Signaling crosstalk

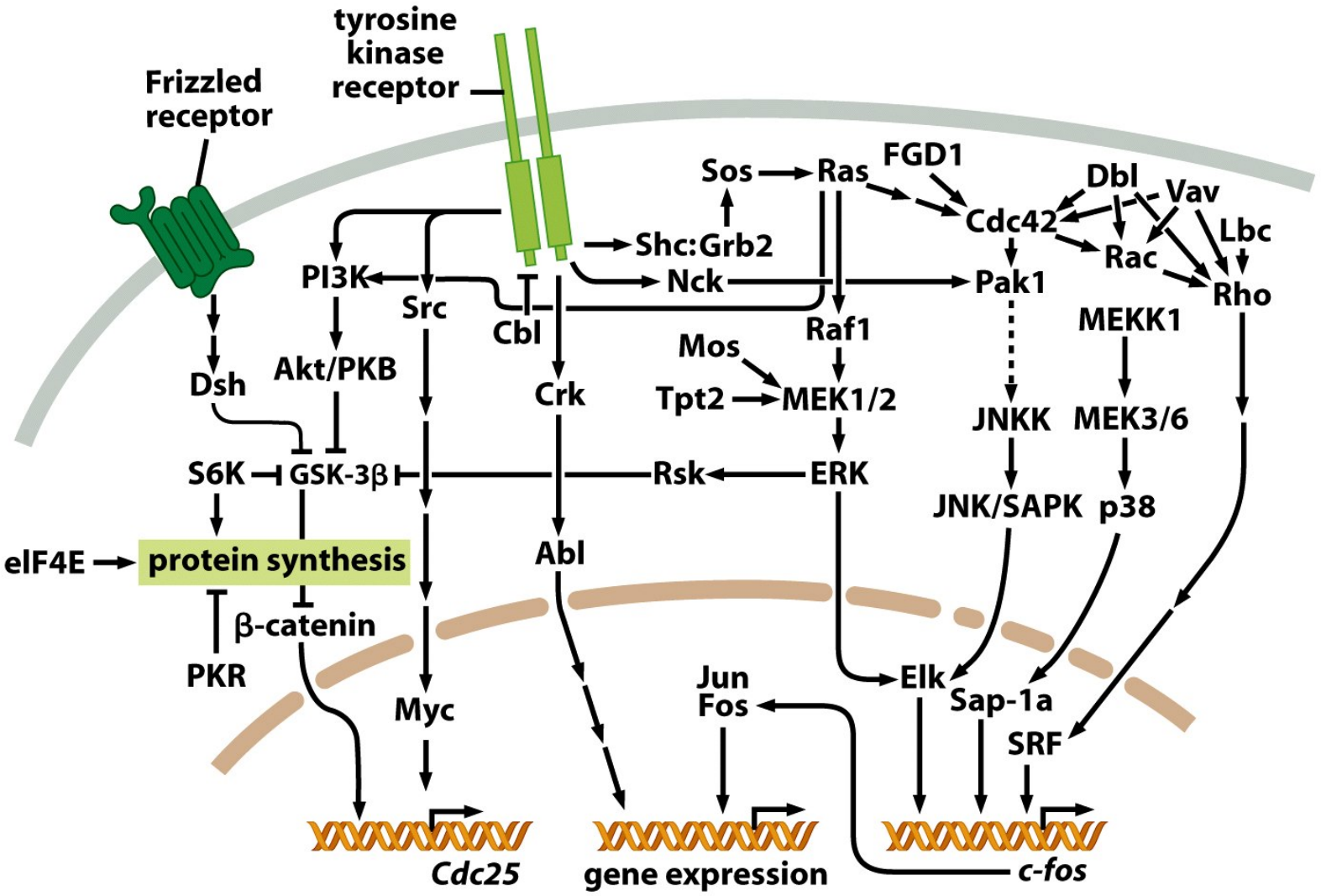
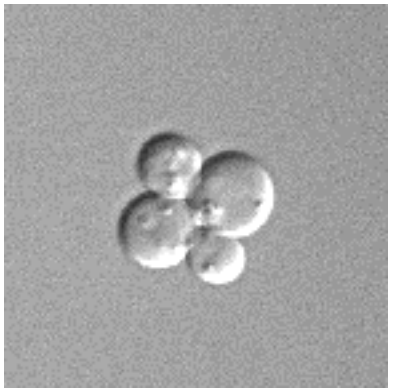


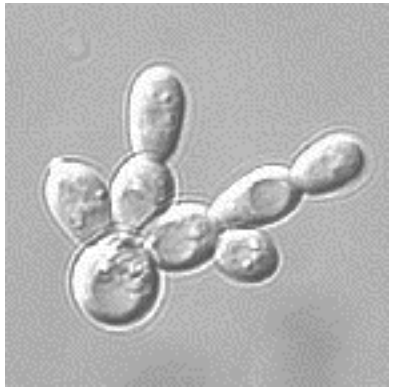
Figure 6.32 *The Biology of Cancer* (© Garland Science 2007)

Yeast Morphogenetic Transitions



mating pheromone

nutrient limitation



MAP Kinase Cascade

MAP kinase kinase kinase (eg Ste11)

Phosphorylates and thereby activates

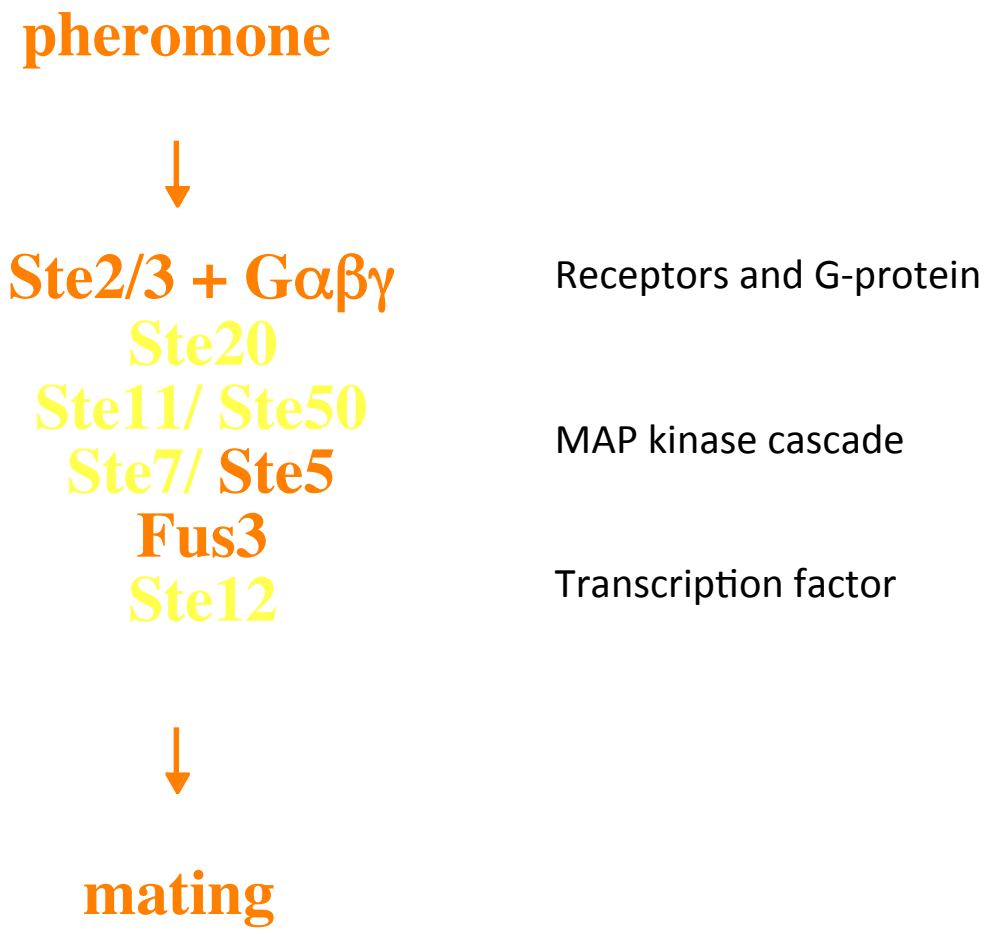
MAP kinase kinase (eg Ste7)

Phosphorylates and thereby activates

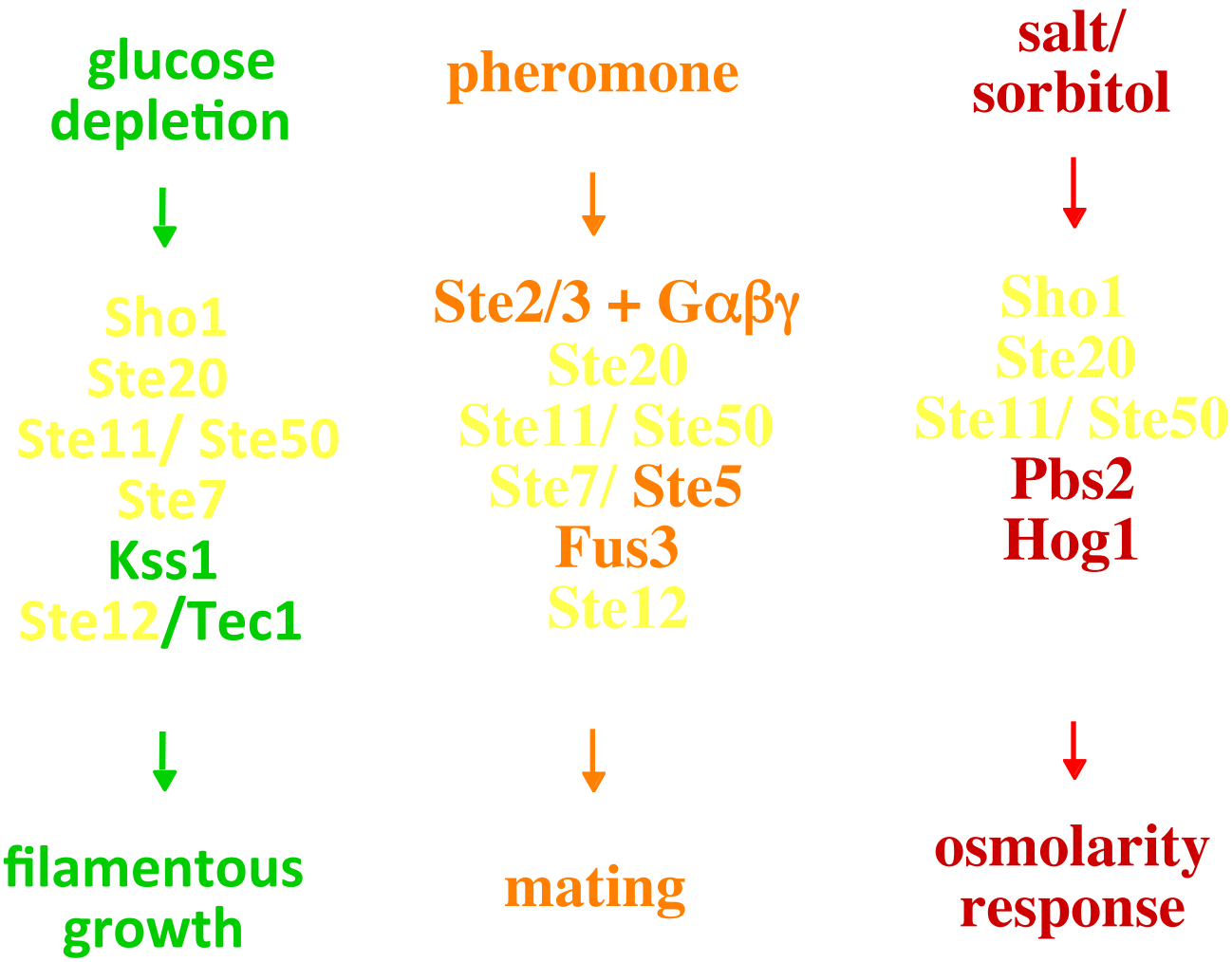
MAP kinase (eg Fus3)

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

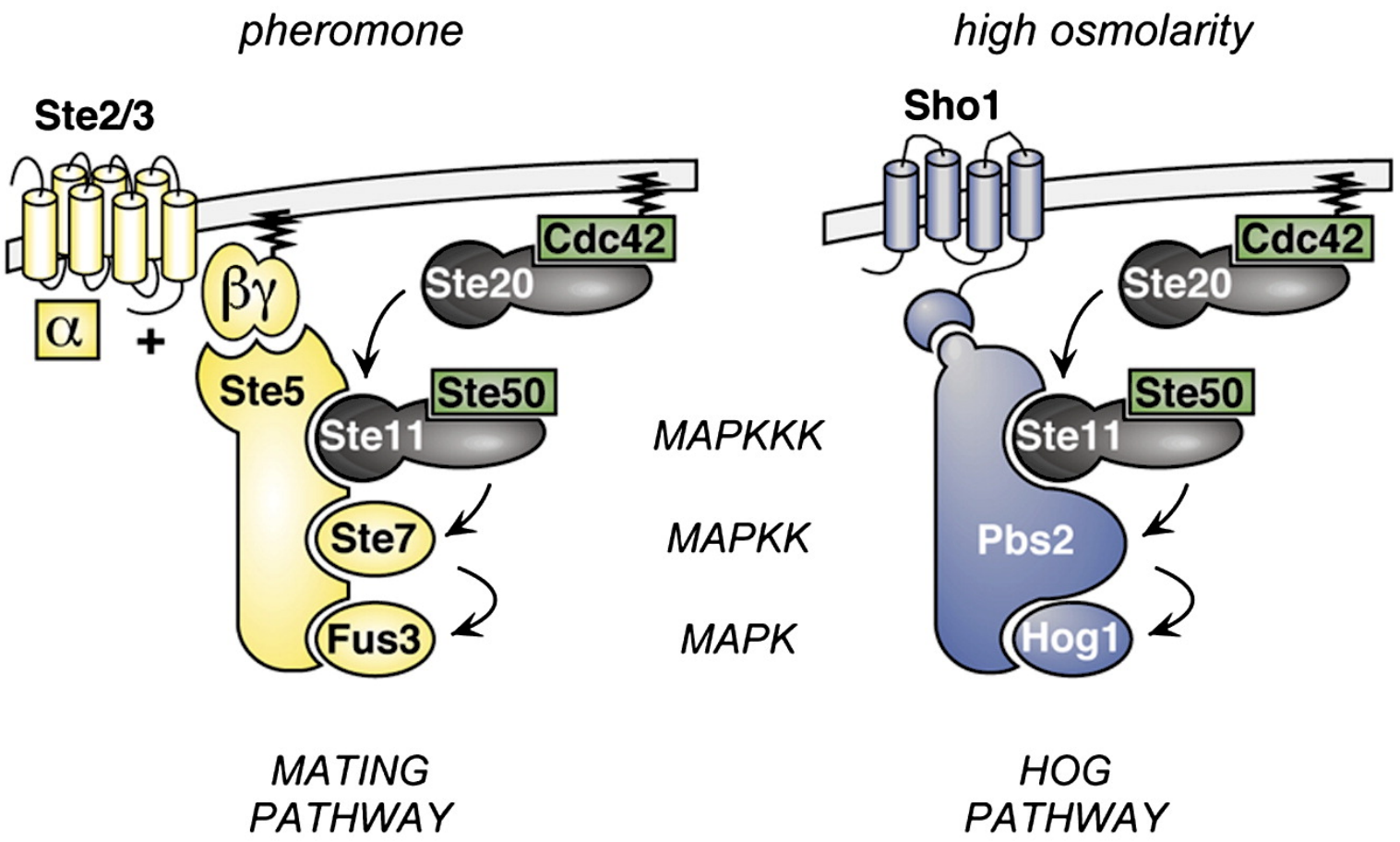
The pheromone response pathway



Signaling pathways share components



Scaffold proteins may help solve some of the specificity problem



Lamson et al. (Pryciak) Current Biology 16:618

Scaffolds also exist in mammalian cells

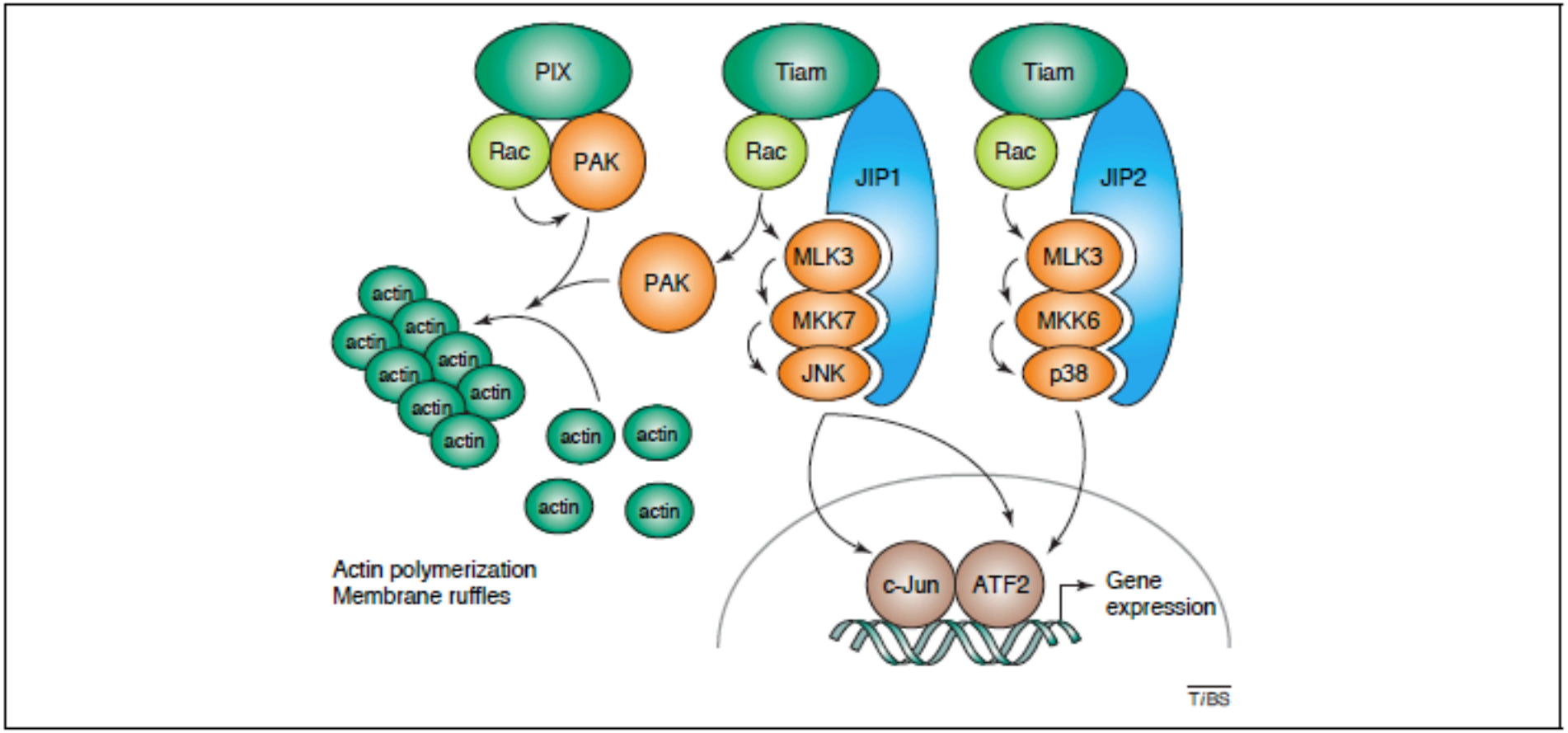
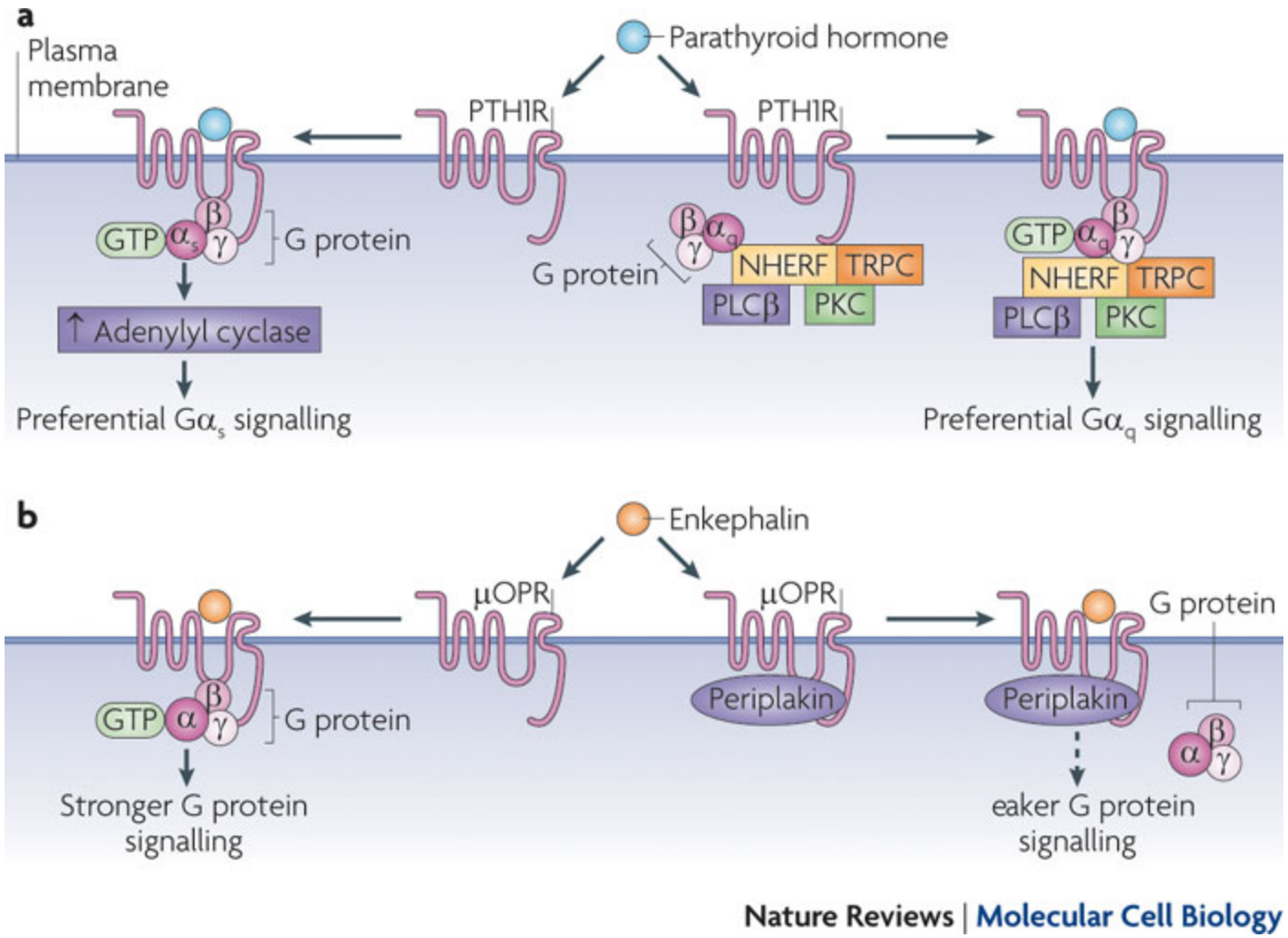


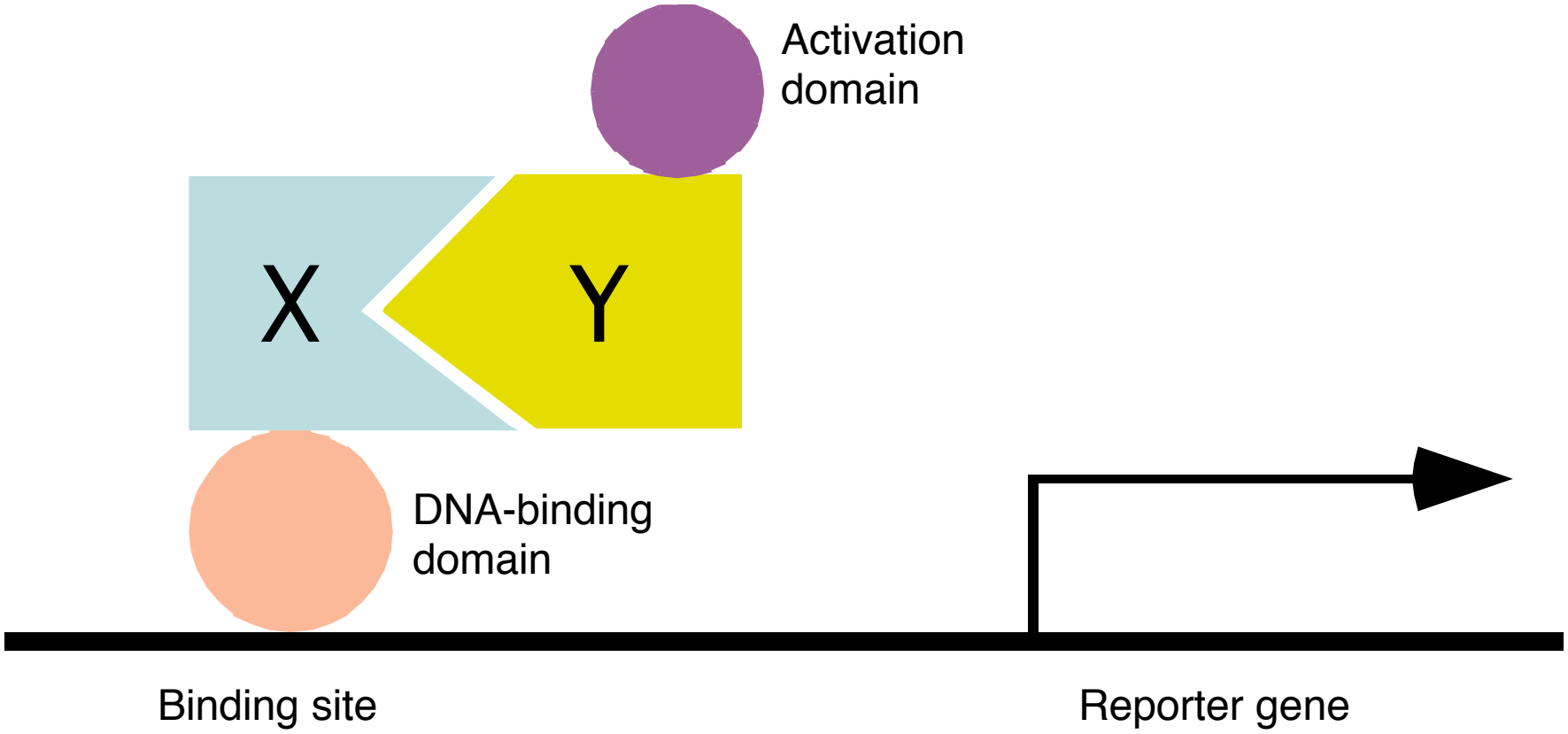
Figure 1. Scaffold proteins govern the selection of signal output upon Rac activation. PIX, a Rac-GEF, forms a molecular complex with the serine/threonine protein kinase Pak1. This facilitates the stimulation of Pak1 upon Rac activation, thereby, promoting the polymerization of actin, which results in 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

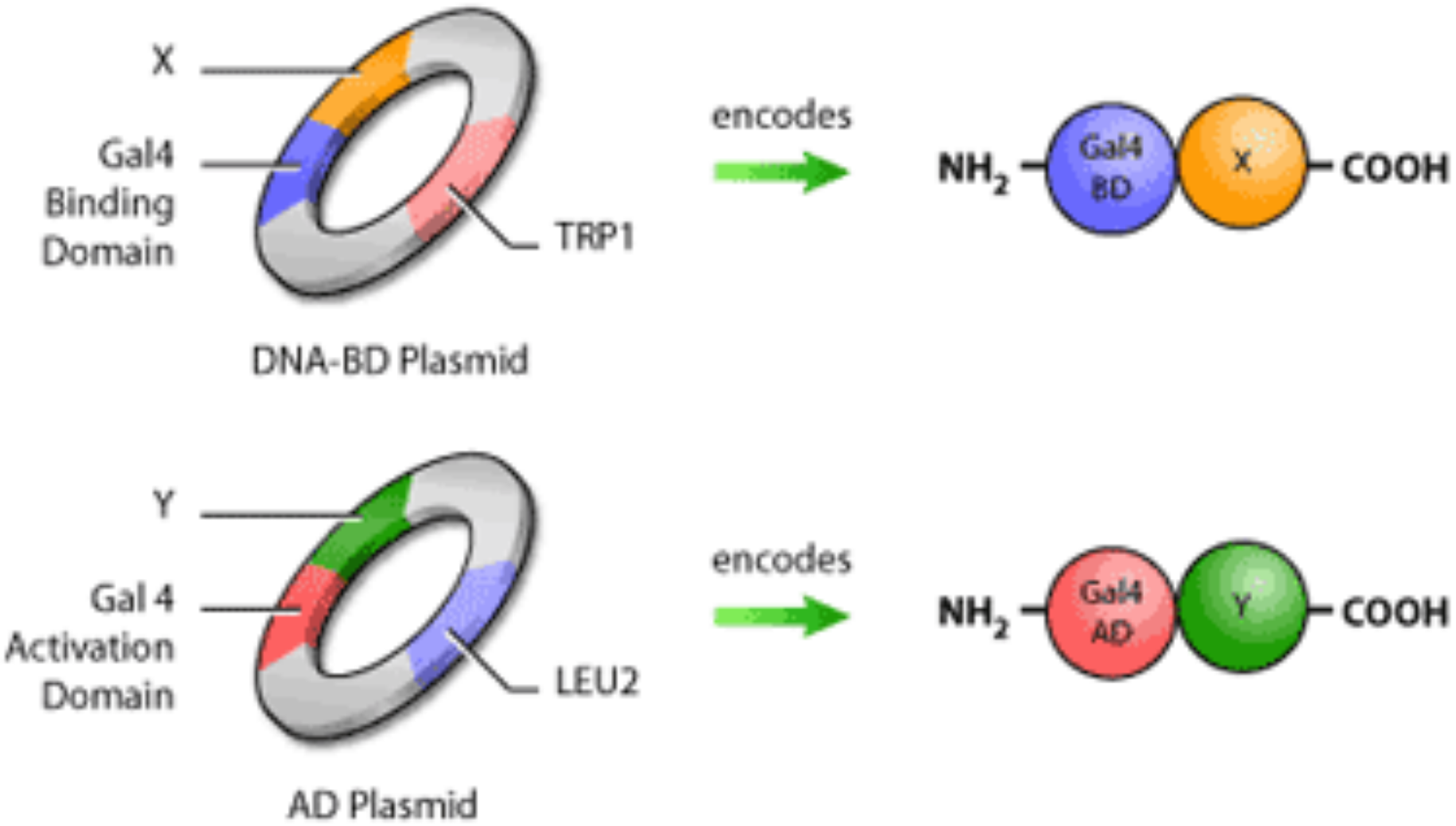


Nature Reviews | Molecular Cell Biology

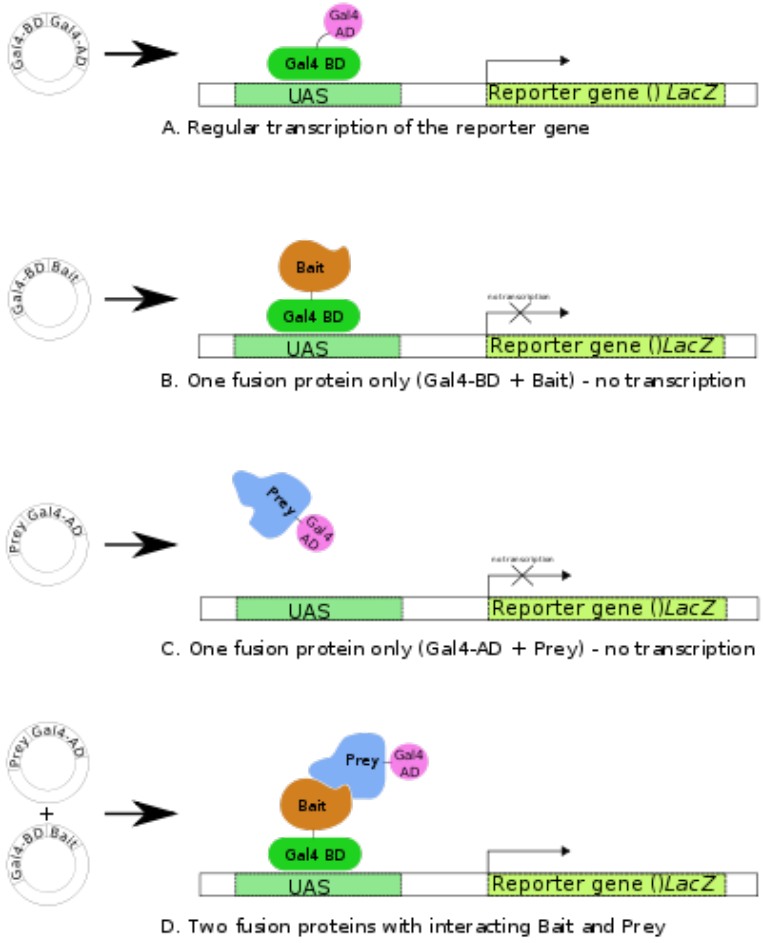
Two-Hybrid System to Detect Protein Interactions



Plasmids for expression of 2-hybrid constructs



Another depiction ofa 2-hybrid experiment



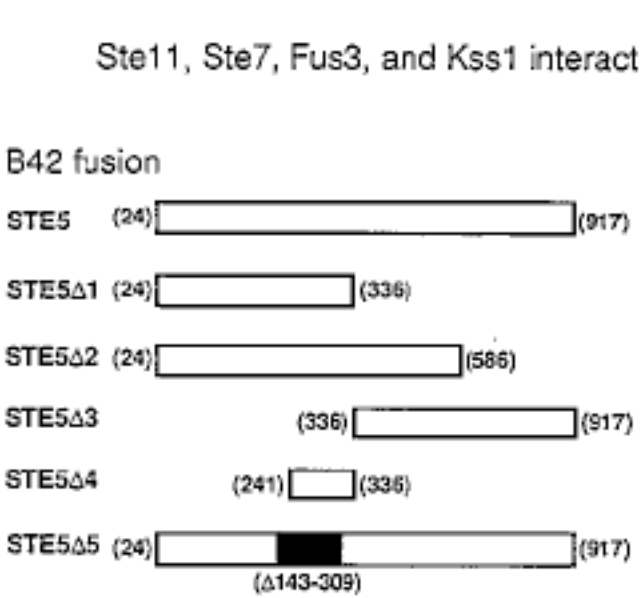
Ste5 interacts with each member of the MAP Kinase cascade

Table 1. β-galactosidase Activity Induced by Interactions between Ste5, Ste20, Ste11, Ste7, Fus3, and Kss1 in a Two-Hybrid System in Ste$^+$ and Ste$^-$ Strains.

			Units β-galactosidase Activity[a] B42 Fusion[b]					
	Strain[c]	LexA Fusion[c]	B42	Ste5	Ste11	Ste7	Fus3	Kss1
I	*STE5*	Ste20	9	33	61	15	20	19
		Ste11	6	5262	103	9	29	2688
		Ste7	20	10699	26	59	1612	12796
		Fus3	43	677	1379	891	61	86
		Kss1	34	1931	2147	2750	110	
		Ste11N	5	681				
		Ste11C	5	4				
		Ste7N	8	6				
		Ste7C	18	2616				
		Bicoid	10	14	14	16	18	20
II	*ste5Δ*	Ste11		4840	99	33	60	3266
		Ste7		4714	45	48	4270	31100
		Fus3		751	593	1675	53	
		Kss1		688	2250	2907		
III	*fus3Δ*	Ste11		17250				
		Ste7		7840				
		Fus3		3200	2000	3750		
		Bicoid		41				
	fus3Δ ste20Δ	Ste11		18050				
		Ste7		7400				
		Fus3		5450	4650	4700		
		Bicoid		25				
	fus3Δ ste11Δ	Ste11		10620				
		Ste7		13260				
		Fus3		3700	1800	4250		
		Bicoid		66				
	fus3Δ ste7Δ	Ste11		11900				
		Ste7		1401				
		Fus3		1138	737	1800		
		Bicoid		40				

2-Hybrid tests localize interaction sites on Ste5

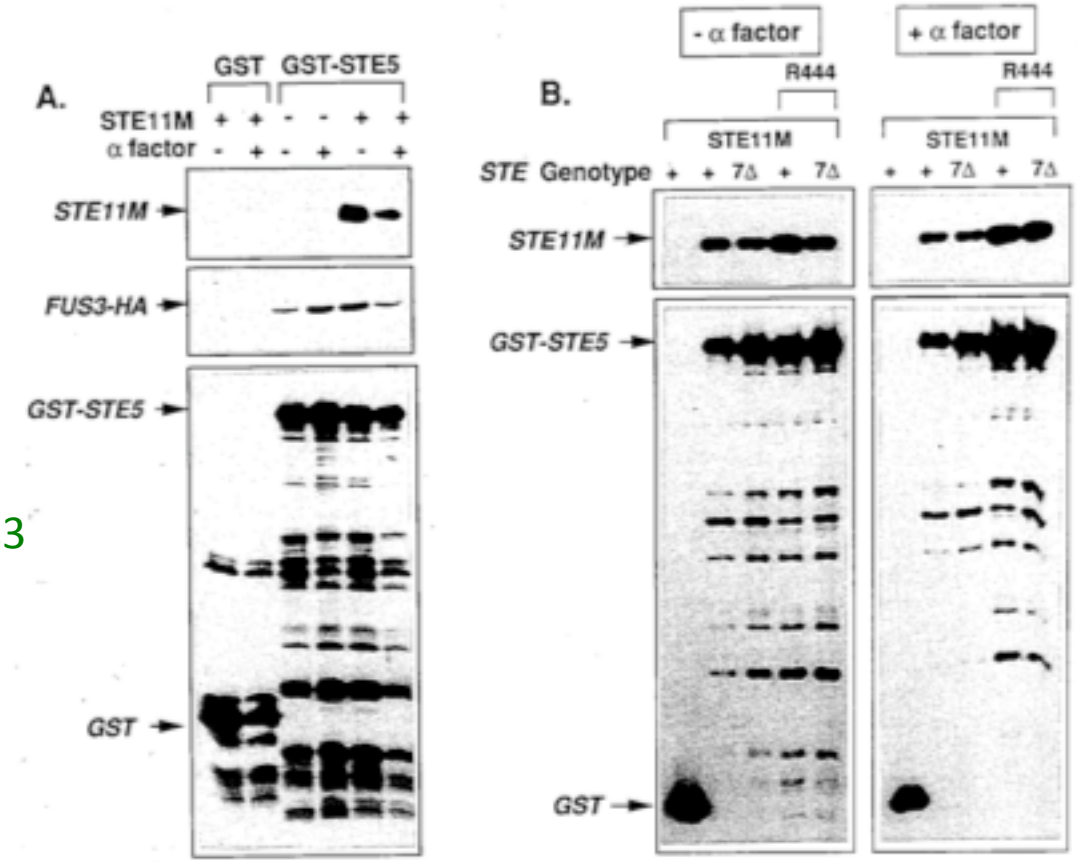
Ste11, Ste7, Fus3, and Kss1 interact with different portions of Ste5



B42 fusion	LexA fusion Ste11	Ste7	Fus3	Kss1
STE5	4840	4717	751	688
STE5Δ1	42	24	295	177
STE5Δ2	3767	14	338	126
STE5Δ3	1556	4691	36	41
STE5Δ4	5	10	262	300
STE5Δ5	5334	17012	42	

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

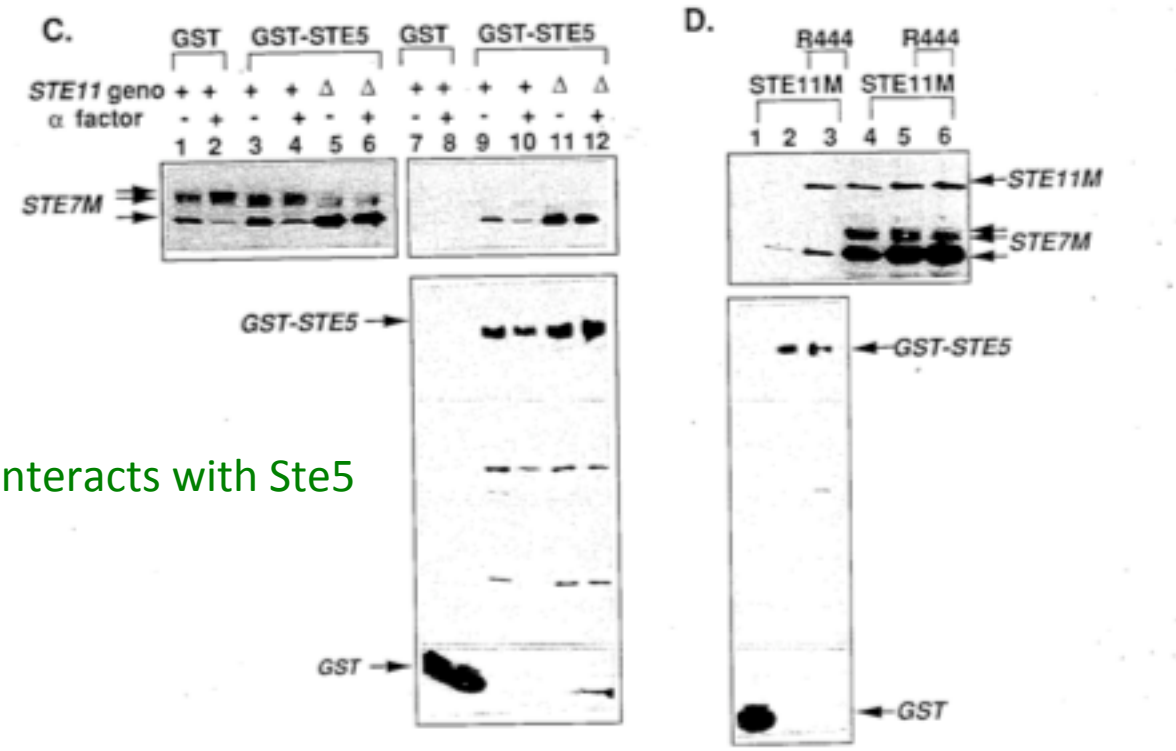
Ste11 and Fus3



Ste5-Ste11 interaction does not require Ste7

IP experiments, continued

Likewise, Ste7 interacts with Ste5



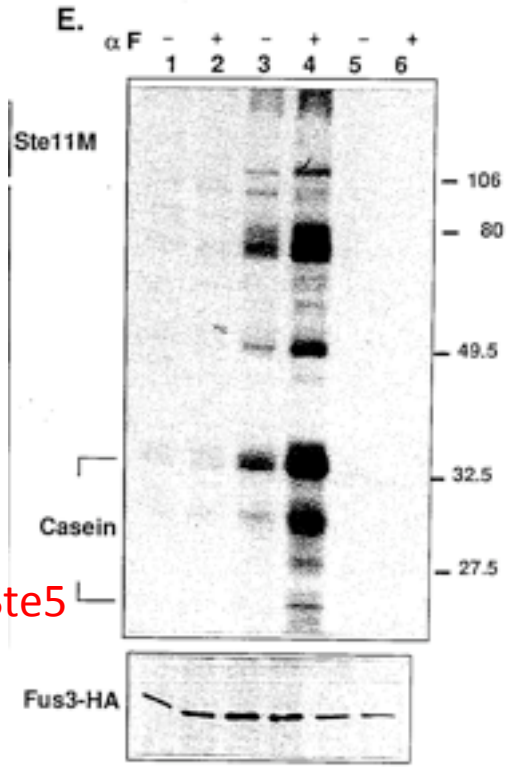
Fus3 is activated by alpha-factor in a Ste5-dependent manner

Fus3 kinase assays

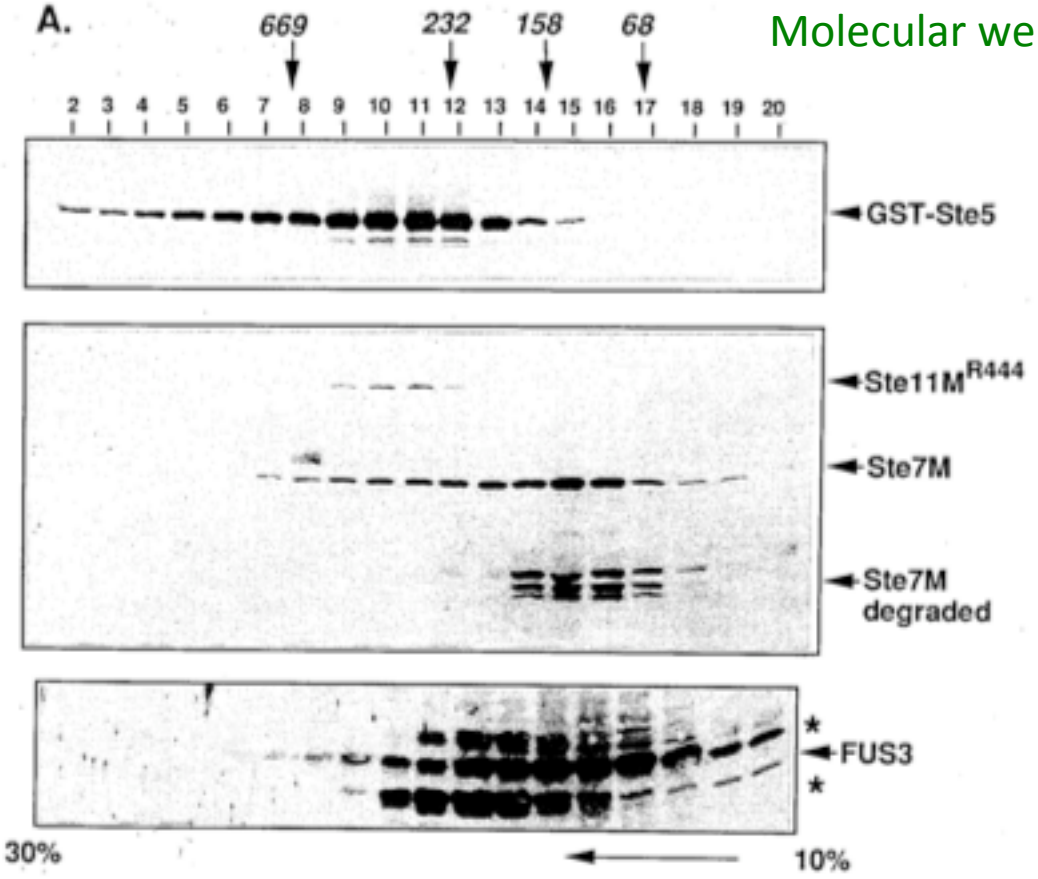
Lanes 1&2: no Ste5

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

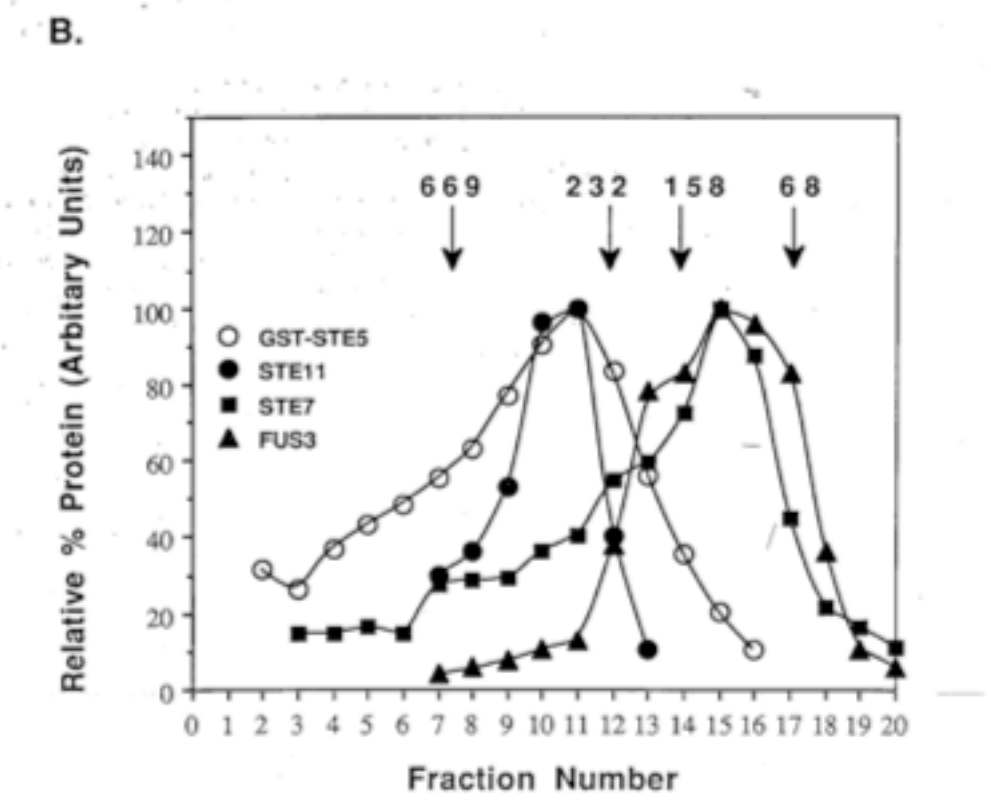
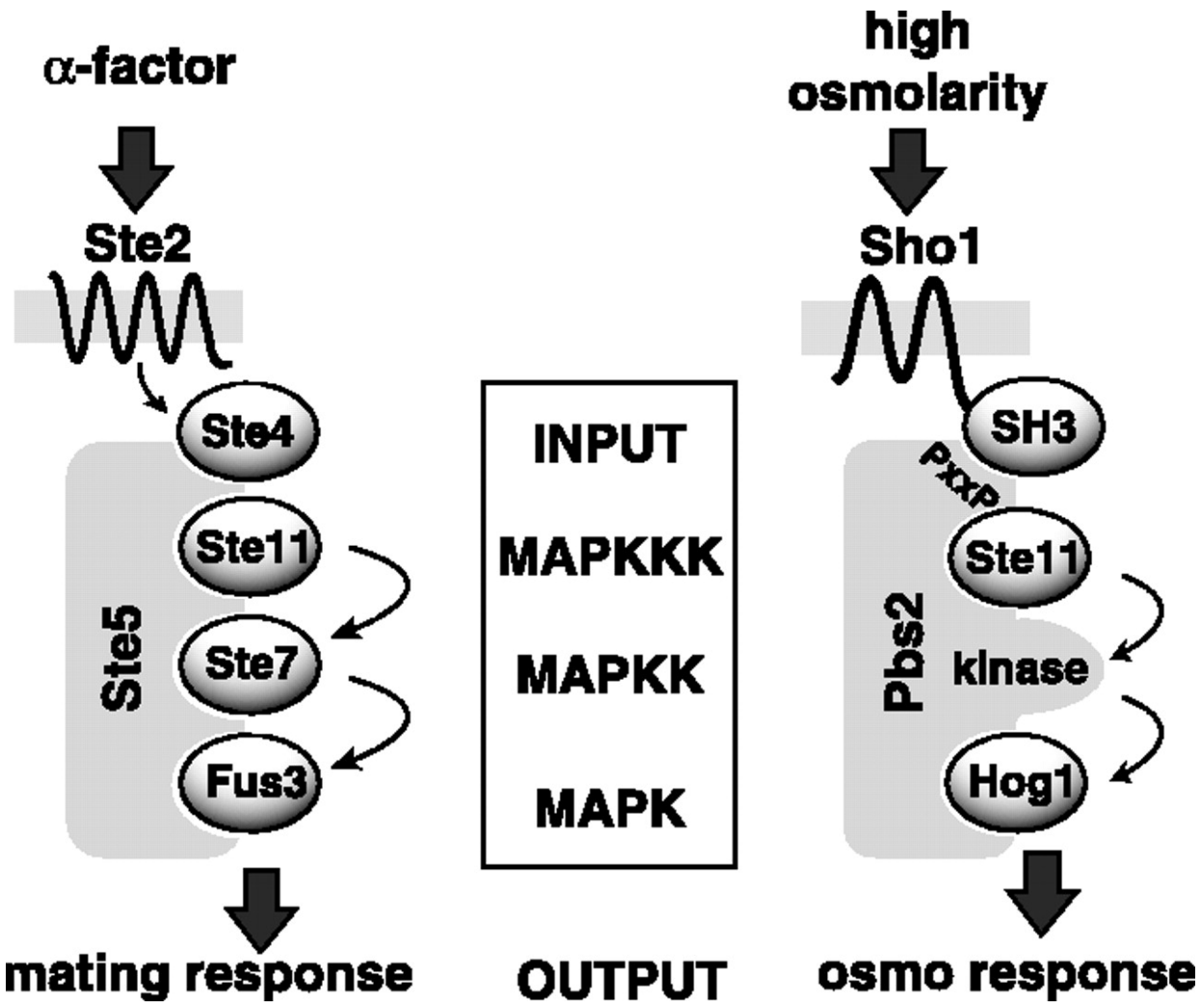
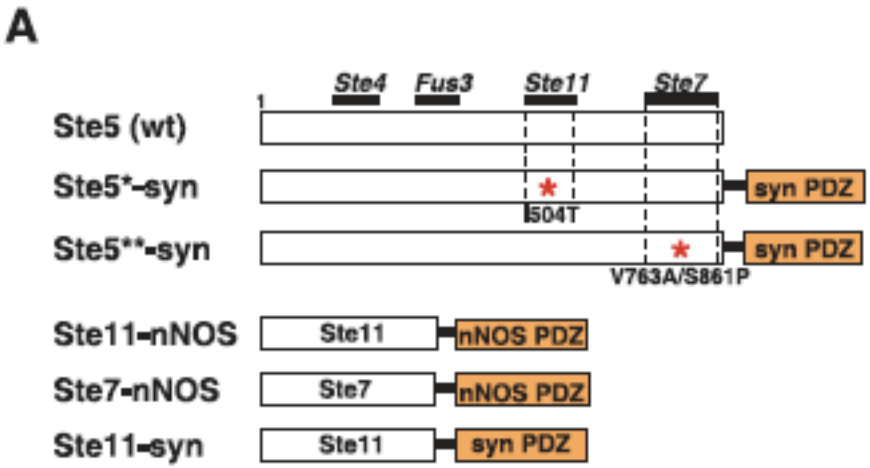


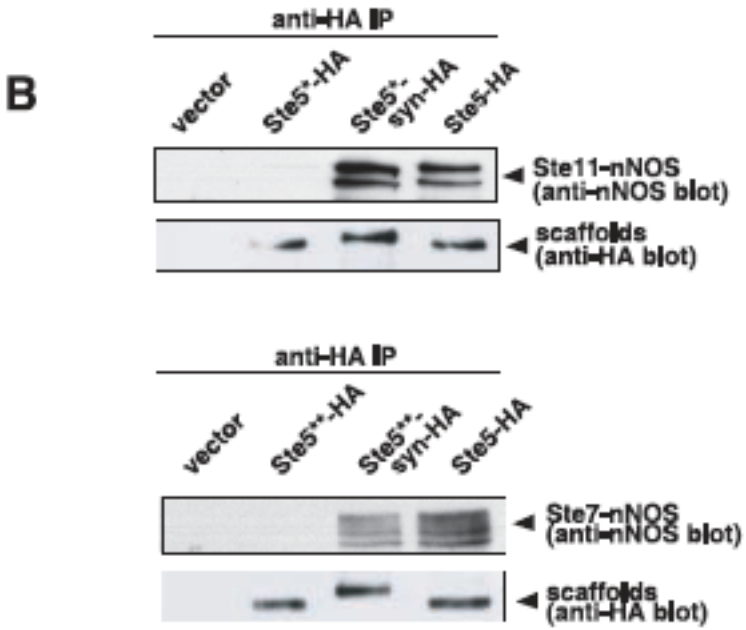
Figure 1 Yeast mating and high-osmolarity MAPK pathways require scaffold proteins Ste5 and Pbs2.



S Park et al. Science 2003;299:1061-1064

Engineered protein-protein interactions can substitute for the native Ste5-Ste7 and Ste5-Ste11 interaction



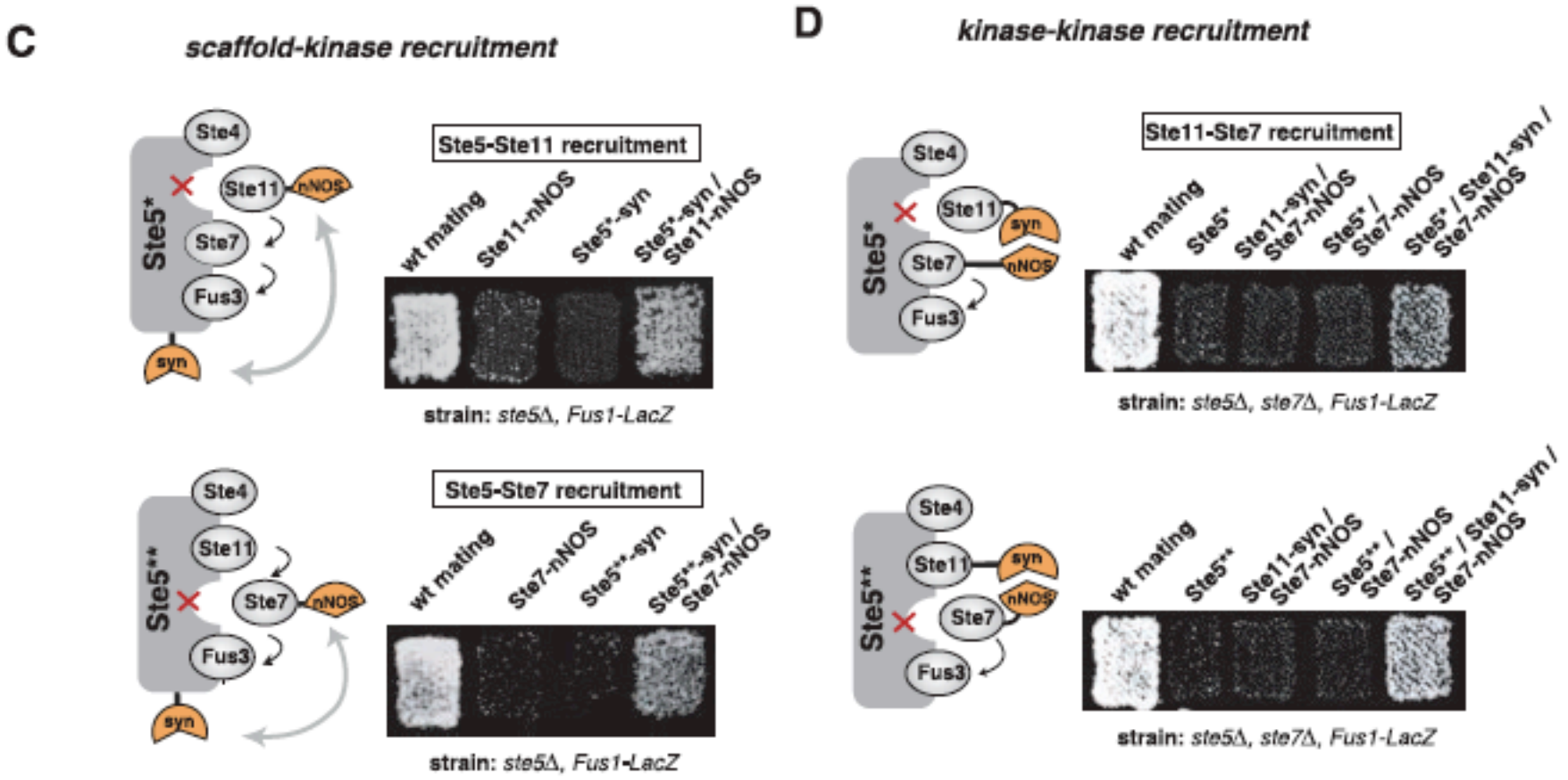


PDZ domains are protein-protein interaction domains

* Indicates mutant binding sites on Ste5

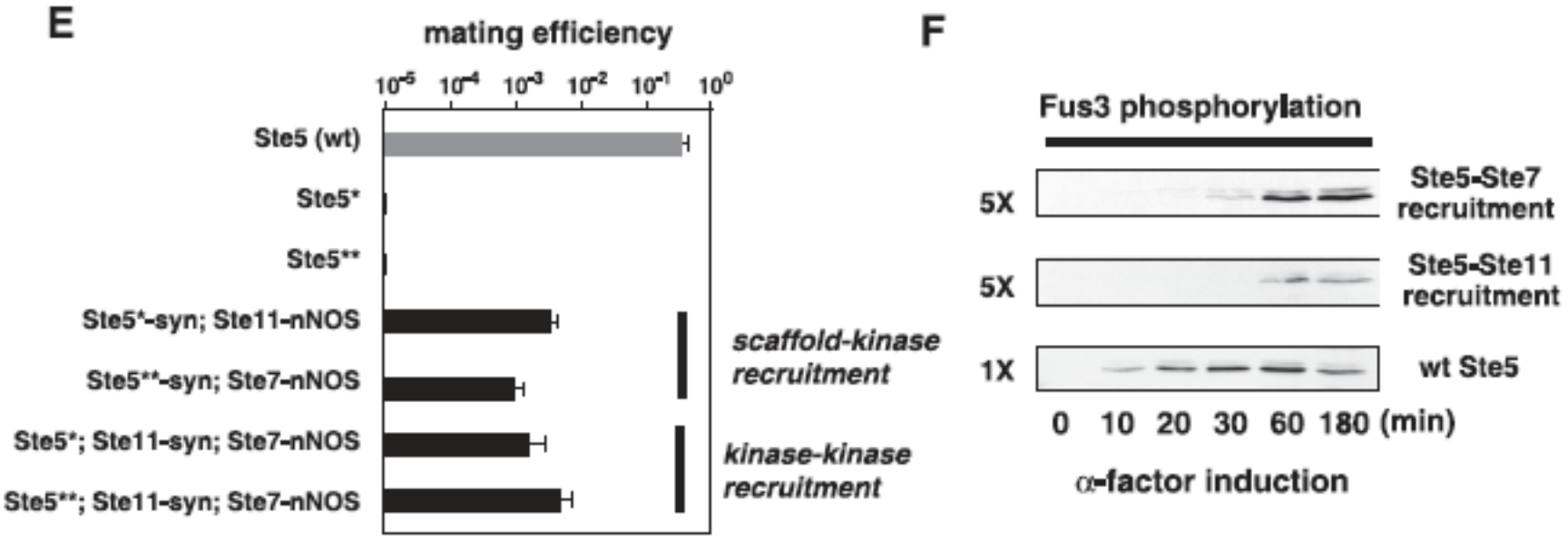
Ste5 is tagged with HA epitope;
IP with anti-HA, probe with anti-NOS

Artificial interactions demonstrated by mating test

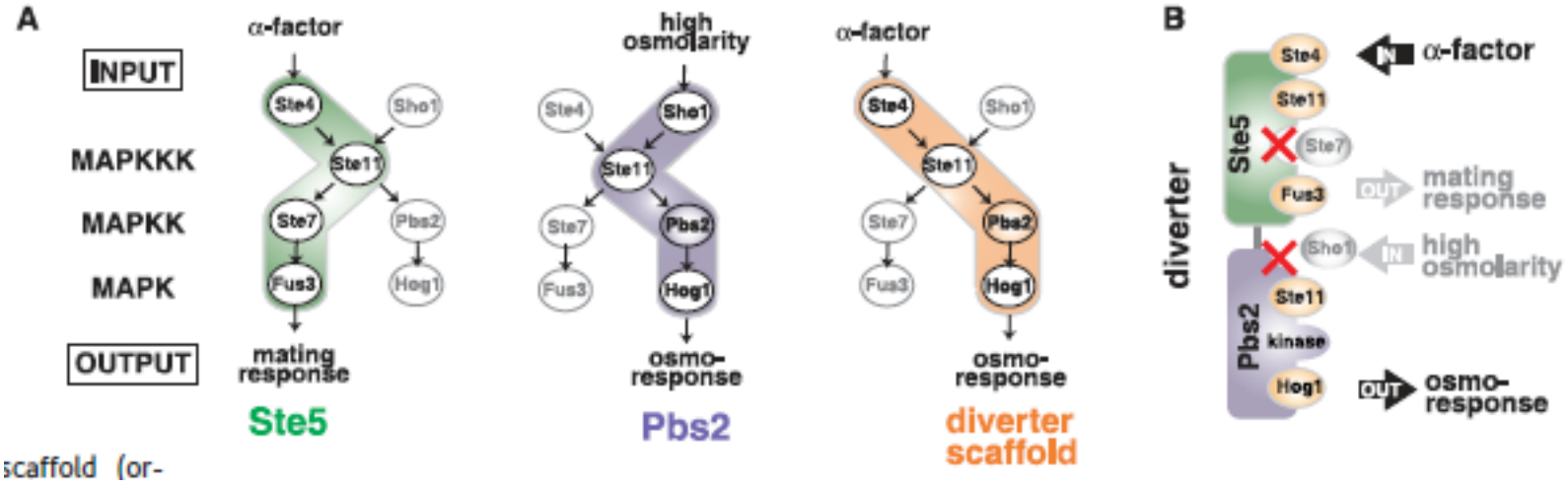


Mating test: strain with Ste5 construct is leu-; test for mating to trp- cells of other mating type. Growth will be observed on minimal medium only if mating has occurred.

… and by quantitative mating tests and phosphorylation of Fus3



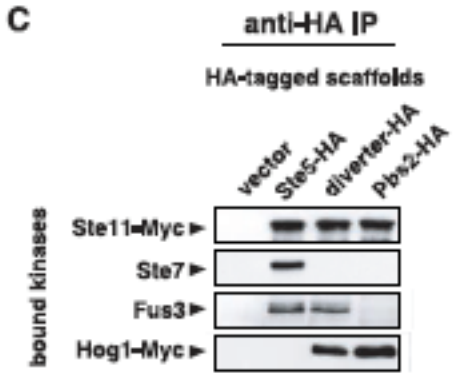
Can one engineer a new scaffold to direct a different output response?



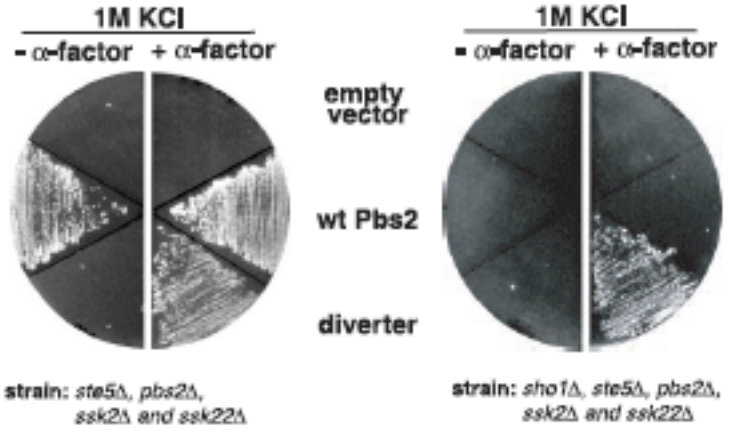
scaffold (or-

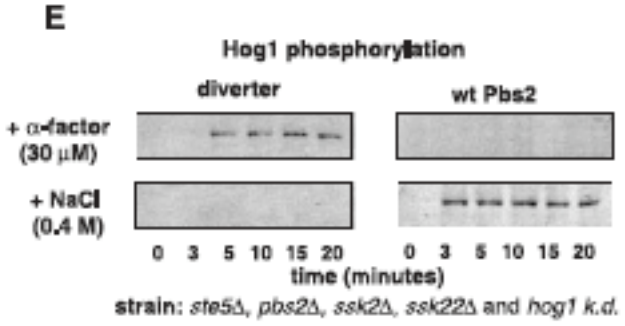
The diverter scaffold works as designed

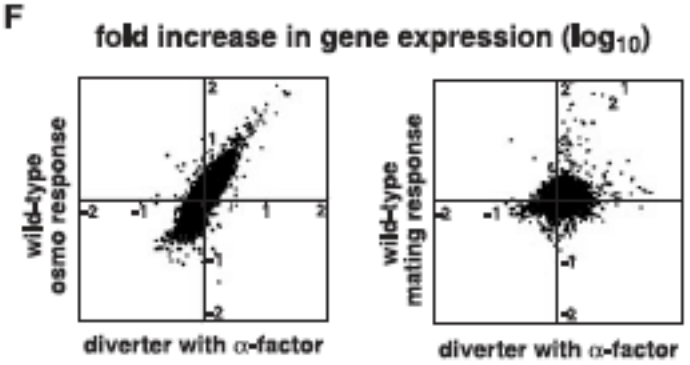
Diverter interacts with appropriate kinases



Growth on salt occurs only in presence of alpha-factor



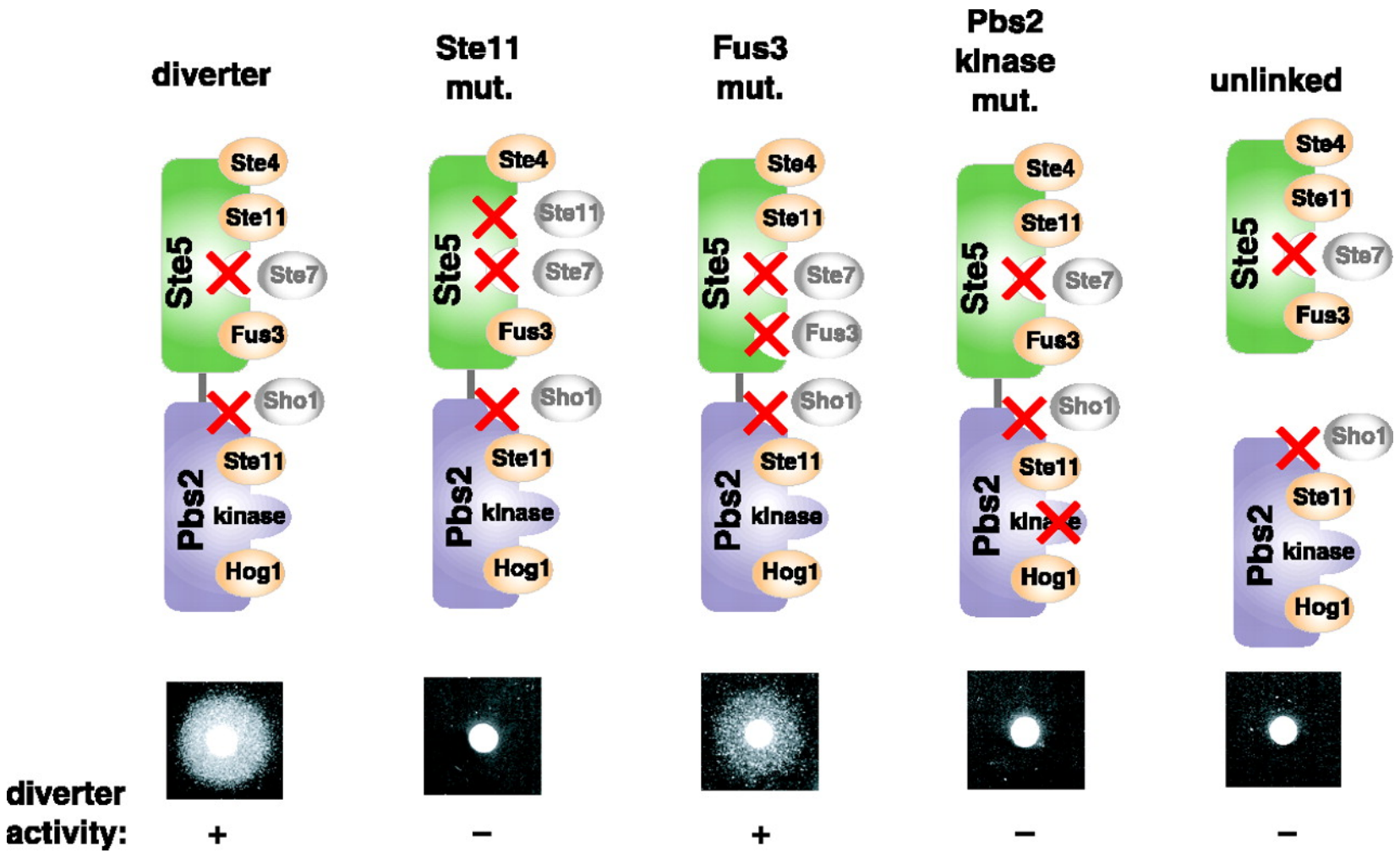




Diverter directs phosphorylation of Hog1

Diverter directs same change in gene expression as wildtype osmo-response

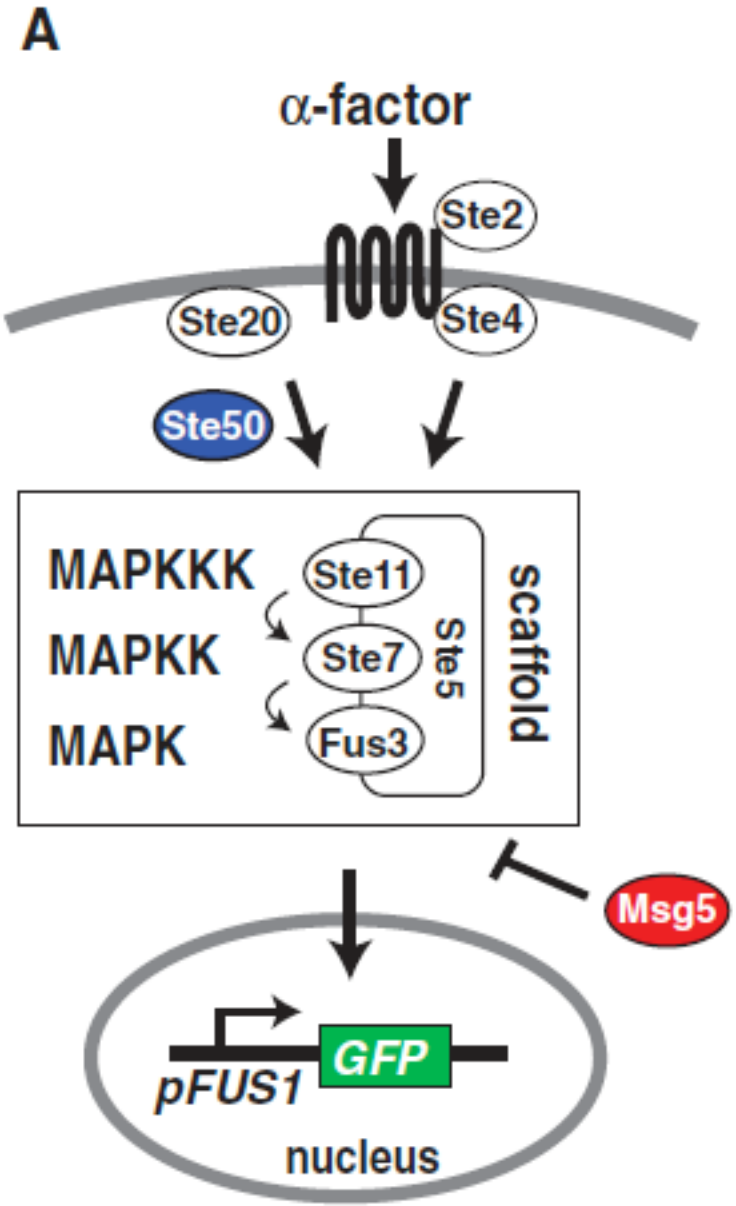
Figure 4 Mutational analysis of diverter scaffold requirements.



S Park et al. Science 2003;299:1061-1064

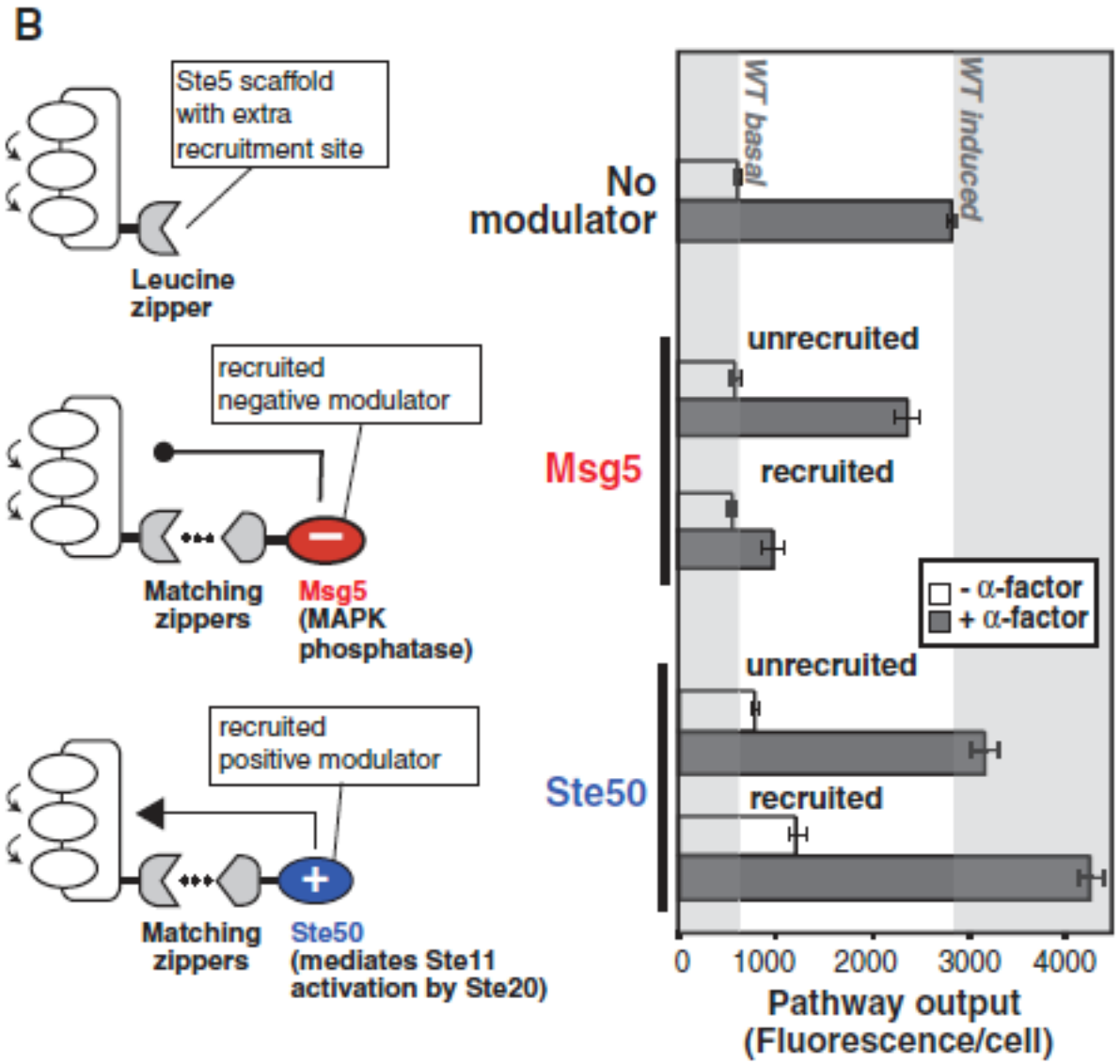
Growth on high salt in presence of α-factor

Additional regulators of pheromone response

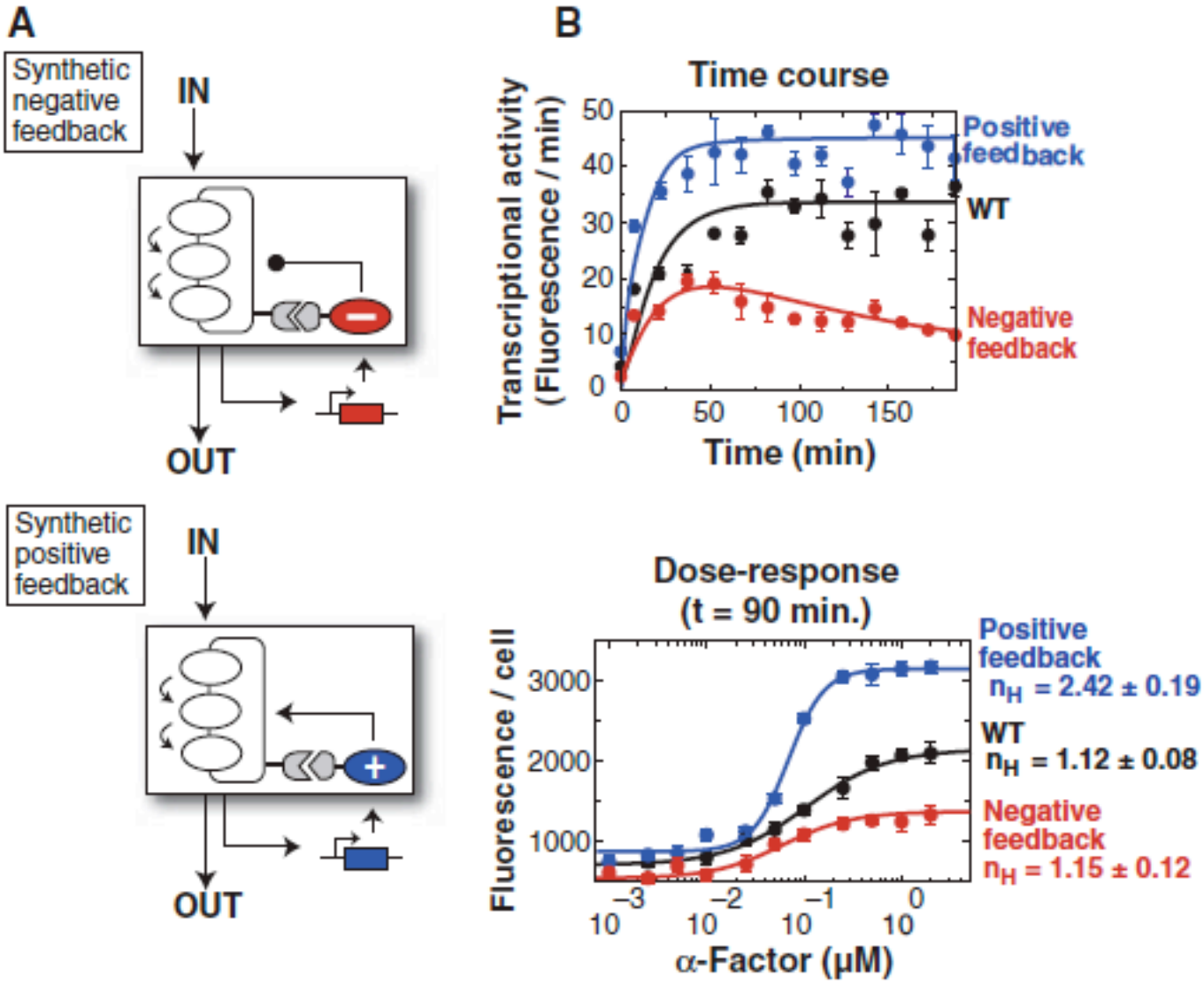


Can a scaffold be engineered to alter kinetics of response to these regulators?

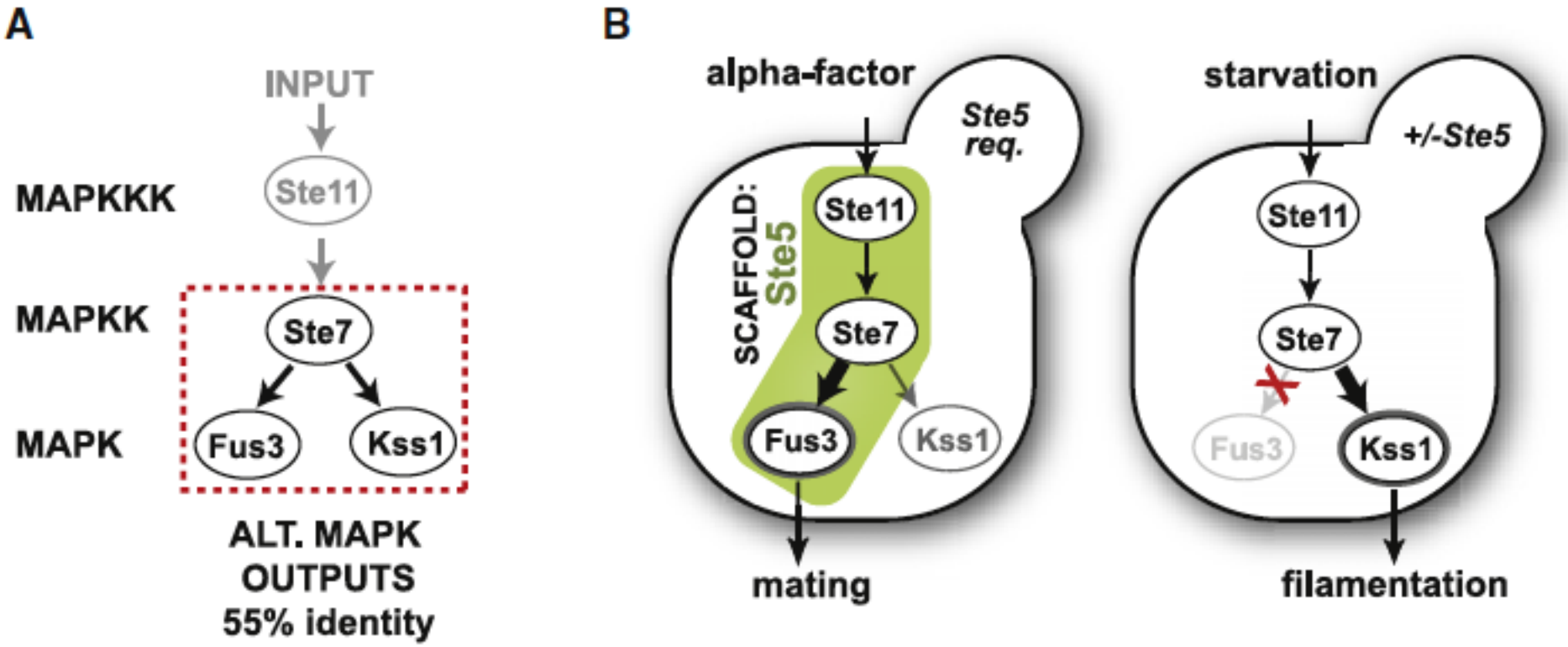
Recruited regulators attenuate or stimulate response



Recruited regulators change time course and dose response

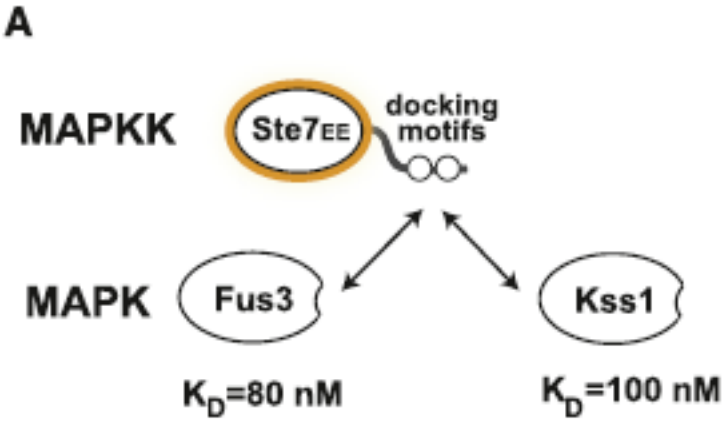


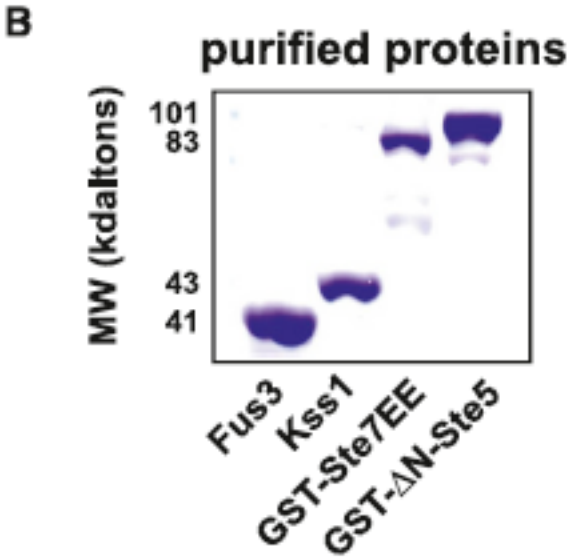
Two MAPK targets for Ste7



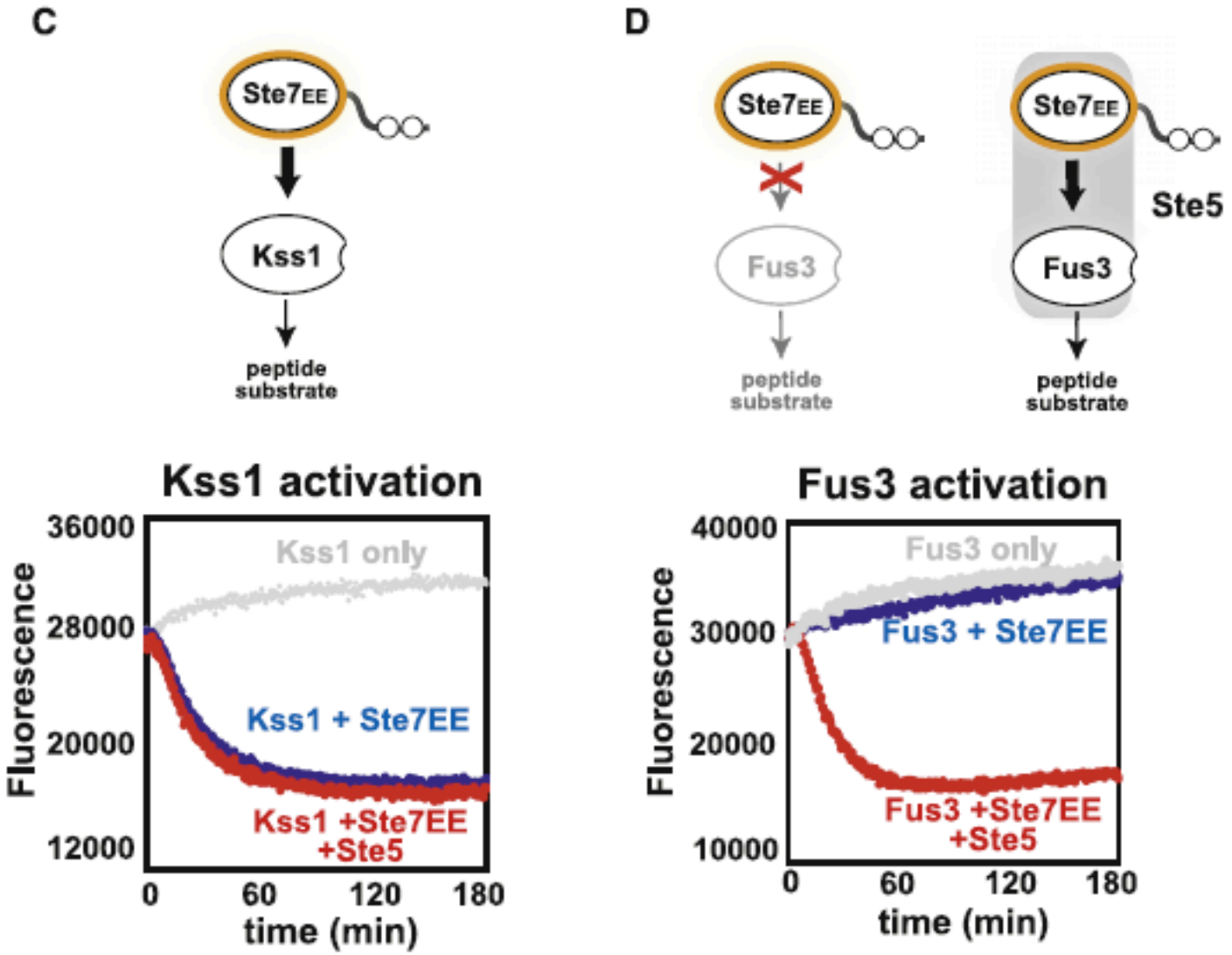
What is role of Ste5 in activation of Fus3

A new role for Ste5

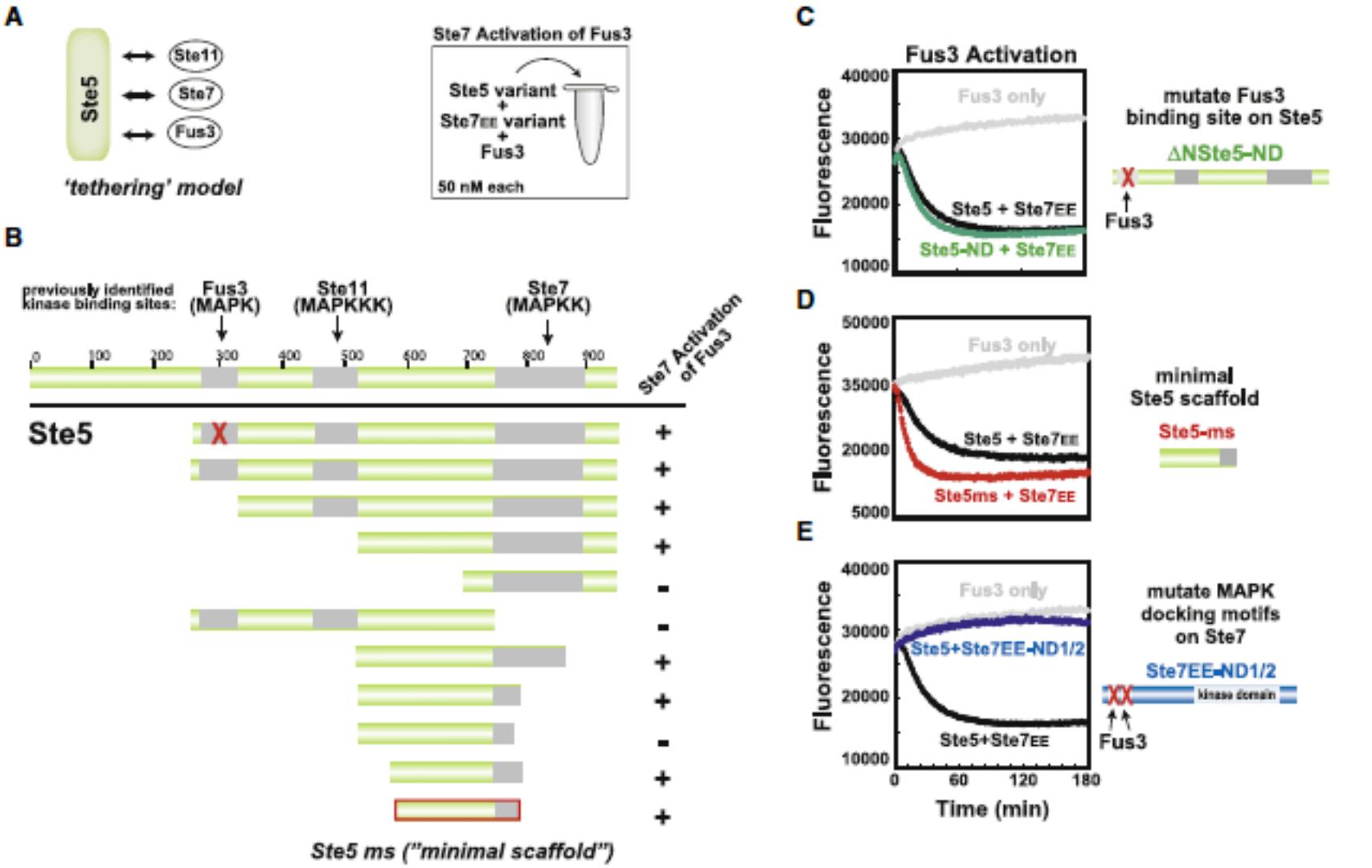




Ste5 is required for activation of Fus3 but not Kss1



Requirements for activation of Fus3 by Ste7

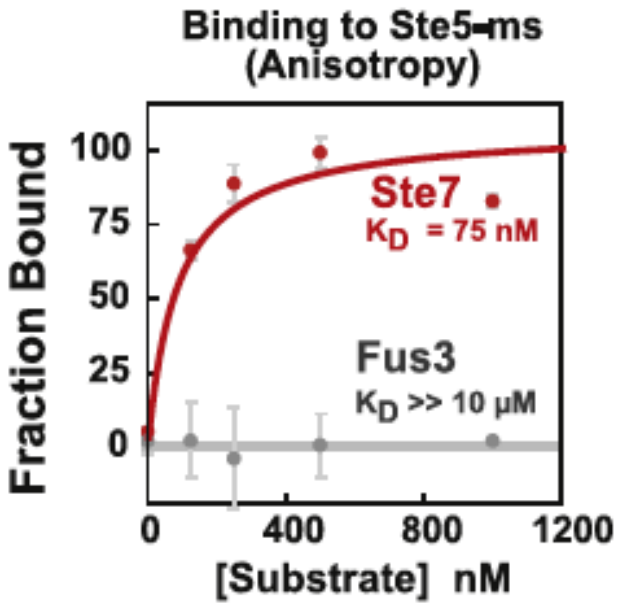


Definition of a minimal scaffold version of Ste5

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

F



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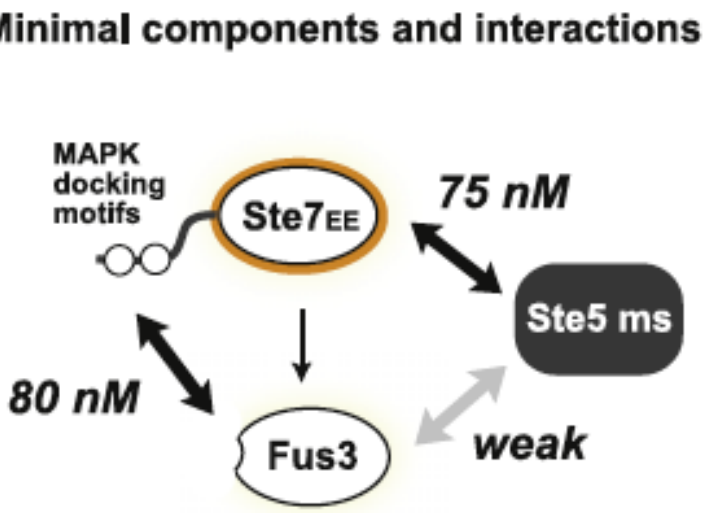


Ste7 tethers Ste5-ms to Fus3

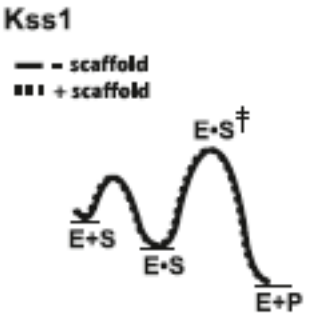
Ste5 changes the Kcat of Ste7 for Fus3, not the Km

A

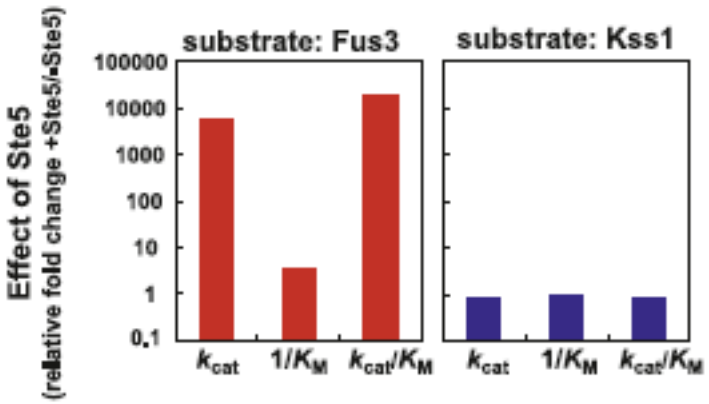
C

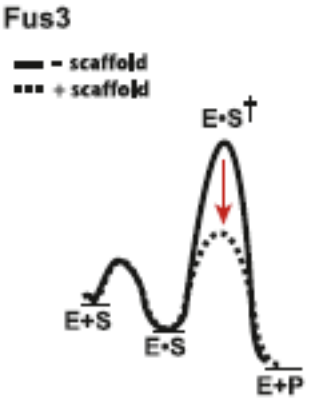
Substrate	Ste5-ms	k_{cat} (s^{-1})	K_M (nM)	k_{cat}/K_M (M^{-1} s^{-1})
Fus3	-	7.4 +/- 1.4 x 10-7	131 +/- 50	5.7
	+	4.3 +/- 1.3 x 10-3	40 +/- 22	108491
Kss1	-	2.1 +/- 0.22 x 10-2	157 +/- 55	135619
	+	1.7 +/- 0.15 x 10-2	156 +/- 61	110933

F

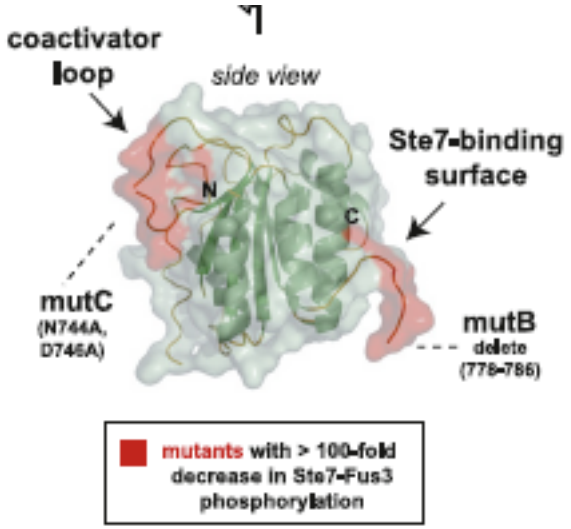


D





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

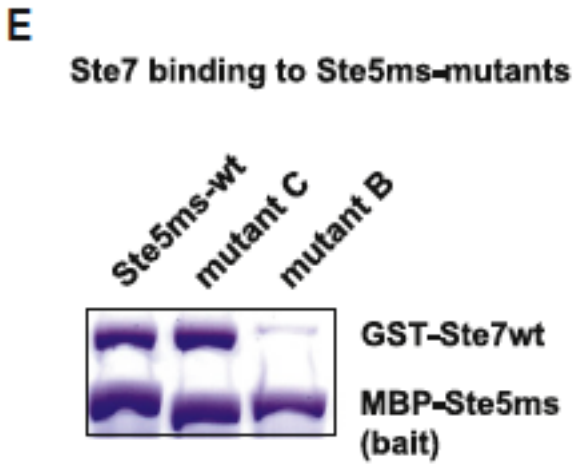


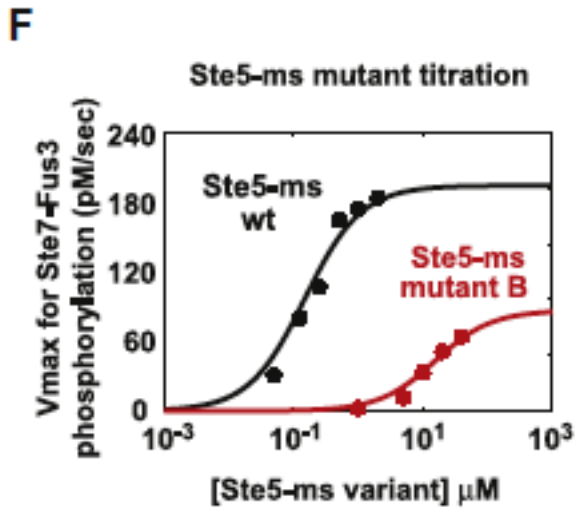
Mutants fall in two regions on Ste5 surface

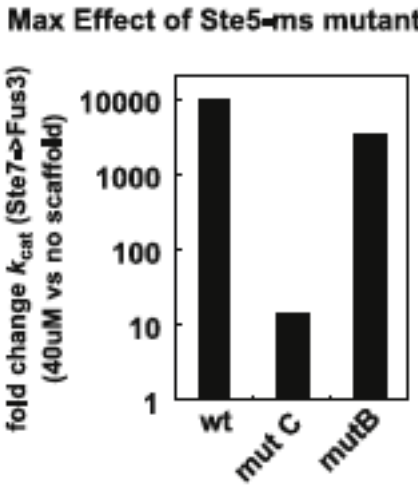
The known Ste7 binding site

A region dubbed the coactivator loop

Ste5ms mutants affect different aspects of phosphorylation of Fus3 by Ste7



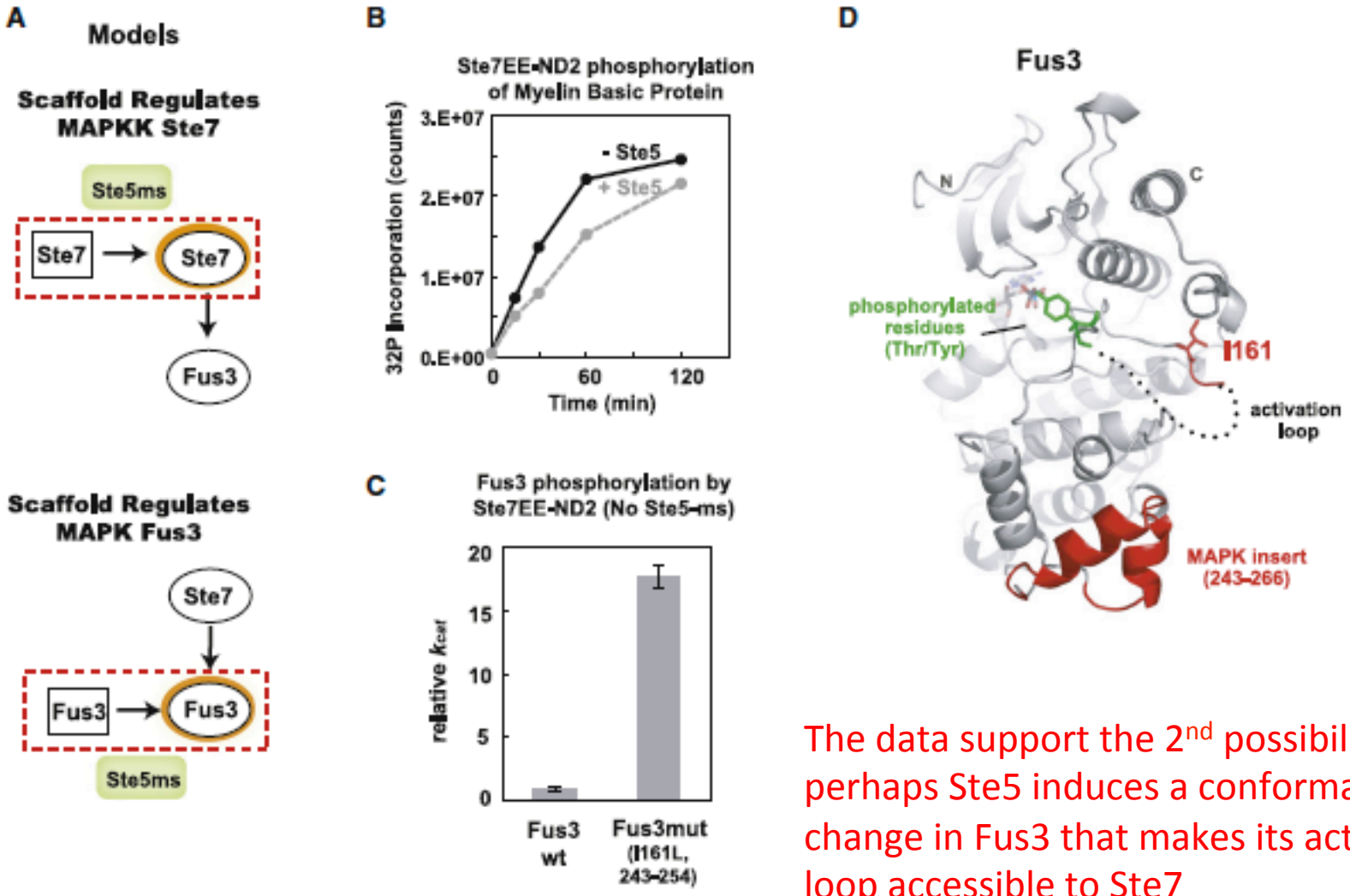




Mutant B affects Ste7 binding

Mutant C affects k-cat

2 possibilities for how Ste5 stimulates phosphorylation of Fus3 by Ste7

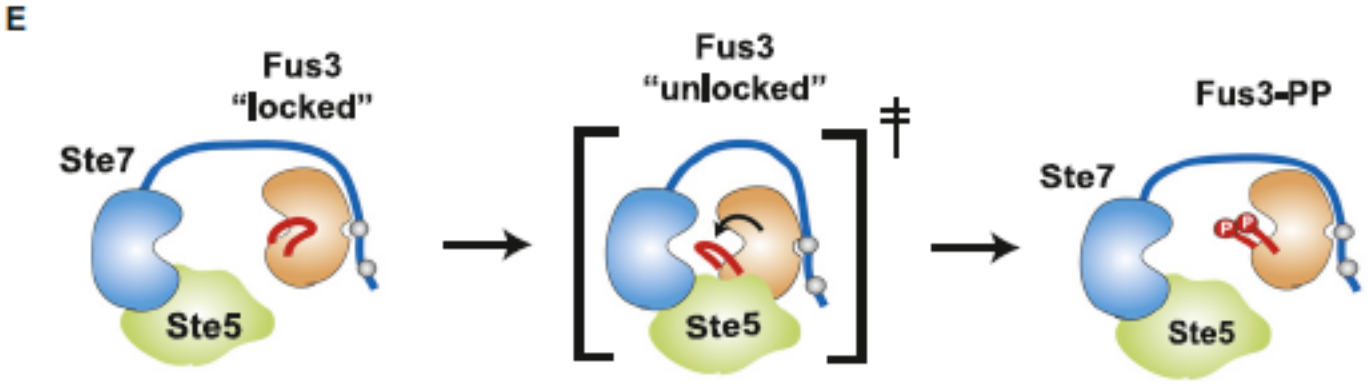


The data support the 2nd possibility; perhaps Ste5 induces a conformational change in Fus3 that makes its activation loop accessible to Ste7

1. Ste5 makes Ste7 a better kinase

2. Ste5 make Fus3 a better substrate

Model


E
Fus3
"locked"
Ste7
Ste5
Fus3
"unlocked"
‡
Ste5
Fus3-PP
Ste7
P
P
Ste5