

Formal Representation of the High Osmolarity Glycerol Pathway in Yeast

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In collaboration with:
Clemens Kuhn, Humboldt University
K. V. S. Prasad, Chalmers University of Technology

Content

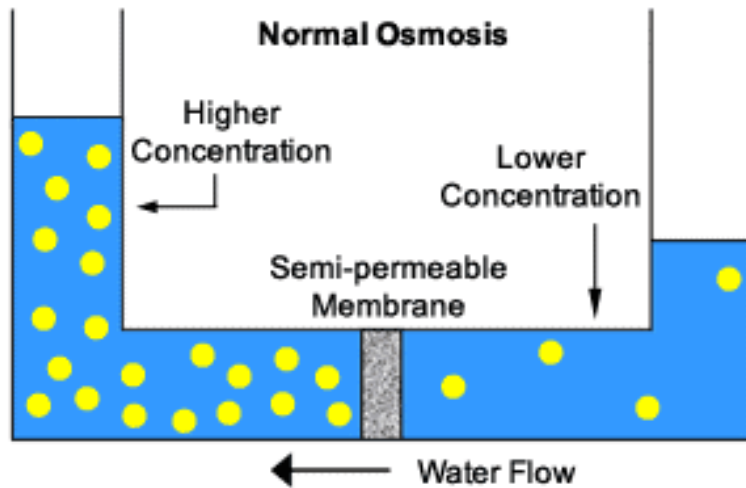
Osmoregulation in yeast

Previous modelling efforts

Rule-based modelling

Discussion

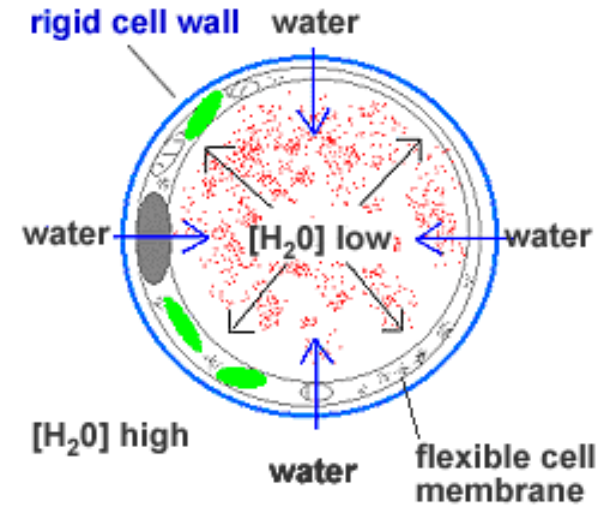
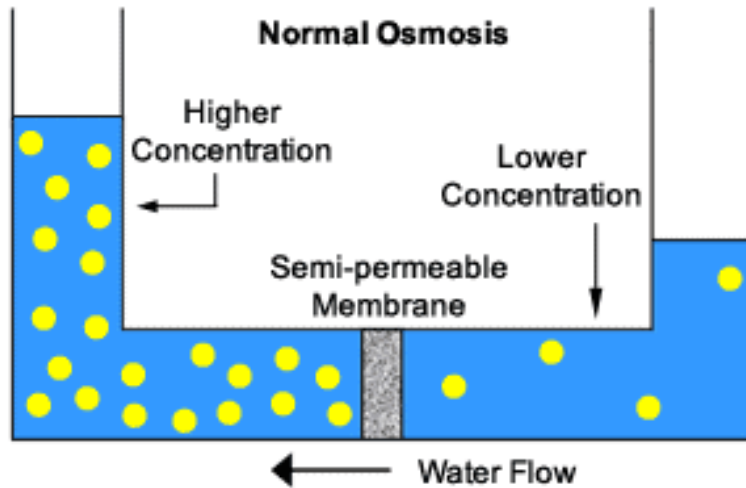
Osmosis



Osmosis: a net flow of water to the region of *lower* chemical potential of water

Flow proportional to the difference in chemical potential of water

Osmosis



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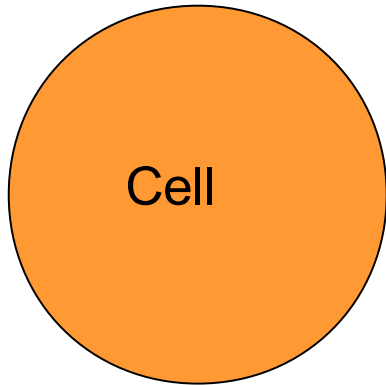
Flow proportional to the difference in chemical potential of water

High osmotic pressure inside the cell

Water tends to flow into the cell

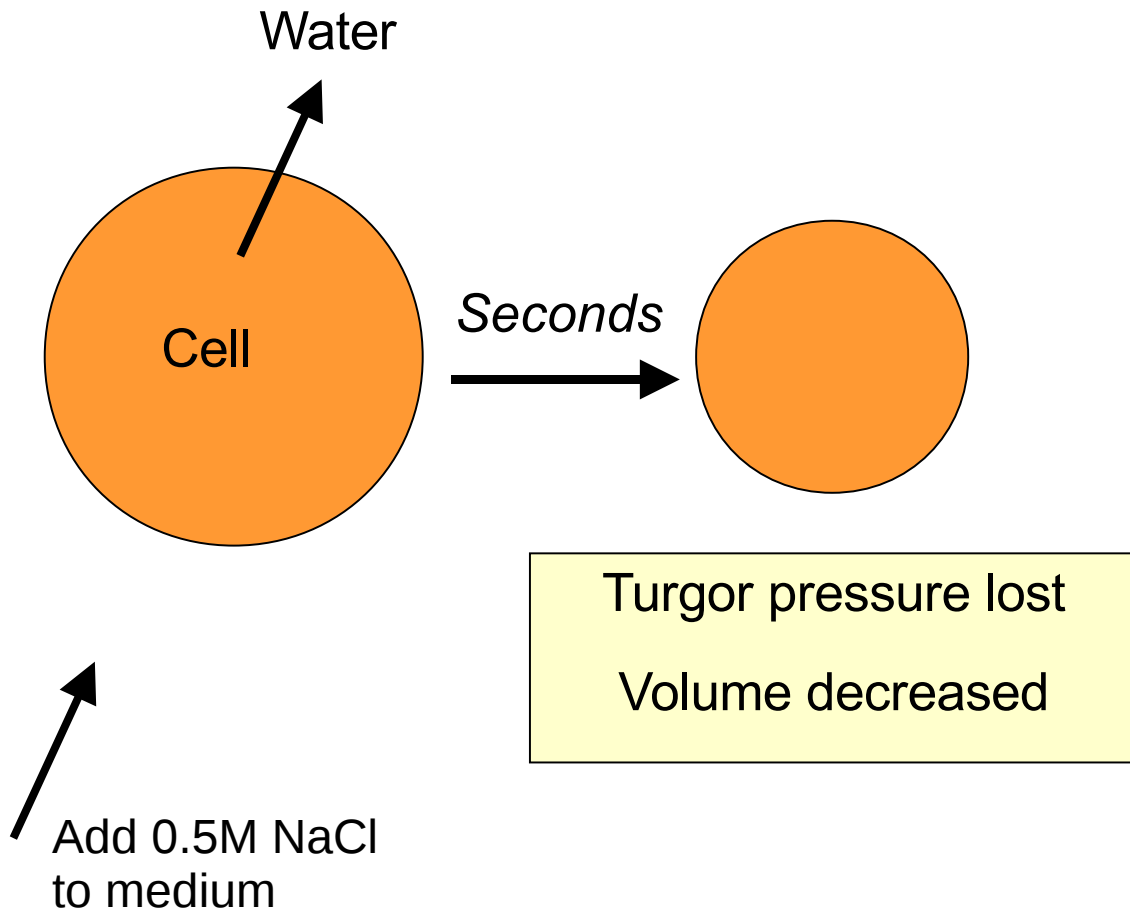
The cell wall counteracts expansion and creates a hydrostatic pressure

Response to osmotic shock in yeast

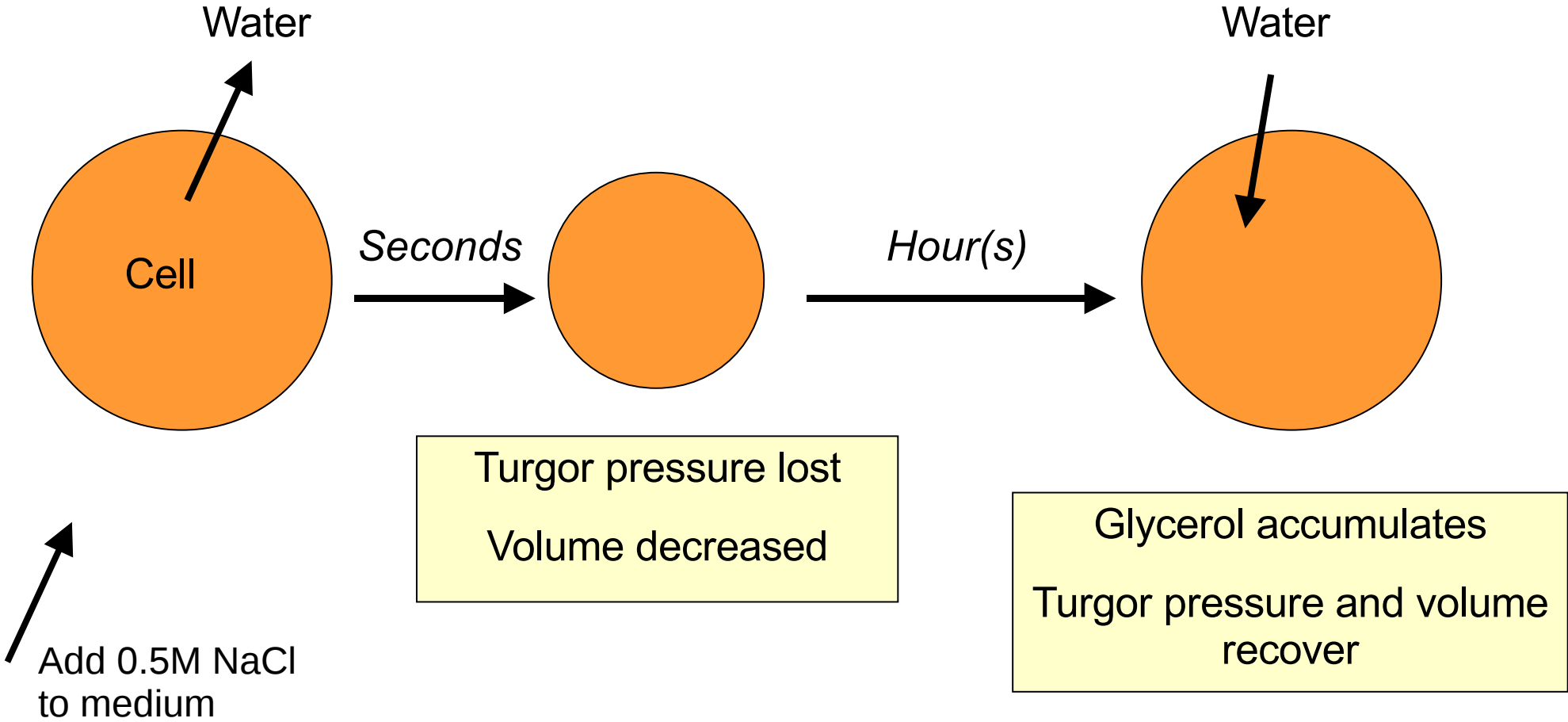


↑
Add 0.5M NaCl
to medium

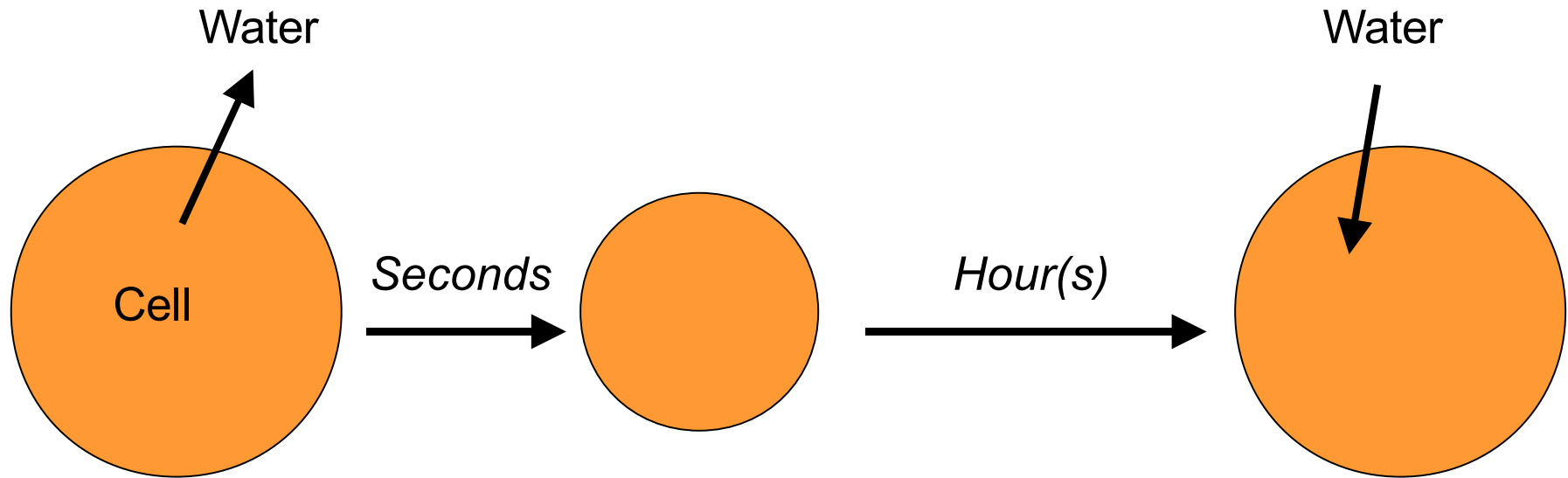
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Response to osmotic shock in yeast



Interesting modelling system

The system is relatively well-characterized, key components identified

Non-trivial complexity, with feedback control on different levels (difficult to understand from a drawing)

Content

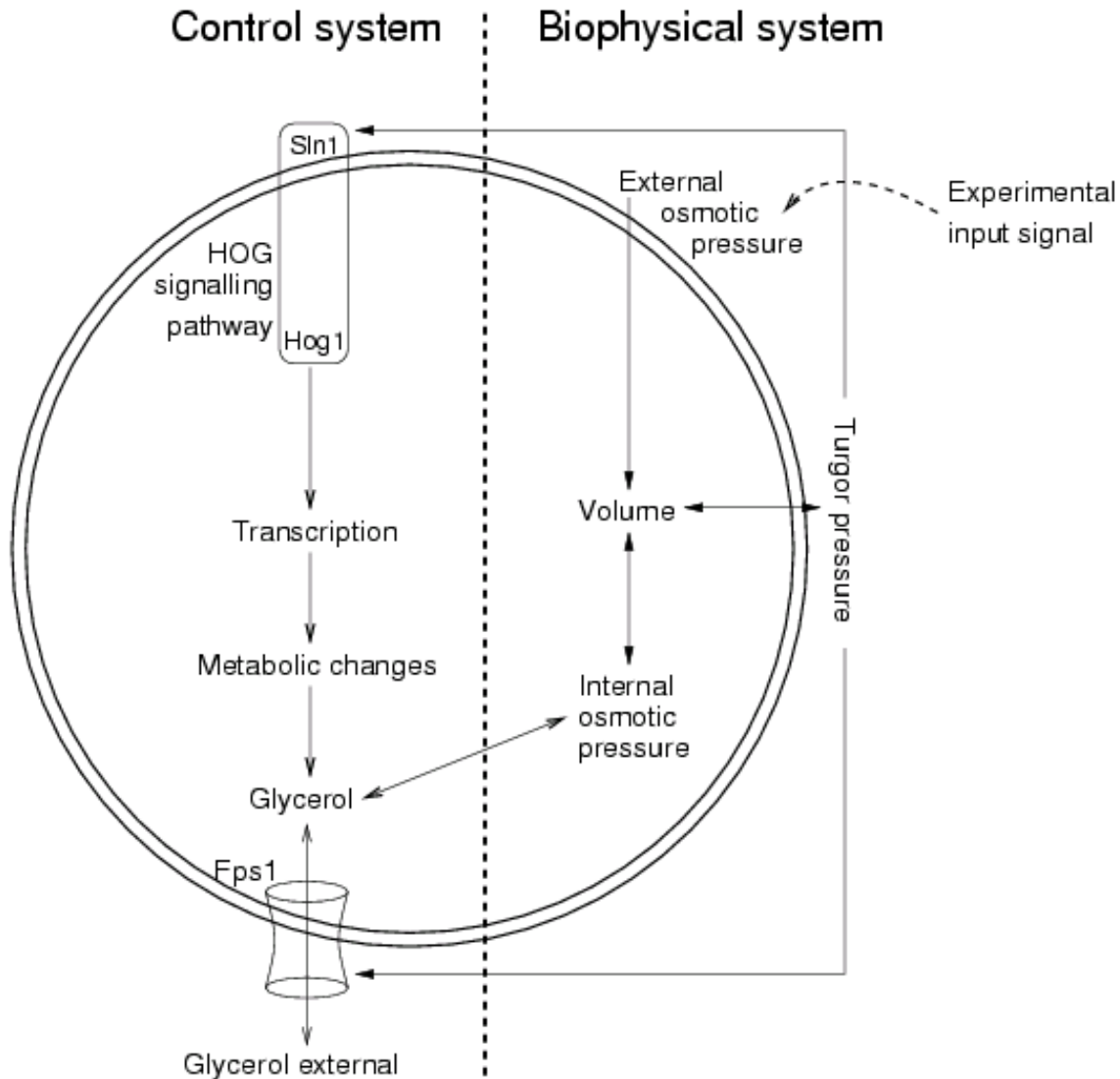
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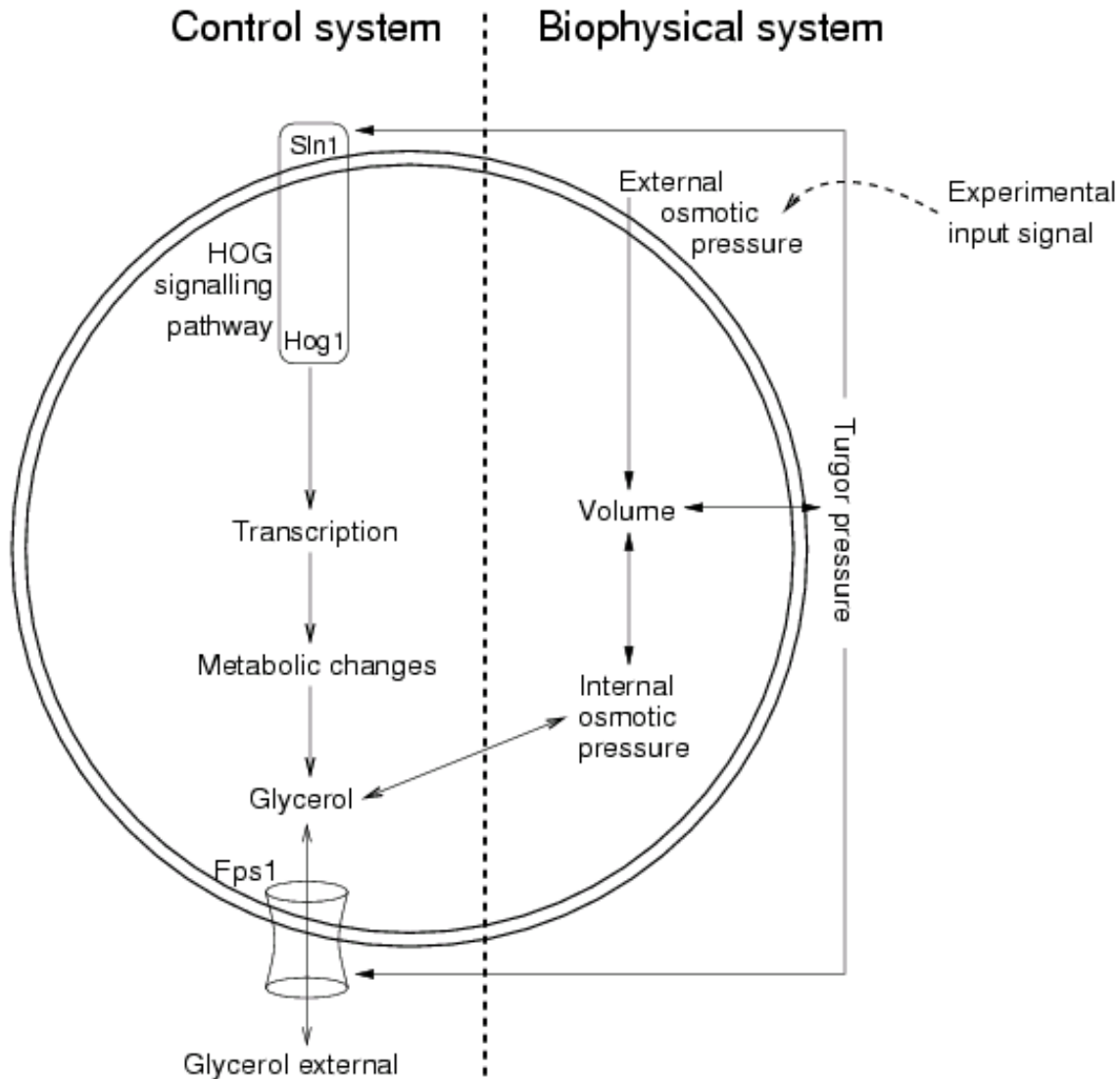
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A simple model of yeast osmoregulation



A simple model of yeast osmoregulation



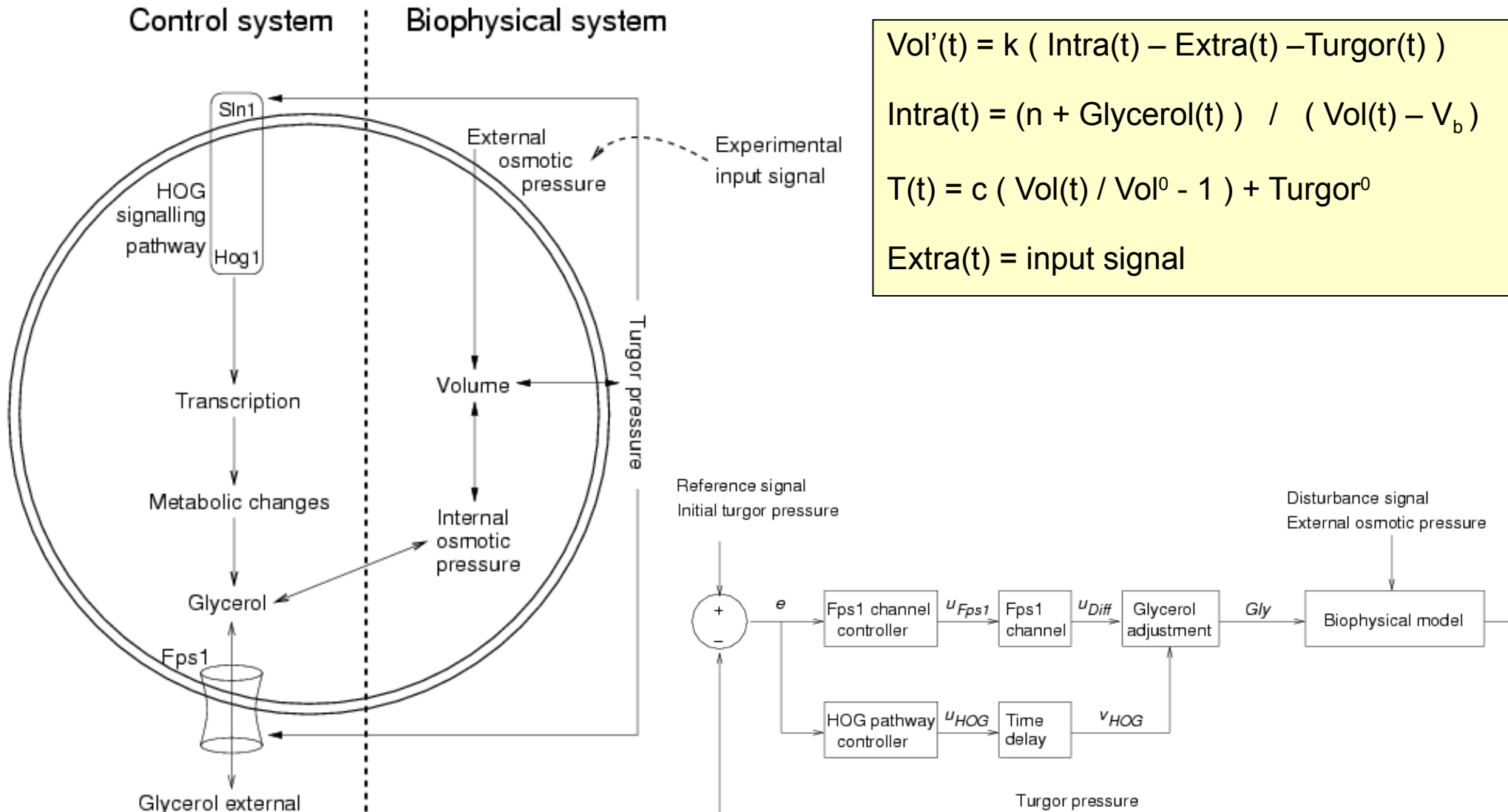
$$\text{Vol}'(t) = k (\text{Intra}(t) - \text{Extra}(t) - \text{Turgor}(t))$$

$$\text{Intra}(t) = (n + \text{Glycerol}(t)) / (\text{Vol}(t) - V_b)$$

$$\text{T}(t) = c (\text{Vol}(t) / \text{Vol}^0 - 1) + \text{Turgor}^0$$

$$\text{Extra}(t) = \text{input signal}$$

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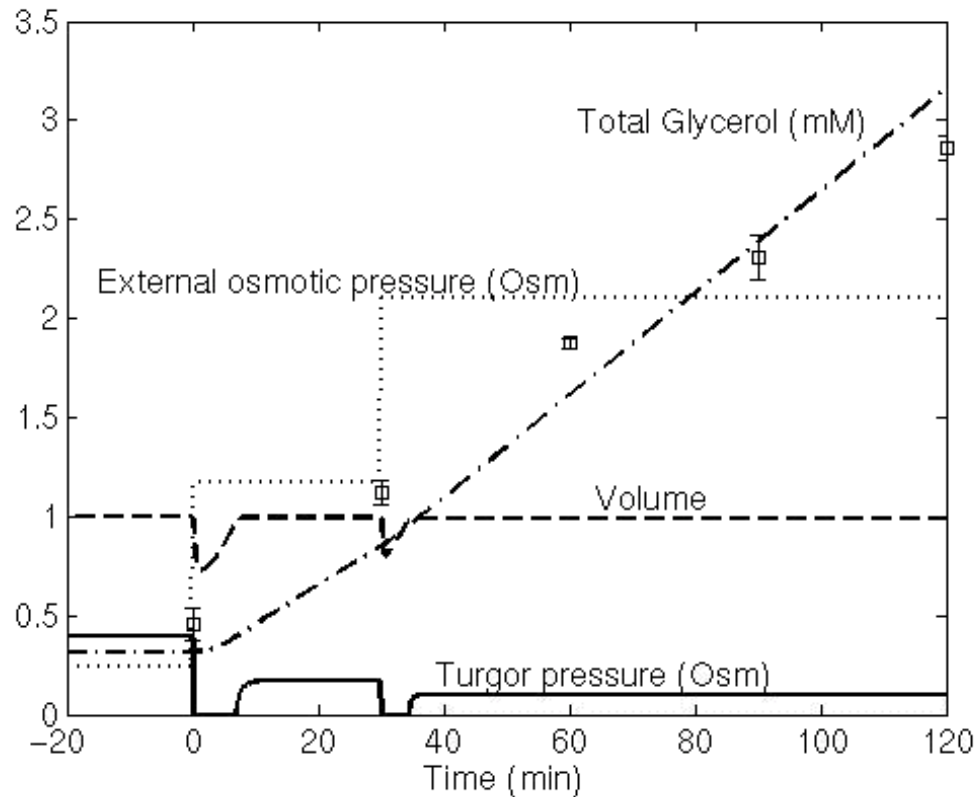
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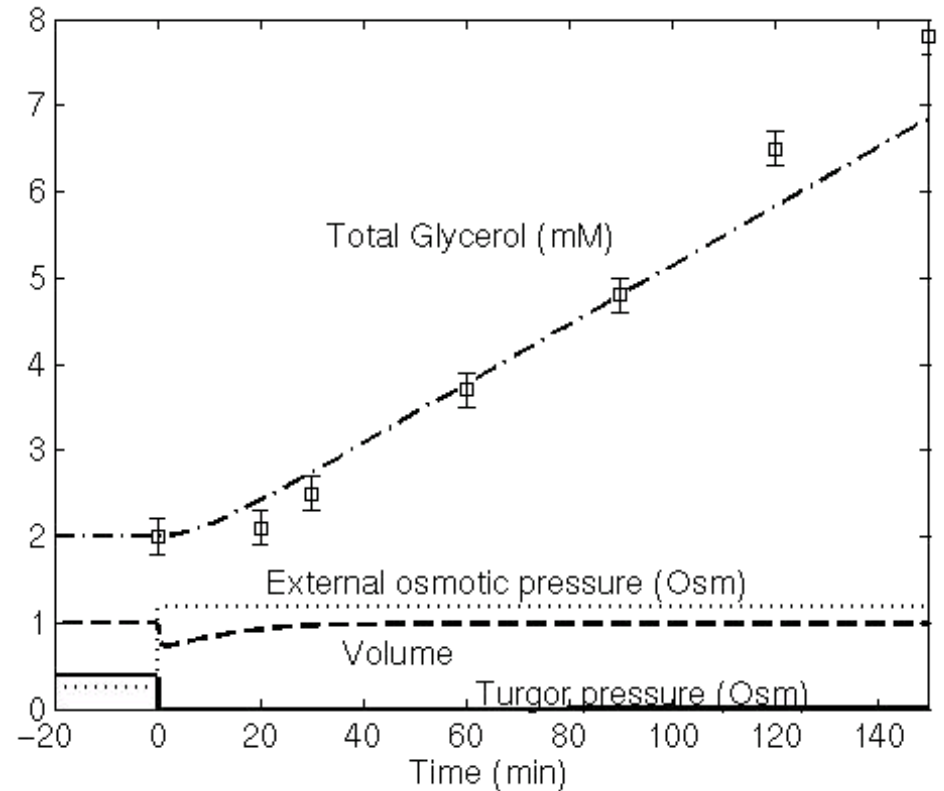
$$Extra(t) = \text{input signal}$$

Simulation, NaCl stress

wild-type



open Fps1



Simple vs. complex models

Simple models:

Contributes to a better understanding of the phenomenon

Easy to understand

Less knowledge required to build the model

Easy to validate, simulate and modify

Better predictions(?)

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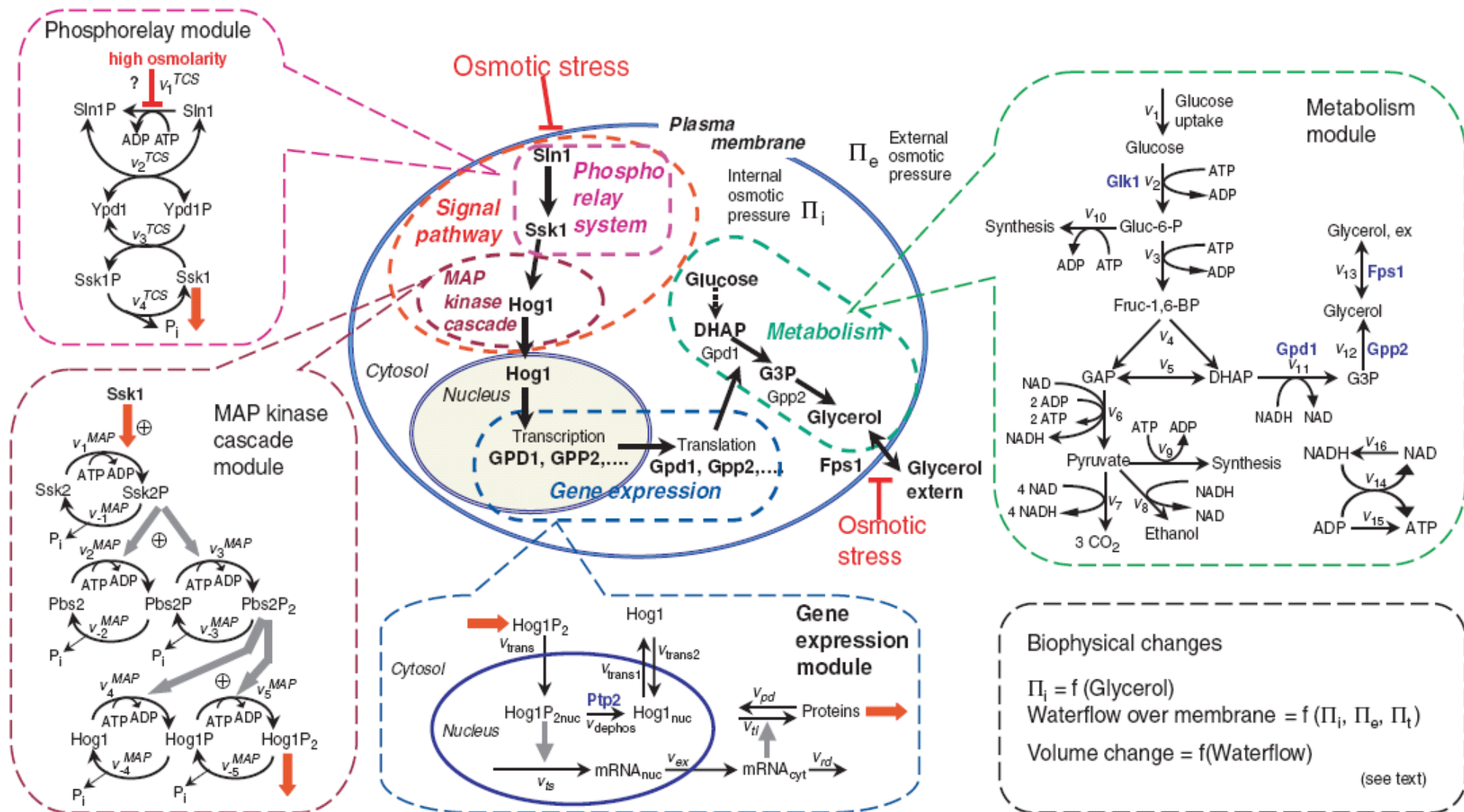
Complex models:

Individual reactions are modelled, no black-box relationships

Easy to communicate details of the model/system

Easy to simulate different mutants

A detailed model (35 ODEs)



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The next generation of osmoregulation models

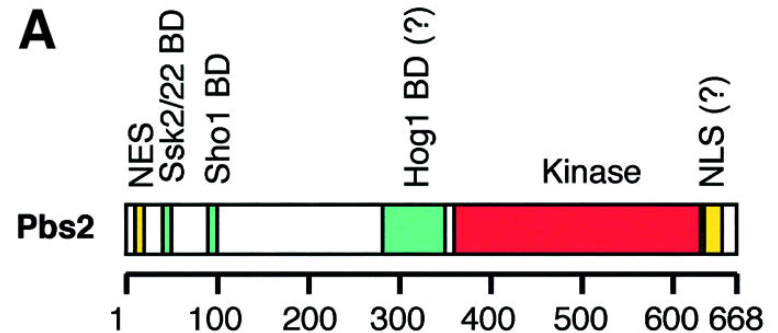
Accumulated evidence for
molecular details
of the HOG pathway

Scaffold proteins are common

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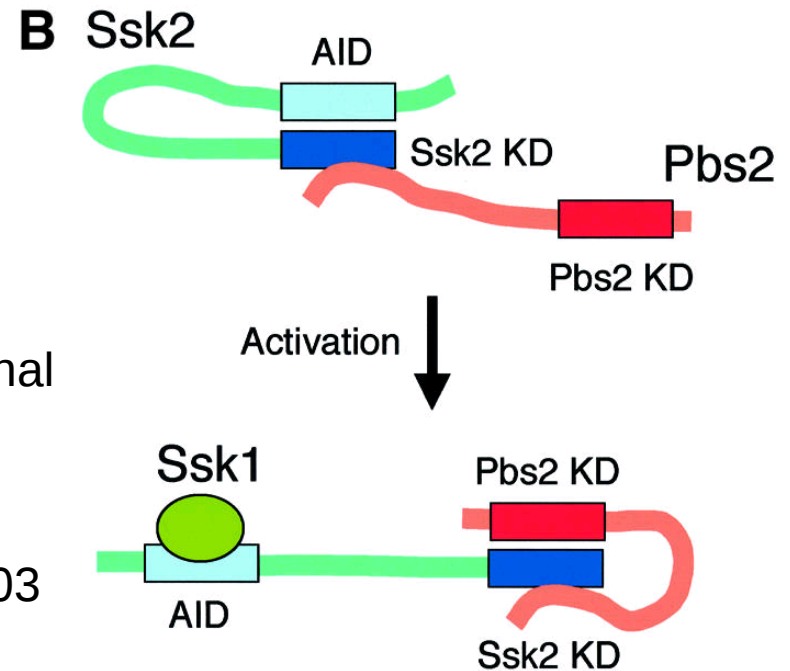
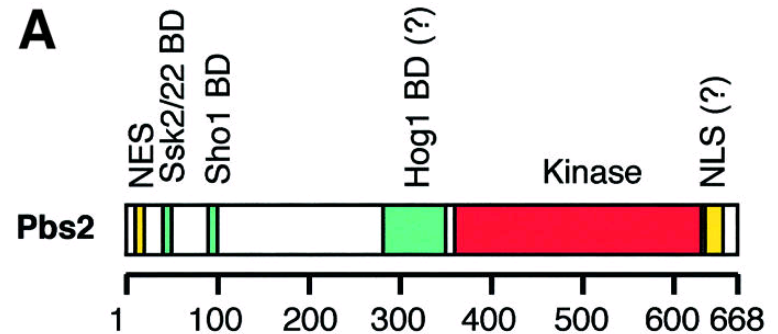
NES, nuclear export signal
BD, binding domain
NLS, nuclear localization signal

Tatebayashi et al. EMBO 2003

The next generation of osmoregulation models

Accumulated evidence for molecular details of the HOG pathway

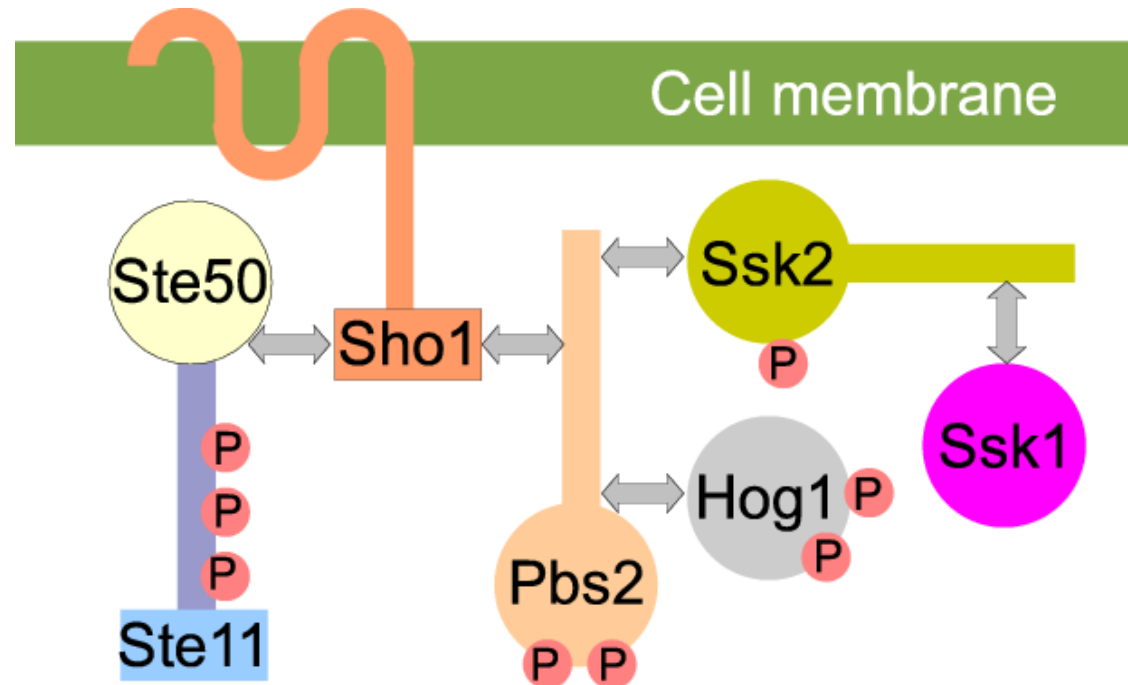
Scaffold proteins are common



NES, nuclear export signal
 BD, binding domain
 NLS, nuclear localization signal
 AID, autoinhibitory domain
 KD, kinase domain

Tatebayashi et al. EMBO 2003

Combinatorial complexity - ODE representation breaks down



8 states for Ste11-Ste50

4 states for Pbs2 and for Hog1

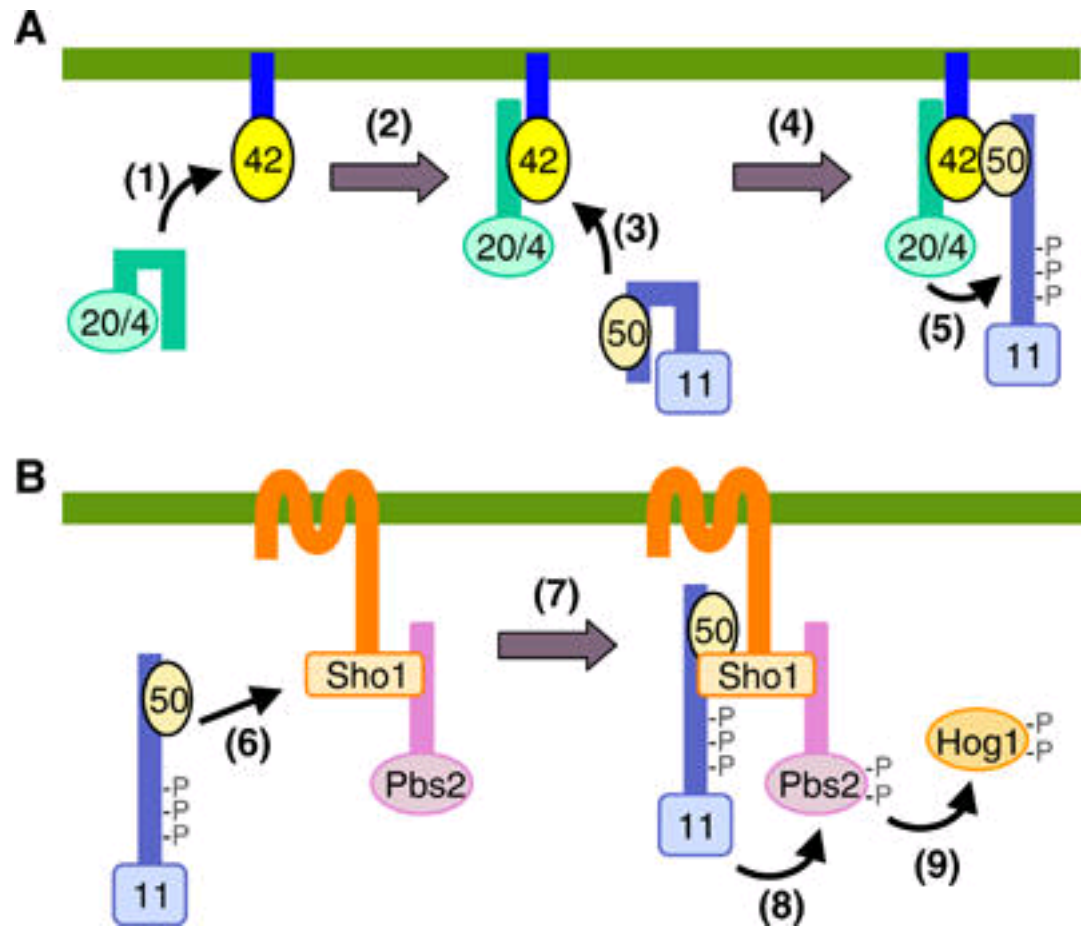
2 states for Ssk2/22

Sho1 can be in an inactive or active state

Some proteins may be absent

→ **1900 states**

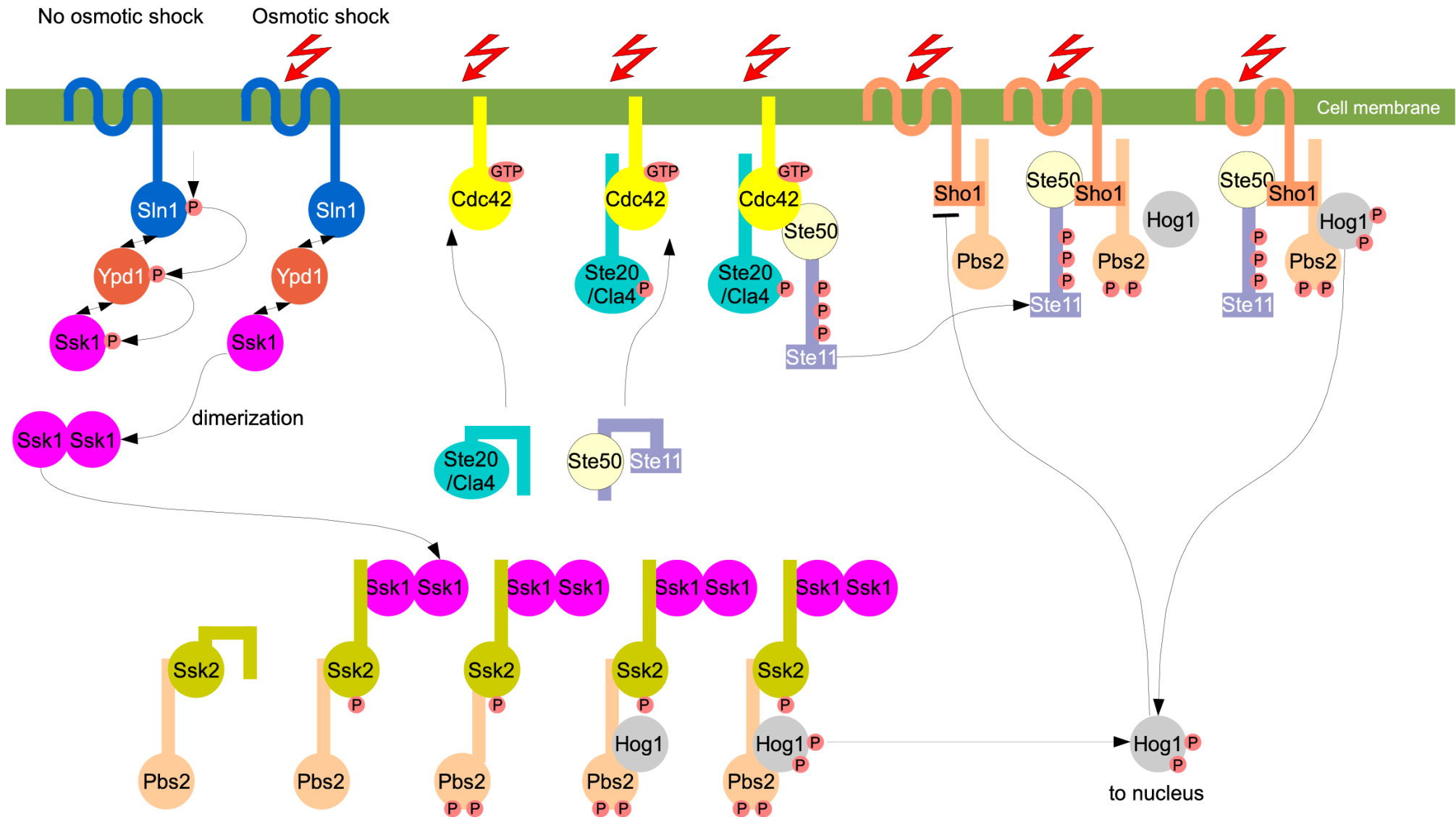
Reactions are dependent on state of the scaffolds



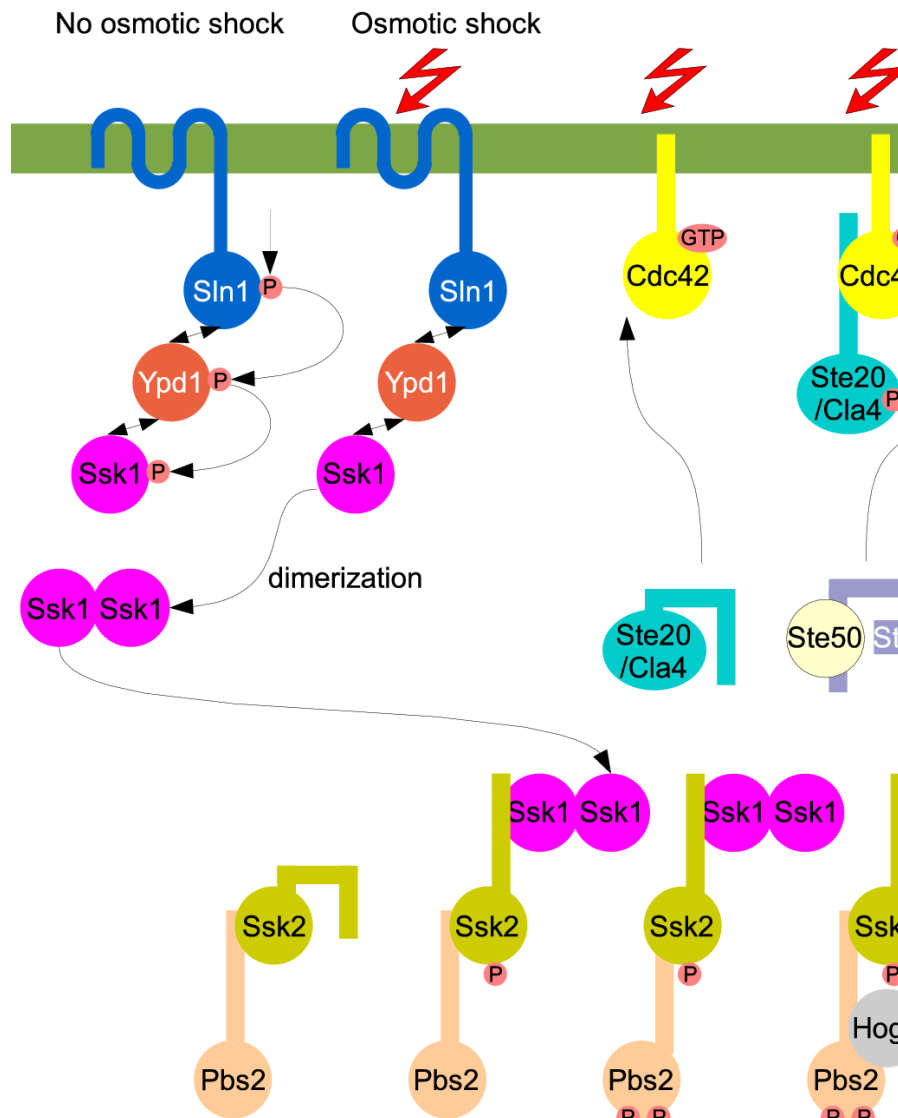
Activation of Ste11 by Ste20 is mediated by indirect docking via Ste50 and Cdc42.

Activation of Pbs2 by Ste11 is mediated by indirect docking via Ste50 and Sho1.

Our own traditional view of the pathway



Our own traditional view of the pathway



Reaction	Modifier(s)	Comment and references
$Cdc42 \rightarrow Cdc42\text{-act}$	Osmostress	Act.[47]
$Cdc42\text{-act} \rightarrow Cdc42$		Deact.
$Cdc42 + Ste20 \rightarrow Cdc42\text{-Ste20}$		Ass. Requires Cdc42-act[47]
$Cdc42\text{-Ste20} \rightarrow Cdc42 + Ste20$		Diss.
$Ste20^U \rightarrow Ste20^P$	Cdc42-act	Phos.[47]
$Ste20^P \rightarrow Ste20^U$	Phosphatases	Dephos.
$Cdc42 + Ste11 \rightarrow Cdc42\text{-Ste11}$		Ass. Requires active Cdc42[47]
$Cdc42\text{-Ste11} \rightarrow Cdc42 + Ste11$		Diss. ¹
$Ste11^U \rightarrow Ste11^P$	$Ste20^P$	Phos. Requires Ste11-Cdc42-Ste20 ^P [47]
$Ste11^P \rightarrow Ste11^U$	Phosphatases	Dephos.
$Sho1 \rightarrow Sho1\text{-act}$	Osmostress	Act.[47]
$Sho1\text{-act} \rightarrow Sho1$		Deact.
$Sho1 + Ste11 \rightarrow Sho1\text{-Ste11}$		Ass. Requires Sho-act[38, 47]
$Sho1\text{-Ste11} \rightarrow Sho1 + Ste11$		Diss.
$Sln1 \rightarrow Sln1^P$	Osmostress	Phos. Inhibited by Osmostress[28]
$Sln1 + Ypd1 \rightarrow Sln1\text{-Ypd1}$		Ass. Requires Sln1 ^U , Ypd1 ^P or Sln1 ^P , Ypd1 ^U ² [39]
$Sln1\text{-Ypd1} \rightarrow Sln1 + Ypd1$		Diss.
$Sln1^P\text{-Ypd1}^U \leftrightarrow Sln1^U\text{-Ypd1}^P$		Phosphotransfer ³
$Ypd1 + Ssk1 \rightarrow Ypd1\text{-Ssk1}$		Ass. Requires Ypd1 ^P , Ssk1 ^U or Ypd1 ^U , Ssk1 ^P ² [39]
$Ypd1\text{-Ssk1} \rightarrow Ypd1 + Ssk1$		Diss.
$Ypd1^P\text{-Ssk1}^U \leftrightarrow Ypd1^U\text{-Ssk1}^P$		Phosphotransfer ³
$Ssk1^P \rightarrow Ssk1^U$	Phosphatases	Depho. ⁴
$Ssk1 + Ssk1 \leftrightarrow Ssk1\text{-Ssk1}$		Dimerization[20]
$Ssk1 + Ssk2 \leftrightarrow Ssk1\text{-Ssk2}$		Ass., Diss. ⁵
$Ssk1^U\text{-Ssk1}^U\text{-Ssk2}^U \rightarrow Ssk1^U\text{-Ssk1}^U\text{-Ssk2}^P$		Phos.
$Ssk2^P \rightarrow Ssk2^U$		Dephos. Requires unbound Ssk2
$Ssk2 + Pbs2 \leftrightarrow Ssk2\text{-Pbs2}$		Ass., Diss.[46]
$Sho1 + Pbs2 \rightarrow Sho1\text{-Pbs2}$		Ass. Requires Sho-act[38, 47]
$Sho1\text{-Pbs2} \rightarrow Sho1 + Pbs2$		Diss.
$Pbs2^{UU} \rightarrow Pbs2^{PP}$	$Ste11^P$	Phos. Requires Ste11 ^P -Sho1-Pbs2[27, 47]
$Pbs2^{PP} \rightarrow Pbs2^{UU}$	$Ssk2^P$	Requires Ssk2 ^P -Pbs2[46]
$Pbs2^{PP} + Hog1 \rightarrow Pbs2^{PP}\text{-Hog1}$	Phosphatases	Dephos.
$Pbs2^{PP}\text{-Hog1} \rightarrow Pbs2^{PP} + Hog1$	$Ssk2^P$	Ass. Requires Ssk2 ^P -Pbs2 ^{PP} [46]
$Pbs2\text{-Hog1} \rightarrow Pbs2 + Hog1$	$Ste11^P$	Requires Ste11 ^P -Sho1-Pbs2 ^{PP} [38]
$Pbs2^{PP}\text{-Hog1}^{UU} \rightarrow Pbs2^{PP}\text{-Hog1}^{PP}$		Diss.
$Pbs2^{PP}\text{-Hog1}^{PP} \rightarrow Pbs2^{PP} + Hog1^{UU}$	$Ssk2^P$	Pho. Requires Ssk2 ^P -Pbs2 ^{PP} [34, 46]
$Hog1^{PP} \rightarrow Hog1^{UU}$	$Ste11^P$	requires Ste11 ^P -Sho1-Pbs2 ^{PP} [38]
	Phosphatases	Dephos. ⁶ [34]

Example of Kappa code

Sho1 has a state called x that is activated (a) and a docking site for Ste11

```
Sho1(x~a,Ste11)
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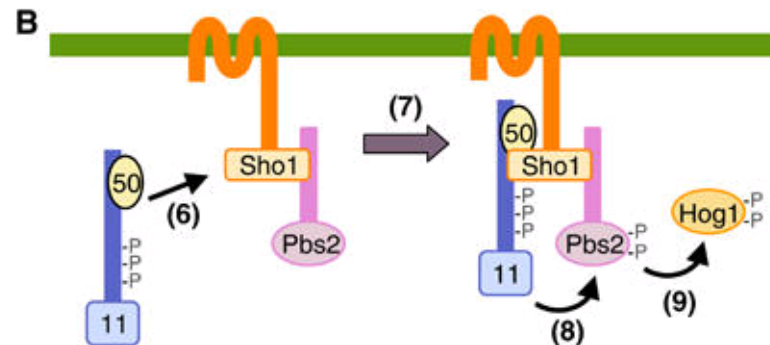
Ste11 is phosphorylated (p) and has docking sites for both Sho1 and Cdc42

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Ste11(x~p,Sho1,Cdc42)
```

Association of active Sho1 to Ste11 requires that Ste11 is not bound to Cdc42. The phosphorylation state of Ste11 is arbitrary

```
Sho1(x~a,Ste11),Ste11(Sho1,Cdc42) ->  
Sho1(x~a,Ste11!1),Ste11(Sho1!1,Cdc42) @ 2.0
```

Example of Kappa code



The phosphorylation of Pbs2 by Ste11 is assumed to require that both Ste11 and Pbs2 are bound to Sho1, and furthermore, that Ste11 is phosphorylated.

$Ste11(x\sim p, Sho1!1), Sho1(Ste11!1, Pbs2!2), Pbs2(x\sim u, y\sim u, Sho1!2) \rightarrow$

$Ste11(x\sim p, Sho1!1), Sho1(Ste11!1, Pbs2!2), Pbs2(x\sim p, y\sim p, Sho1!2) @ 1.0$

Model available at www.cellucodate.com

The screenshot displays the Cellucodate website interface. At the top, there is a navigation bar with links for Home, Bookshelf, Discussions, Contacts (0), and Profile (Peter Gennemark, logout). The user is identified as Clemens Kuehn, and the current model is HogPW_2009_03_31. A search bar is located on the right.

The main content area is titled "Ste11 pho". It features four panels:

- Diagram & Kappa**: Shows a reaction scheme where Cdc42 binds to Ste11 and Ste20. The forward rate is 1.0 s⁻¹. The Kappa string is: $\text{Cdc42}(\text{ste11}|2, \text{ste20}|1), \text{Ste20}(\text{x}\sim\text{p}, \text{cdc42}|1), \text{Ste11}(\text{x}\sim\text{u}, \text{cdc42}|2) \rightarrow \text{Cdc42}(\text{ste11}|2, \text{ste20}|1), \text{Ste20}(\text{x}\sim\text{p}, \text{cdc42}|1), \text{Ste11}(\text{x}\sim\text{p}, \text{cdc42}|2)$.
- Contact Map**: A network diagram showing interactions between Sho1, Ste11, Ste20, and Cdc42. Sho1 is connected to Ste11 and Ste20, which are both connected to Cdc42. A 'stress' node is also connected to Cdc42.
- Annotations (0)**: A panel for model annotations, currently empty.
- Influence Map**: A diagram showing the influence of various parameters on the 'Ste11 pho' node. Parameters include 'Hog1 *pp by Ras2,Ste11,Sho1', 'Sho1 Ste11 association', 'Cdc42 Ste11 association', 'Ste11 Ras2 phos', and 'Ste11 depho'.

A left sidebar contains a "Jump to Page" field and a list of model components, with "Ste11 pho" selected.

Our rule-based model

Represents current knowledge compactly and without the ambiguity of traditional pathway diagrams

Takes complexes into account without enumerating all states

Captures phosphorylation reactions known to be strictly dependent on the set of species bound to a certain complex

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The model includes dynamic information, allowing simulation

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Desired properties of tools

Integration with other cellular processes like gene regulation and metabolism, and with the environment

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Compartments like the cytosol, nucleus and vacuole have individual properties and species concentrations

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Compartments like the cytosol, nucleus and vacuole have individual properties and species concentrations

Biological processes at the molecular level are stochastic, and deterministic simulation is not always an adequate approximation. Clearly, hybrid simulation methods are of interest when combining signalling pathways with metabolic systems in one model

Some tools

Cellucidate, cellucidate.com

Rule-based, stochastic simulation

No full enumeration of ODEs

No compartments, algebraic equations

Strong GUI, intuitive language

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PottersWheel, potterswheel.de

ODEs and deterministic simulation
A range of analysis tools
Full set of ODEs is automatically generated
Code is less compact, requires additional manual work
Algebraic equations, compartments
No GUI for rule-based modelling

Acknowledgements

Clemens Kuhn, Jörg Schaber, Edda Klipp (Humboldt University)

K. V. S. Prasad (Chalmers)

Vincent Danos (Edinburgh)

Benchmarks for ODE identification

www.odeidentification.org

[Home](#) [Introduction](#) [The benchmark problems](#) [Best solutions](#) [Run online](#) [Documentation](#)

The benchmark problems

The problems are grouped after the original system that they are based on, an overview is shown in the following table with links to more detailed descriptions and problem files:

Systems based on chemical rate equations (simulated data)

Source system/model	Problem	#var	#exp	#pts	Noise	Best error
simpleLin	simpleLin1	3	3	13	0%	8
	simpleLin2		8	13	10%	140.2
simpleFb	simpleFb1	3	4	7	0%	7
	simpleFb2		4	7	5%	42.14
	simpleFb3		1	7	0%	7
	simpleFb4		1	7	~5% ¹	7.071
osc	osc1	3	1	41	0%	6.149
	osc2		1	41	3%	87.34
metabol	metabol1	5	12	7	0%	30
	metabol2		12	21	10%	751.6