Osmosensing and HOG MAPK

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Introduction to Yeast Osmoregulation

1.Maintenance of the cellular water balance, control of cell shape, turgor and relative water content.

2.Osmotic shock:

- Hyper-osmotic stress (water efflux, cell shrinkage)
- Hypo-osmotic stress (water influx, cell swelling)

3.The strategies of cellular adaptation are conserved from bacteria to human. Yeast is a suitable model system to study osmoregulation. A lot of information is available on osmotic shock induced signal transduction, control of gene expression and accumulation of osmolytes.

4.Central to the osmoregulation is the high osmolarity glycerol (HOG) signaling system.

Hyper-osmotic stress (phenotype&genotype)

1.Yeast cells shrink within seconds.

2.Within less than 1 min the glycerol export channel Fps1 closes rapidly to prevent glycerol leakage and enables the accumulation of this compatible solute.

3.Within the same time frame, the HOG signal transduction system is activated . The initial responses mediated by the active Hog1:

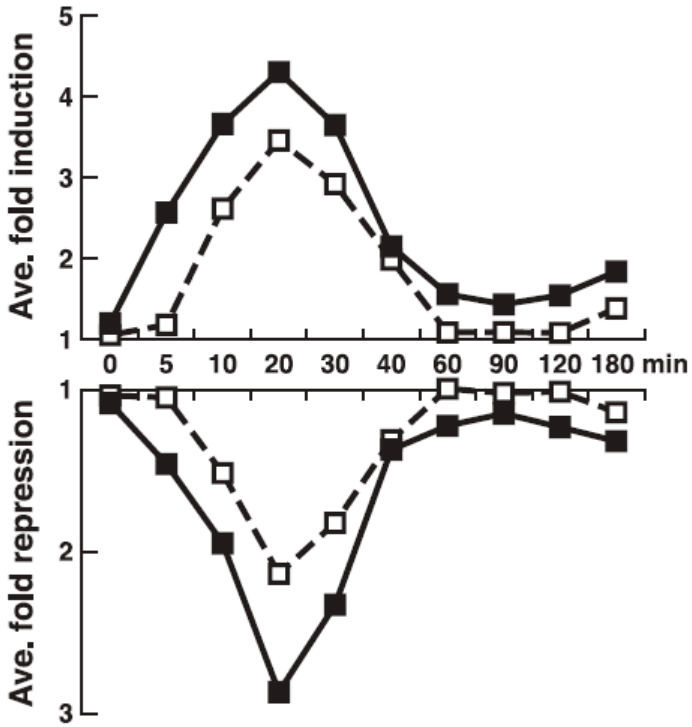
- stimulation of ion export
- arrest of the cell cycle
- diminishing of translational capacity
- stimulation of glycolysis to enhance production of glycerol.

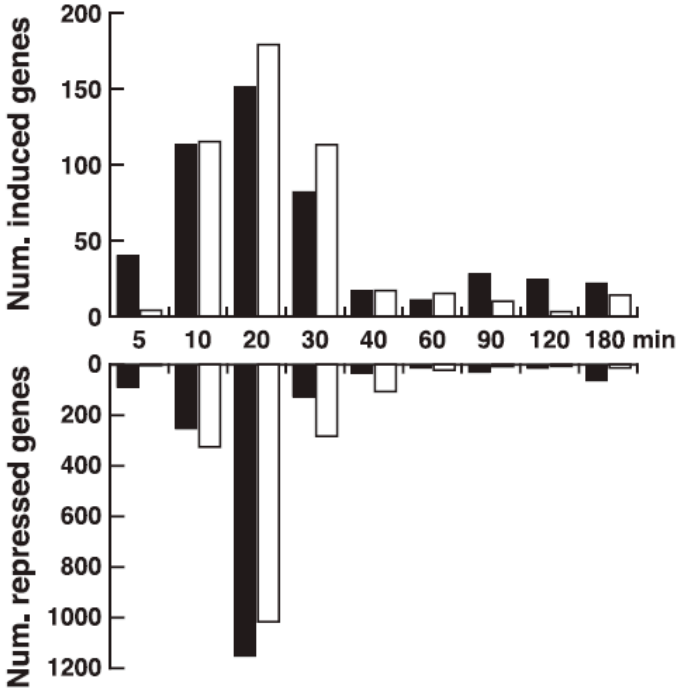
4.A significant portion of active Hog1 accumulates in the nucleus where it is recruited to target promoters to control gene expression (Hot1, Sko1, Smp1, Msn1, Msn2/4).

- Genes encoding enzymes in glycerol production and active glycerol uptake are among those upregulated in a Hog1-dependent manner.
- Bulk of the transcriptional change seems to revolve around metabolism and is similar to that caused by other stresses.
- Adaptation depends on glycerol accumulation, and once the intracellular glycerol level has reached about one-third of its maximum, Hog1 is dephosphorylated and gene expression responses attenuate.

High osmolarity regulated genes

- Treated with 0.5M KCL(black bars/squares) or 1M sorbitol(open bars/squares).
- Only genes that were regulated twofold or more in at least two time points in either the KCl or sorbitol time course were included.
- There are 488 osmo-induced and 1789 osmo-repressed genes.

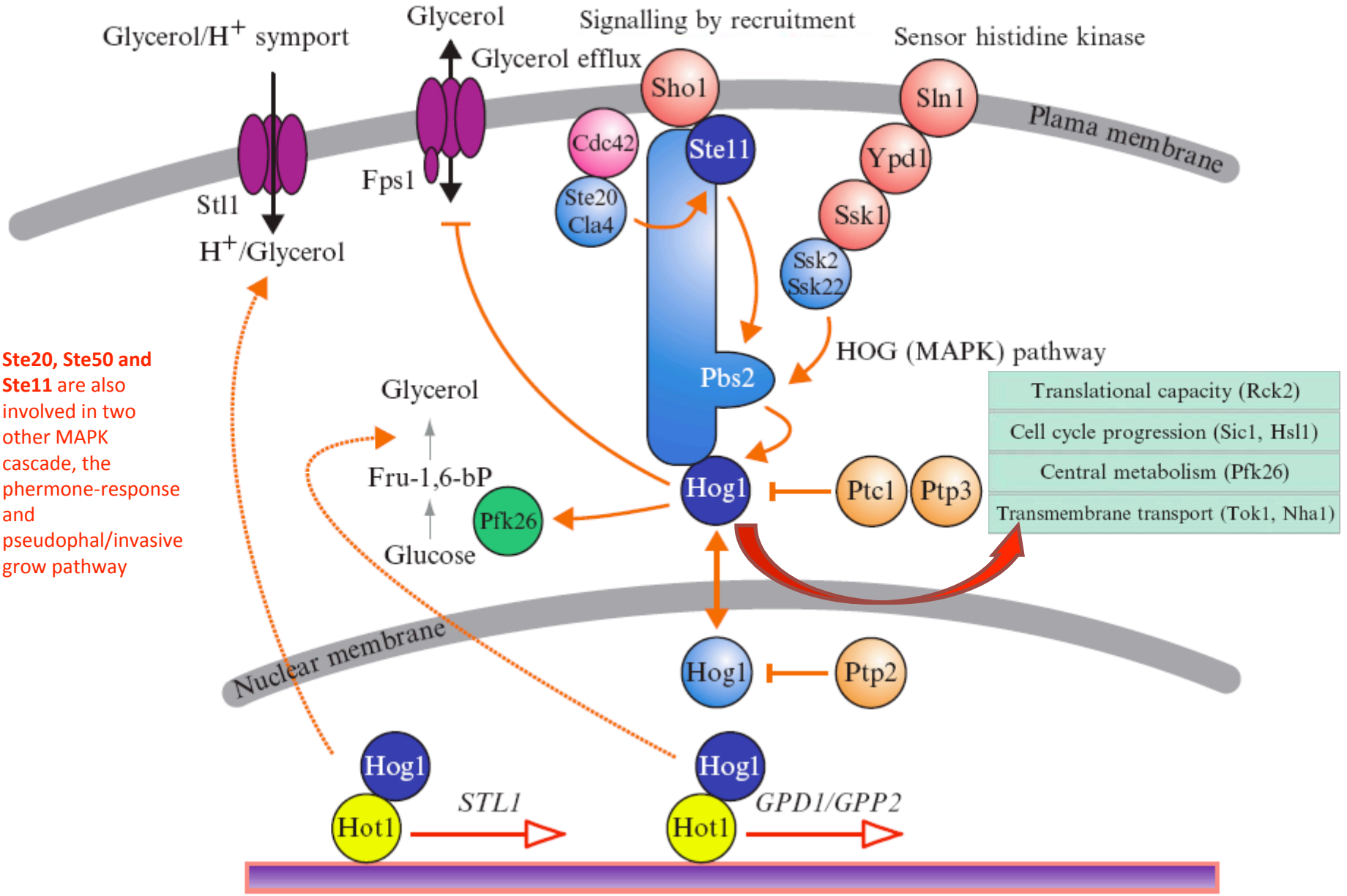




Genome expression profiling (WT vs *hog1* mutant)

	Functional Description
Cluster 1	Genes that were inappropriately induced in the *hog1* mutant. This cluster includes the pheromone-induced genes, *FUS1, STE2, KAR5, AGA1, and PRM1* and several genes associated with filamentous growth, *TEC1, PHD1,* and *PGU1.*
Cluster 2	Nine ORFs that required *HOG1* for basal expression and in some cases for very modest induction. Two genes encoding proteins with known or predicted biochemical roles are *RHR2,* which encodes a glycerol-3-phosphatase that produces glycerol and phosphate from glycerol 3-phosphate, and *YKL161c,* which codes for a MAPK. Three genes encoding cell wall proteins also required *HOG1* for their basal expression: *CWP1, SED1, and PIR3.*
Cluster 3	32 genes exhibited very strong induction in wild-type cells but little in the *hog1* mutant. Most of these genes' function are unknown, but some genes have known or predicted functions: *ENA1* plays a role in ion homeostasis, *STL1* codes for a putative hexose transporter, *TKL2* codes for a transketolase, *DAK1* codes for a putative dihydroxyacetone kinase, and *THI4* codes for a putative enzyme involved in vitamin B1 biosynthesis. *HBT1* is involved in polarized morphogenesis.
Cluster 4	Genes induced in both wild-type and *hog1* mutants in response to high osmolarity with different time duration. The response in wild-type cells was transient, peaking at 20 min and decreasing to near uninduced levels by 40–90 min. In cells lacking *HOG1,* some of these genes were still induced normally, although the down-regulation that occurred in wildtype cells was delayed or absent. Many genes in this cluster are induced in response to diverse environmental stresses such as heat shock, protein-damaging agents, nitrogen starvation, and stationary phase
Cluster 5	These genes exhibit transient reductions in transcript levels in the wild-type strain in response to osmotic stress but greatly prolonged reductions in the *hog1* mutant. Among genes of this group, 96 code for ribosomal protein subunits. Finding that ribosomal subunit-coding genes are down-regulated during osmotic stress is not surprising. But the observations indicate that Hog1 plays a role in reestablishment of expression of these genes after stress.

The HOG signaling system



Ste20, Ste50 and Ste11 are also involved in two other MAPK cascade, the phermone-response and pseudophal/invasive grow pathway

Questions regarding HOG MAPK pathways remain unanswered.

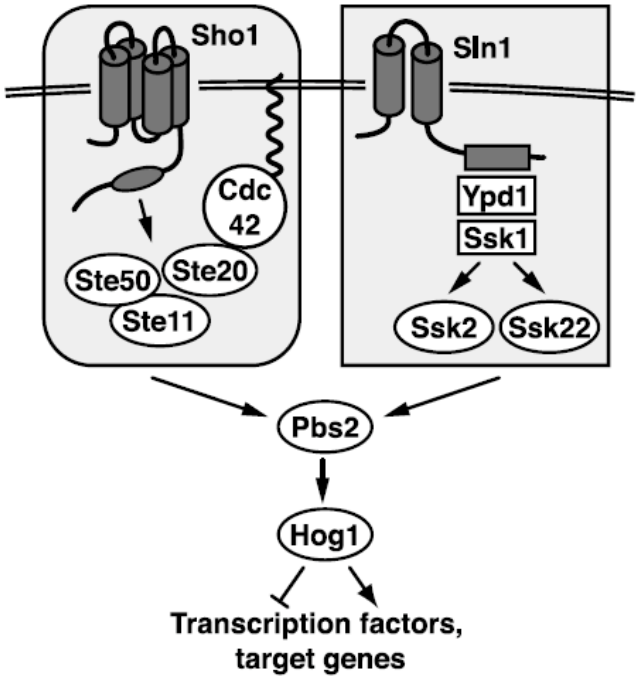
What mechanisms the cell use to detect changes in osmolarity?

What is the benefit of having multiple upstream branches to the HOG pathway?

How to avoid cross-talk among different MAPK system since there are common signaling molecules?

The HOG pathway: two upstream branches

- Two transmembrane proteins, Sho1 and Sln1, act on downstream proteins to ultimately regulate the MAPK Hog1.
- Sho1 and Sln1 have been termed osmosensors, but recent studies indicate that the single transmembrane-spanning domain protein Msb2 may contribute additional osmolarity-sensing.
- Sho1 and Sln1 have very different functional domains and communicate directly to different downstream effector proteins.



- The two upstream branches of the HOG pathway are often thought to be redundant.
  - For growth on high osmolarity medium, they are redundant.
  - However, Hog1 phosphorylation in response to low solute concentrations is more dependent on the Sln1 branch. Global gene regulation in response to modest osmotic stress (e.g. 0.0625 or 0.125 M KCl) specifically requires the Sln1 branch of the HOG pathway; the Sho1 branch is dispensable.

Sln1 branch

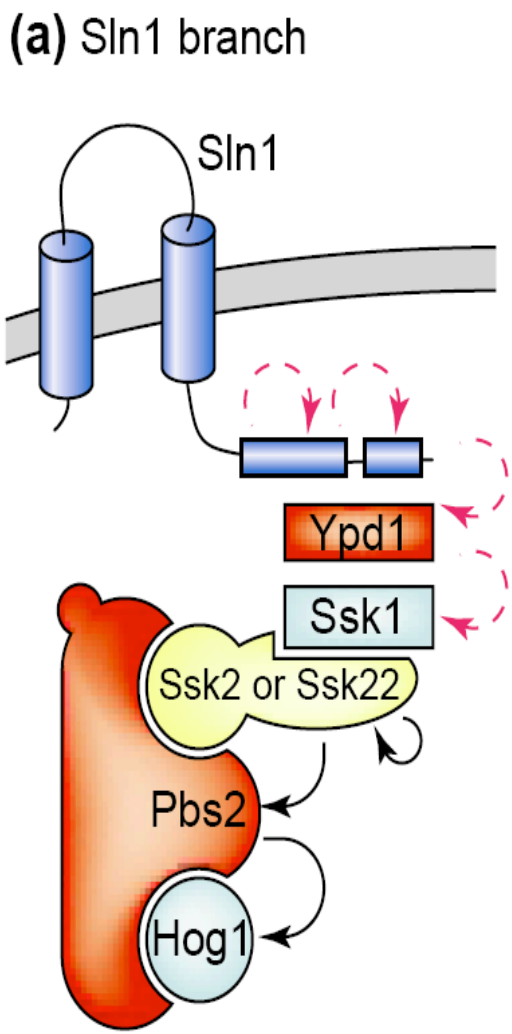
➢Sln1 has two transmembrane regions and an intracellular histidine kinase domain that signals to two other proteins, Ypd1 and Ssk1, which together form a phosphorelay system. Homologs of Sln1 are found in bacterial two-component systems.

➢Sln1 is constitutively active during normal conditions, leading to phosphorylation of the downstream target protein, Ypd1, which transfers its phosphate group to the response regulator Ssk1. Such phosphorylation of Ssk1 is thought to prevent interaction of Ssk1 with the MAPKKKs Ssk2 and Ssk22 and, consequently, the downstream components of the pathway remain inactive.

➢After exposure to increased external osmolarity, the histidine kinase activity of Sln1 is thought to be inhibited. Thus, Ypd1 and Ssk1 are dephosphorylated, and this enables binding of Ssk1 to MAPKKK Ssk2, triggering Ssk2 autophosphorylation, subsequent phosphorylation of Pbs2 and activation of Hog1.

➢The method by which Sln1 actually senses changes in osmotic pressure also remains elusive. Also, the levels of intracellular glycerol appear to regulate Sln1-dependent signaling.

➢YPD1, represents a bifurcation point between the SLN1–YPD1–SSK1 pathway responsible for osmotic stress responses and the SLN1–YPD1–SKN7 pathway involved in cell wall biosynthesis and cell cycle control



Sho1 branch

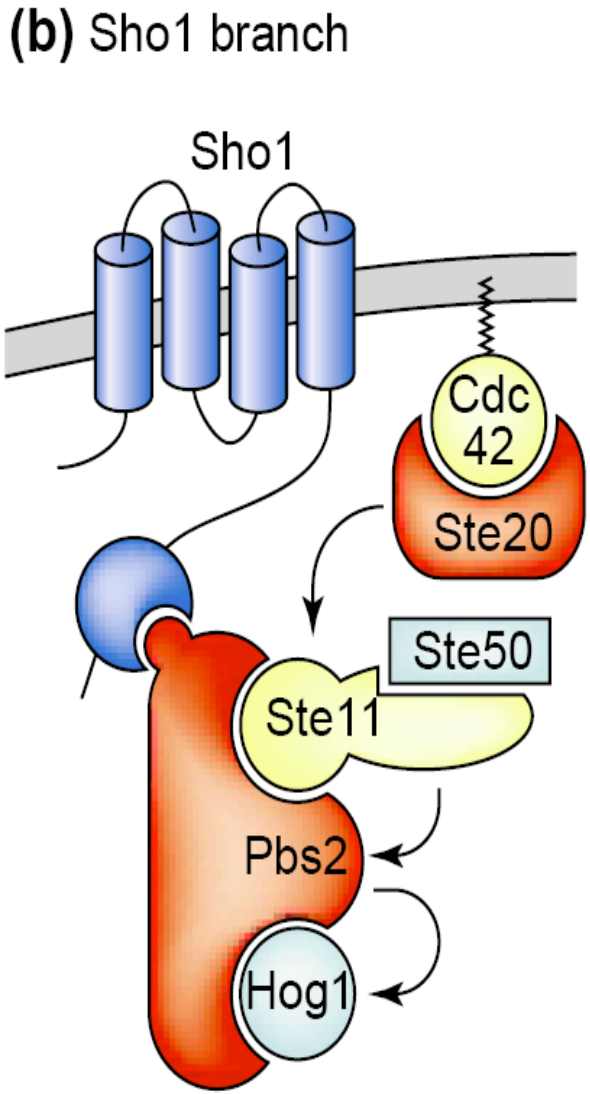
➢The Sho1 protein is composed of four transmembrane segments and a C-terminal SH3 domain, through which it interacts with downstream signaling element Pbs2 in the HOG pathway.

➢Sho1 homologs have only been identified in fungi.

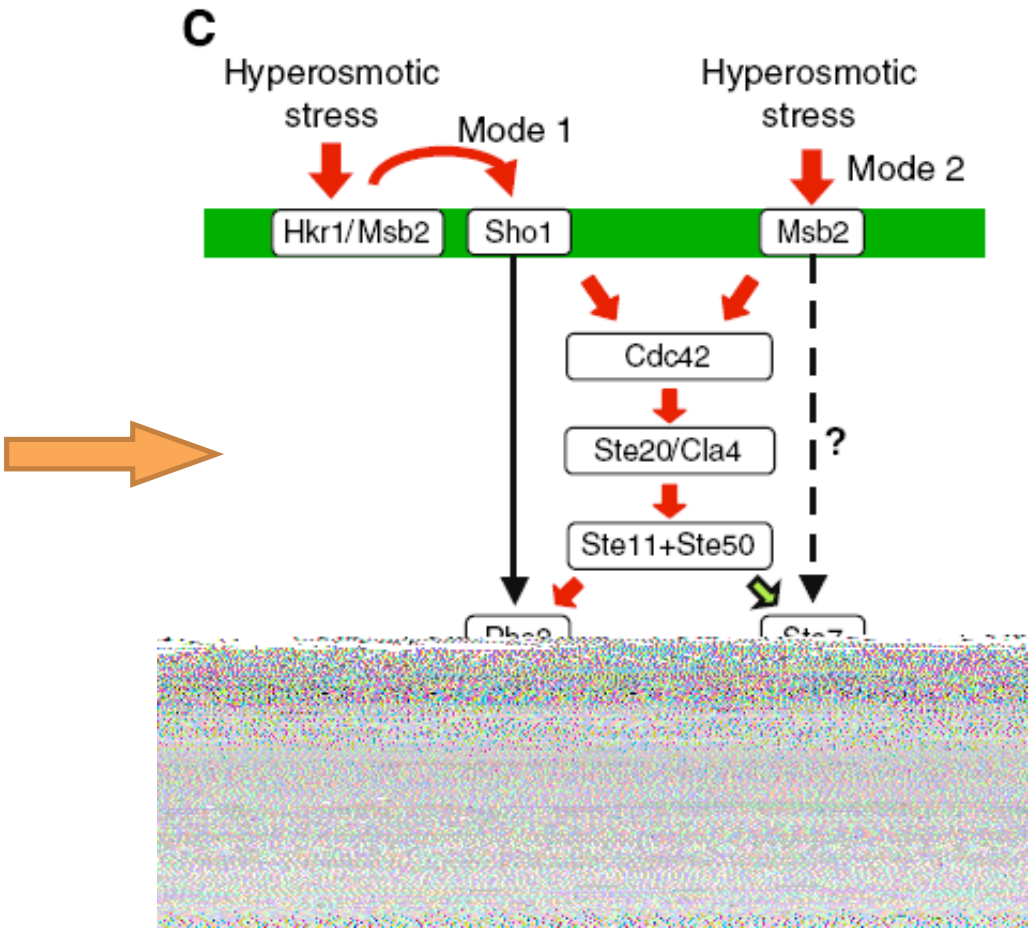
➢The physiological role of Sho1 as an osmosensor has been called into question and, thus, the activation mechanism of this branch of the pathway is unclear.

➢Plasma membrane localization of the SH3 domain is required, and Sho1 might not sense osmolarity directly but might instead provide a docking site (via its SH3 domain) for downstream proteins.

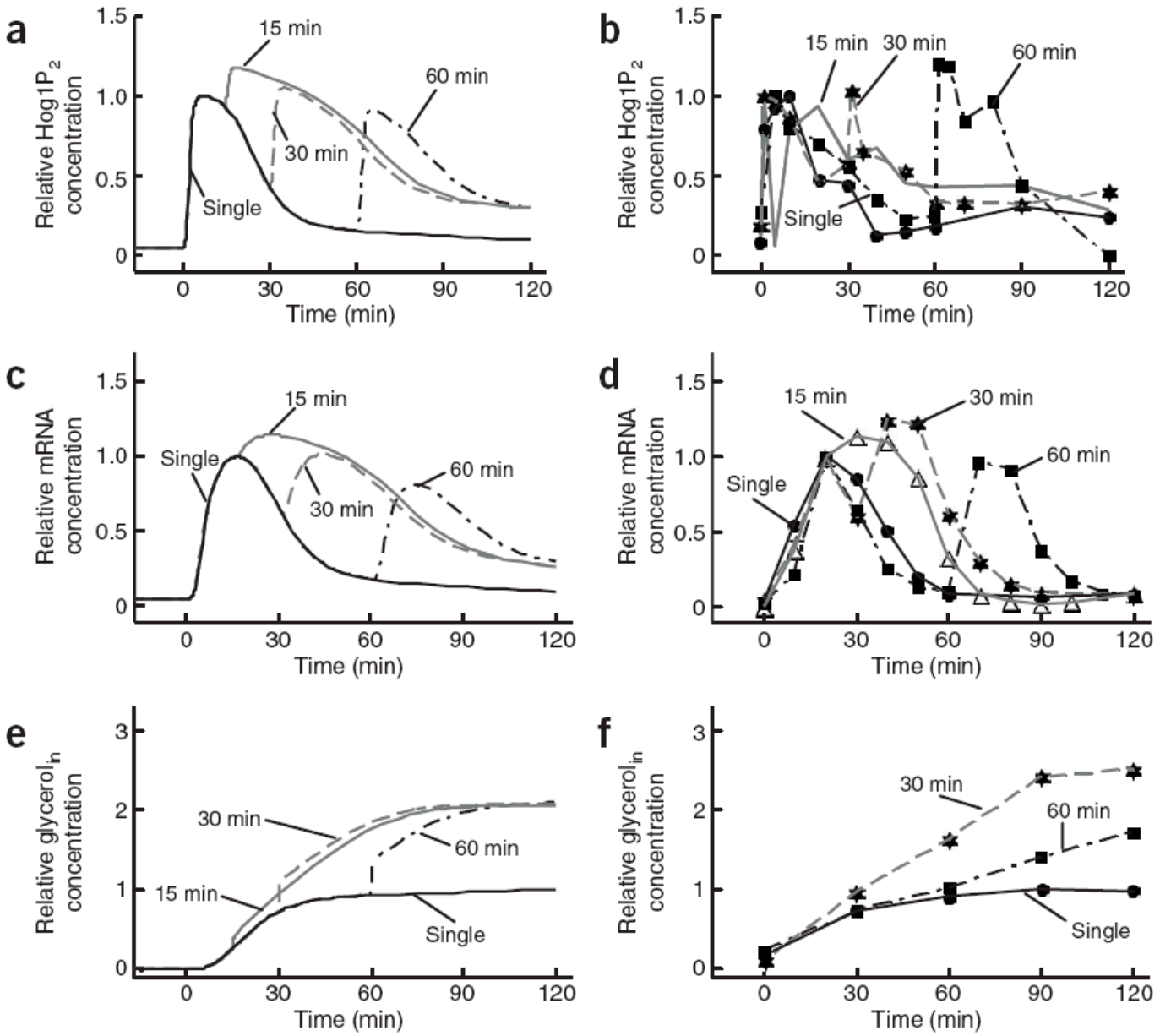
➢The Sho1 branch uses proteins that are also components of the pheromone-response and filamentous MAPK pathways. This branch includes Ste20, Ste50 and the MAPKKK Ste11. Pbs2 has been proposed to act as a scaffold, linking Sho1 to Ste11 activation and possibly limiting cross-talk to other Ste11-dependent MAPK pathways.



Novel osmosensor – Msb2?

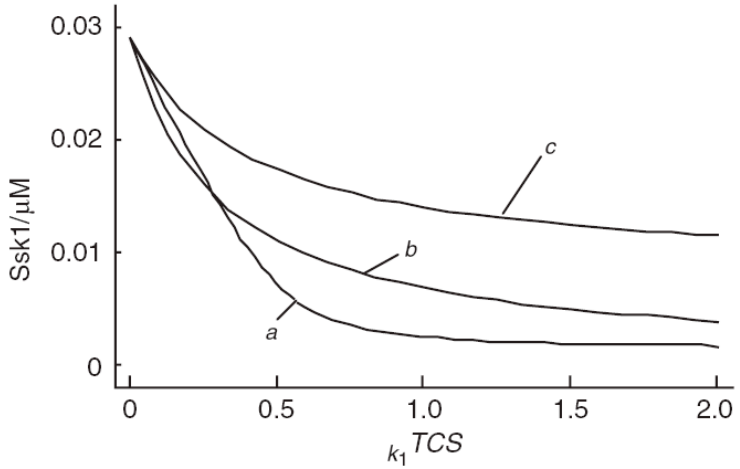


Pathway reactivation

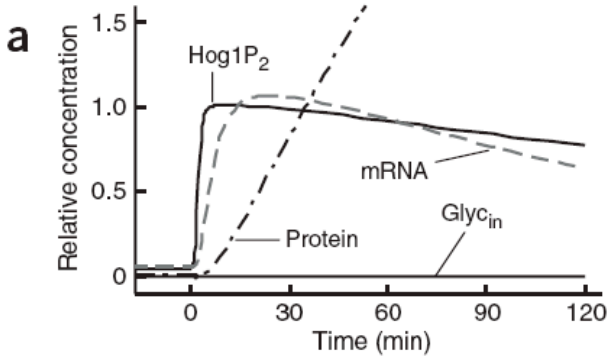


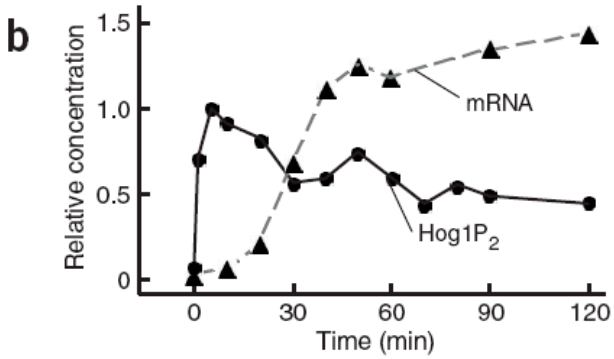
Some novel information from the results of this simulation

Sln1-Ypd1-Ssk1 allows the system to attain a sharper, switch-like behavior such that it remains responsible until a certain threshold.

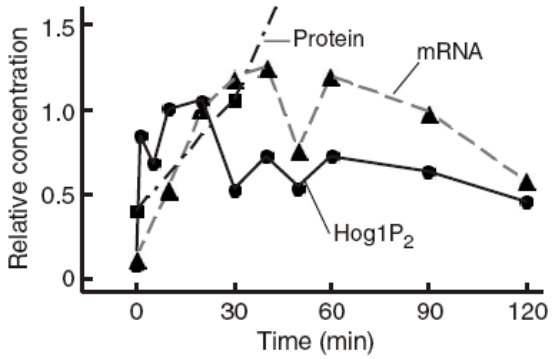


The basal glycerol production level combined with rapid closure of Fps1 is suffient to explain an initial glycerol accumulation after osmotic shock, which may in turn account for HOG down-regulation. The main role of phosphatase is to constantly counteract pathway activation to set thresholds and reduce noise instead of providing a pathway-intrinsic feedback loop.





Mutant that cannot produce glycerol



Unregulated Fps1

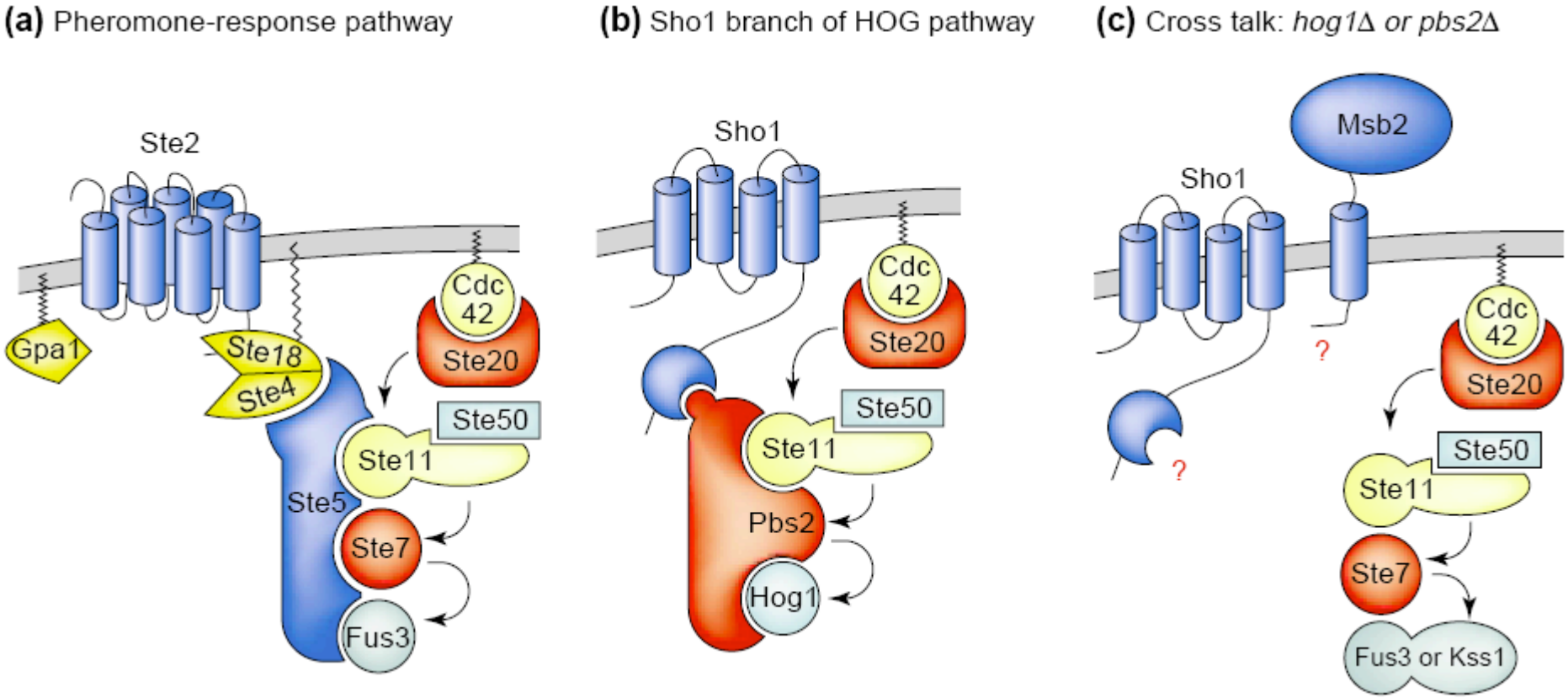
Things that can be further developed:

- Two branches of osmosensing:
  -- Different degrees of osmotic shock

- Cellular compartmentation of signaling molecules

- What results in the transient activity of HOG system (activation/inhibition mechanism)

Cross-talk to other MAPK pathways



•In wild-type cells, there is little cross-activation of the pheromoneresponse or filamentous MAPK pathways by increased osmolarity.

•Cross-talk is evident, however, when *HOG1 or PBS2 is mutated*. The cross-talk circuit that operates in *hog1 and pbs2 mutants was traced from the* transmembrane proteins Sho1 and Msb2 to the

•MAPKs Fus3 and Kss1. In *hog1 and pbs2 mutants, Sho1 and Msb2 stimulate Ste50,* Ste20, Ste11, Ste7, the MAPKs Fus3 and Kss1, and Ste12; this ultimately induces transcriptional targets of the pheromone-response and filamentous pathways.

How to maintain pathway specificity?

- Pbs2 acts as a scaffold protein and thus tethers multiple components of the HOG pathway to limit cross-talk.

- Activation of the HOG pathway triggers the activity of phosphatases, which then feedback to inhibit other MAPK pathways (not yet demonstrated).

- Another mechanism has been proposed involving Hog1 feedback that inhibits the Sho1 branch after osmotic stress.