Yeast Morphogenetic Transitions

mating pheromone

nutrient limitation
MAP Kinase Cascade

MAP kinase kinase kinase (eg Ste11)
Phosphorylates and thereby activates

MAP kinase kinase (eg Ste7)
Phosphorylates and thereby activates

MAP kinase (eg Fus3)

Which then phosphorylates and regulates downstream targets (eg transcription factors and Cdk regulators) that together achieve the appropriate outcome
The pheromone response pathway

pheromone

↓

Ste2/3 + Gαβγ
Ste20
Ste11/ Ste50
Ste7/ Ste5
Fus3
Ste12

MAP kinase cascade

Receptors and G-protein

Transcription factor

mating
Signaling pathways share components

- **glucose depletion**
  - Sho1
  - Ste20
  - Ste11/ Ste50
  - Ste7
  - Kss1
  - Ste12/Tec1
  - filamentous growth

- **pheromone**
  - Ste2/3 + Gαβγ
  - Ste20
  - Ste11/ Ste50
  - Ste7/ Ste5
  - Fus3
  - Ste12
  - mating

- **salt/sorbitol**
  - Sho1
  - Ste20
  - Ste11/ Ste50
  - Pbs2
  - Hog1
  - osmolarity response
Scaffold proteins may help solve some of the specificity problem.
Scaffolds also exist in mammalian cells

**Figure 1.** Scaffold proteins govern the selection of signal output upon Rac activation. PIX, a Rac-GEF, forms a molecular complex with the serine/threonine protein kinase Pak1. This facilitates the stimulation of Pak1 upon Rac activation, thereby, promoting the polymerization of actin, which results in rapid changes in the actin-based cytoskeleton and the formation of membrane ruffles known as lamellipodia. Two other guanine-nucleotide exchange factors for Rac (RacGEFs), Tiam1 and Ras-GRF1 (not shown), bind to the scaffold proteins JIP2 and JIP1, leading to the preferential activation of p38 and probably JNK by Rac, respectively, and the consequent phosphorylation of nuclear transcription factors that regulate gene expression. Arrows represent activation events either by direct binding or by phosphorylation as in the case of the kinases (orange) and transcription factors (brown).
Another example of scaffolds and specificity
Two-Hybrid System to Detect Protein Interactions

- DNA-binding domain
- Activation domain
- Reporter gene
- Binding site
Plasmids for expression of 2-hybrid constructs

DNA-BD Plasmid

AD Plasmid

X

Gal4 Binding Domain

TRP1

Y

Gal4 Activation Domain

LEU2

encodes

NH₂

Gal4 BD

X

COOH

encodes

NH₂

Gal4 AD

Y

COOH
Another depiction of a 2-hybrid experiment

A. Regular transcription of the reporter gene

B. One fusion protein only (Gal4-BD + Bait) - no transcription

C. One fusion protein only (Gal4-AD + Prey) - no transcription

D. Two fusion proteins with interacting Bait and Prey
Ste5 interacts with each member of the MAP Kinase cascade.

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<th>LexA Fusion</th>
<th>Units β-galactosidase Activity^</th>
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2-Hybrid tests localize interaction sites on Ste5

Ste11, Ste7, Fus3, and Kss1 interact with different portions of Ste5

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IP experiments reveal that Ste5 interacts with each member of the MAPK cascade.

Ste5-Ste11 interaction does not require Ste7.
IP experiments, continued

Likewise, Ste7 interacts with Ste5
Fus3 is activated by alpha-factor in a Ste5-dependent manner

Fus3 kinase assays

Lanes 1&2: no Ste5

Lanes 3&4: wildtype Ste5

Lanes 5&6: mutant form of Ste5 unable to bind Fus3
Glycerol gradient centrifugation reveals a multi-protein complex

Molecular weight standards
Quantitation of protein levels across gradient
Figure 1 Yeast mating and high-osmolarity MAPK pathways require scaffold proteins Ste5 and Pbs2.

S Park et al. Science 2003;299:1061-1064
Engineered protein-protein interactions can substitute for the native Ste5-Ste7 and Ste5-Ste11 interaction.

**PDZ domains are protein-protein interaction domains**

* Indicates mutant binding sites on Ste5

Ste5 is tagged with HA epitope; IP with anti-HA, probe with anti-NOS
Artificial interactions demonstrated by mating test

Mating test: strain with Ste5 construct is leu-; test for mating to trp- cells of other mating type. Growth will be observed on minimal medium only if mating has occurred.
... and by quantitative mating tests and phosphorylation of Fus3
Can one engineer a new scaffold to direct a different output response?
The diverter scaffold works as designed

Diverter interacts with appropriate kinases

Growth on salt occurs only in presence of alpha-factor

Diverter directs phosphorylation of Hog1

Diverter directs same change in gene expression as wildtype osmo-response
Figure 4 Mutational analysis of diverter scaffold requirements.

Growth on high salt in presence of α-factor
Additional regulators of pheromone response

Can a scaffold be engineered to alter kinetics of response to these regulators?
Recruited regulators attenuate or stimulate response
Recruited regulators change time course and dose response
Two MAPK targets for Ste7

What is role of Ste5 in activation of Fus3
A new role for Ste5
Ste5 is required for activation of Fus3 but not Kss1
Requirements for activation of Fus3 by Ste7

Definition of a minimal scaffold version of Ste5

Fus3 binding site on Ste5 is not required but docking sites on Ste7 are
The idea is, Ste7 tethers Fus3 to the Ste5 minimal scaffold
Ste5 changes the Kcat of Ste7 for Fus3, not the Km

A

\[
\text{Ste7EE + MAPK} \xrightarrow{k_1} \text{Ste7EE MAPK} \xrightarrow{k_{\text{cat}}} \text{Ste7EE + MAPK-PP}
\]

C

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<th>Substrate</th>
<th>Ste5-ms</th>
<th>( k_{\text{cat}} ) (s(^{-1}))</th>
<th>( K_M ) (nM)</th>
<th>( \frac{k_{\text{cat}}}{K_M} ) (M(^{-1}) s(^{-1}))</th>
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<td>1.7 +/- 0.15 ( \times 10^{-2} )</td>
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Effect of Ste5

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Ste5 mutants with greater than 100-fold reduction in phosphorylation of Fus3 by Ste7

Mutants fall in two regions on Ste5 surface

- The known Ste7 binding site
- A region dubbed the coactivator loop
Ste5ms mutants affect different aspects of phosphorylation of Fus3 by Ste7

Mutant B affects Ste7 binding

Mutant C affects k-cat
2 possibilities for how Ste5 stimulates phosphorylation of Fus3 by Ste7

1. Ste5 makes Ste7 a better kinase
2. Ste5 makes Fus3 a better substrate

The data support the 2nd possibility; perhaps Ste5 induces a conformational change in Fus3 that makes its activation loop accessible to Ste7
Model