunoprecipitation (Fig. 3C) and glycerol gradients (18) confirmed that the diverter scaffold recruited the predicted non-native combination of kinases.

Cells expressing the diverter scaffold displayed the targeted signaling behavior. A

of the diverting scaffold strain with α -factor and the wild-type osmoresponse program (left). No correlation with the wild-type mating response is observed (right). Maximal changes in expression are plotted on a log scale.

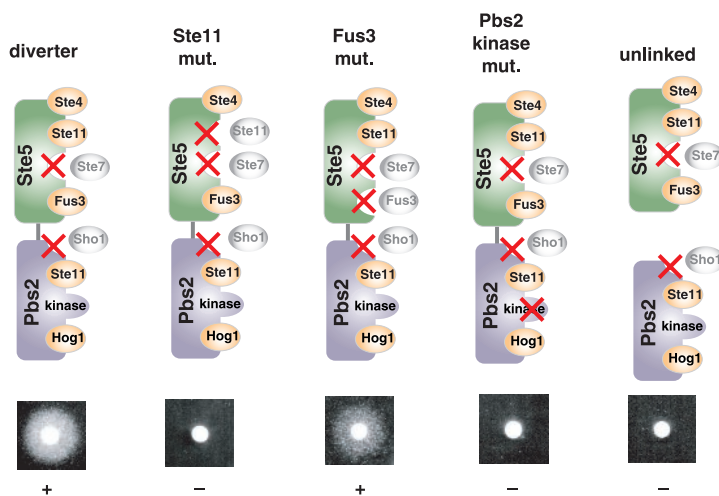


Fig. 4. Mutational analysis of diverter scaffold requirements. Modular functions in the diverter scaffold were individually disrupted (above) and the resulting diverter variants were tested by α -factor disc assay (below) for their ability to mediate α -factor-induced osmoresistance [see (28) for specific mutation and assay]. Growth surrounding the disc indicates α -factor-dependent osmoresistance (i.e., diverter function). Although not shown here, a deletion of the nuclear localization signal in a portion of Ste5 (Δ 49-66) resulted in loss of diverter function, consistent with the hypothesis that nuclear shuttling is required for scaffold function (29).

REPORTS

strain lacking the wild-type Ste5 and Pbs2 scaffolds but expressing this diverter scaffold survived on 1 M KCl only in the presence of α -factor (Fig. 3D). This conditional osmoresistance was independent of the osmosensor Sho1, indicating that α -factor is the only required input. This designed pathway was extremely specific: Cells bearing the diverter scaffold were sterile (mating efficiency $< 10^{-5}$; fig. S2) and did not yield the osmoresponse (Hog1 phosphorylation) upon salt stimulation. Instead, Hog1 phosphorylation was only observed upon stimulation with α -factor (Fig. 3E). The magnitude of the diverted response, as measured by Hog1 phosphorylation, was comparable to the normal osmoresponse. Microarray analysis revealed that the global transcriptional response elicited by the new pathway was nearly identical to that of the wild-type osmolarity response, but clearly distinct from the wild-type mating response (Fig. 3F). Thus, rewiring by the diverter scaffold is efficient and specific.

Mutagenesis revealed that the diverter-mediated response was dependent on the specific set of interactions consistent with pathway connectivity (Fig. 4). Mutation of the Ste11 binding site on the Ste5 fragment of the diverter destroyed its function, whereas mutation of binding sites for kinases downstream of Ste11 (Ste7 and Fus3) did not. Similarly, on the Pbs2 fragment, mutation of binding sites for components upstream of Ste11 (Sho1) had no effect on function, although mutation of activities downstream from Ste11 (Pbs2 kinase activity) destroyed function (mutations that selectively block Ste11 and Hog1 binding to Pbs2 have not been identified). In addition, covalent linkage between the Ste5 and Pbs2 fragments of the diverter scaffold was absolutely required. These specific requirements are inconsistent with indirect mechanisms of Hog1 activation, including simple α -factor-dependent targeting of Pbs2 to the membrane or buildup of high steady-state levels of activated Ste11 caused by disruption of negative feedback (21).

Although signaling by the diverter scaffold was efficient and specific, when wild-type Ste5 and the diverter scaffold were coexpressed, only a slightly attenuated mating response was observed (18). The apparent dominance of wild-type Ste5 over the diverter may result in part because Ste5 functions as an oligomer, or because of cross-pathway negative feedback. Wild-type efficiency may require more fine-tuned evolutionary refinement.

Our findings indicate that scaffolds such as Ste5 are conceptually similar to promoters: Both are modular and flexible organizing centers that can control the flow of information in signaling or transcription, respectively. Similarly, the regulation of a transcriptional response can be modulated by simple alterations in the presence or arrangement of

diverse transcription factor docking sites (22, 23). Both of these organizing structures thus appear to be optimized for evolvability, a property that may provide increased fitness in the face of constantly changing environmental challenges and signaling needs. Conversely, just as promoter engineering can be used to control cellular behavior and to create useful tools (e.g., yeast two-hybrid systems) (24), these and other related results (25) indicate that scaffold engineering may allow for systematic manipulation of cytoplasmic signaling pathways.

References and Notes

1. T. Pawson, J. D. Scott, *Science* **278**, 2075 (1997).
2. T. P. Garrington, G. L. Johnson, *Curr. Opin. Cell Biol.* **11**, 211 (1999).
3. A. J. Whitmarsh, R. J. Davis, *Trends Biochem. Sci.* **23**, 481 (1998).
4. W. R. Burack, A. S. Shaw, *Curr. Opin. Cell Biol.* **12**, 211 (2000).
5. J. A. Printen, G. F. Sprague Jr., *Genetics* **138**, 609 (1994).
6. K. Y. Choi, B. Satterberg, D. M. Lyons, E. A. Elion, *Cell* **78**, 499 (1994).
7. S. Marcus, A. Polverino, M. Barr, M. Wigler, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7762 (1994).
8. F. Posas, H. Saito, *Science* **276**, 1702 (1997).
9. J. E. Ferrell Jr., *Science's STKE* (www.stke.org/cgi/content/full/OC_sigtrans;2000/52/pe1) (2000).
10. E. A. Elion, *J. Cell Sci.* **114**, 3967 (2001).
11. C. Sette, C. J. Inouye, S. L. Stroschein, P. J. Iaquina, J. Thorne, *Mol. Biol. Cell* **11**, 4033 (2000).
12. C. Inouye, N. Dhillon, T. Durfee, P. C. Zambryski, J. Thorne, *Genetics* **147**, 479 (1997).
13. B. J. Hillier, K. S. Christopherson, K. E. Prehoda, D. S. Bredt, W. A. Lim, *Science* **284**, 812 (1999).

14. B. Z. Harris, B. J. Hillier, W. A. Lim, *Biochemistry* **40**, 5921 (2001).
15. J. Chevesich, A. J. Kreuz, C. Montell, *Neuron* **18**, 95 (1997).
16. S. Tsunoda et al., *Nature* **388**, 243 (1997).
17. B. Z. Harris, W. A. Lim, *J. Cell Sci.* **114**, 3219 (2001).
18. S.-H. Park, A. Zarrinpar, W. A. Lim, data not shown.
19. A. J. Bardwell, L. J. Flatauer, K. Matsukuma, J. Thorne, L. Bardwell, *J. Biol. Chem.* **276**, 10374 (2001).
20. S.-H. Park, A. Zarrinpar, W. A. Lim, unpublished data.
21. S. M. O'Rourke, I. Herskowitz, *Genes Dev.* **12**, 2874 (1998).
22. K. Struhl, *Annu. Rev. Genet.* **29**, 651 (1995).
23. M. Ptashne, A. Gann, *Curr. Biol.* **8**, R812 (1998).
24. S. Fields, O. Song, *Nature* **340**, 245 (1989).
25. K. Harris et al., *Curr. Biol.* **11**, 1815 (2001).
26. T. Maeda, M. Takekawa, H. Saito, *Science* **269**, 554 (1995).
27. T. Maeda, S. M. Wurgler-Murphy, H. Saito, *Nature* **369**, 242 (1994).
28. See supporting data on Science Online.
29. S. K. Mahanty, Y. Wang, F. W. Farley, E. A. Elion, *Cell* **98**, 501 (1999).
30. We thank S. O'Rourke and the Lim lab for advice and assistance, and H. Bourne, I. Herskowitz, R. Bhattacharyya, H. Madhani, E. O'Shea, and J. Weissman for helpful discussions. S.-H.P. is a Jane Coffin Childs Memorial Fund postdoctoral fellow. Supported by grants from NIH, the Packard Foundation, and the Kirsch Foundation (W.A.L.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/1076979/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 and S2
References

5 August 2002; accepted 27 November 2002
Published online 2 January 2003;
10.1126/science.1076979
Include this information when citing this paper.

DNA Damage-Induced Replication Fork Regression and Processing in *Escherichia coli*

Justin Courcelle,* Janet R. Donaldson, Kin-Hoe Chow, Charmain T. Courcelle

DNA lesions that block replication are a primary cause of rearrangements, mutations, and lethality in all cells. After ultraviolet (UV)-induced DNA damage in *Escherichia coli*, replication recovery requires RecA and several other *recF* pathway proteins. To characterize the mechanism by which lesion-blocked replication forks recover, we used two-dimensional agarose gel electrophoresis to show that replication-blocking DNA lesions induce a transient reversal of the replication fork in vivo. The reversed replication fork intermediate is stabilized by RecA and RecF and is degraded by the RecQ-RecJ helicase-nuclease when these proteins are absent. We propose that fork regression allows repair enzymes to gain access to the replication-blocking lesion, allowing processive replication to resume once the blocking lesion is removed.

Irradiation of cells with near-UV light induces DNA lesions that block replication (1). In *E. coli*, replication is transiently inhibited after a

Department of Biological Sciences, Box GY, Mississippi State University, Mississippi State, MS 39762, USA.

*To whom correspondence should be addressed. E-mail: jcourcelle@biology.msstate.edu

moderate dose of UV irradiation, but it recovers efficiently at a time that correlates with the removal of the lesions from the genome by the nucleotide excision repair proteins (1–3). Cells deficient in lesion removal are severely impaired in their ability to recover replication and exhibit elevated levels of recombination, genomic rearrangements, and cell lethality (4–7).