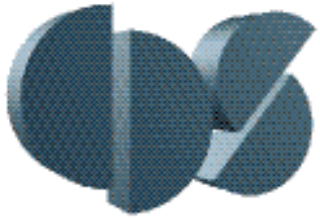

# Lecture 3: Examples



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**Goals:**

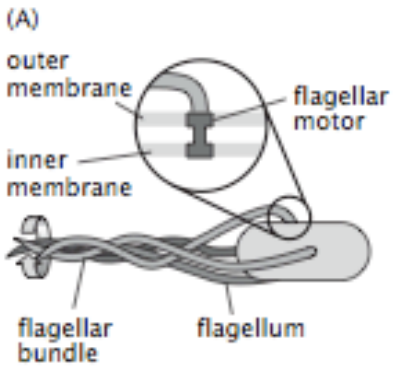
- Describe some principles of biological circuits via specific examples
- Provide enough detail to be able to read through reference articles
- Describe modeling techniques, tools and challenges

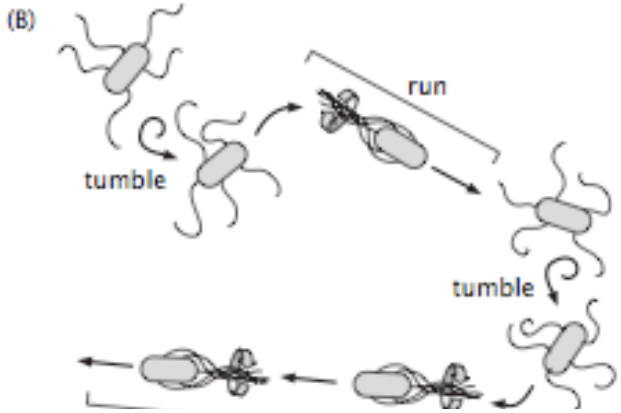
**Examples to be covered**

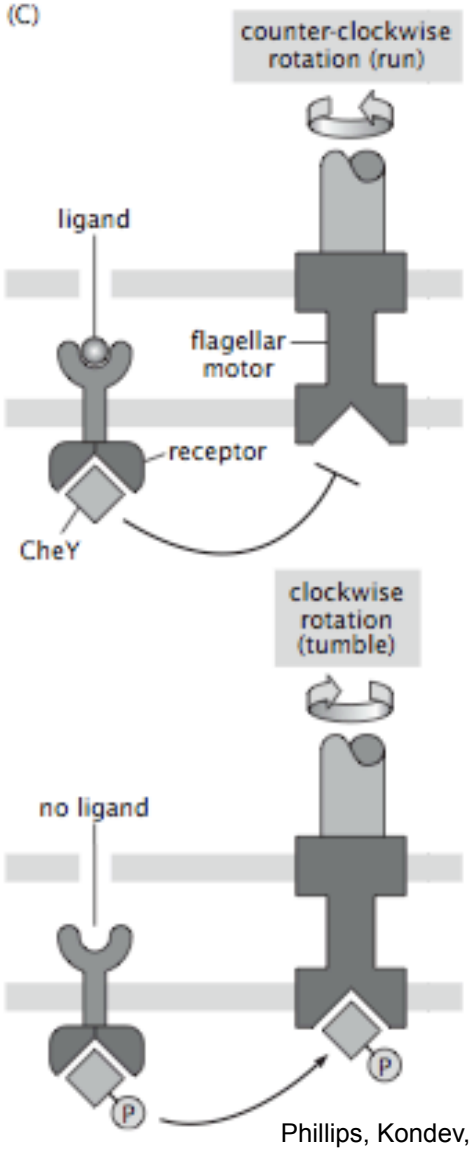
- *Chemotaxis*: what are the sensing, actuation and feedback mechanisms that control movement of bacteria in the presence of nutrient gradients
- *Heat shock*: how does the cell protect itself against environmental disturbances
- *Yeast mating response*: how do yeast respond to pheromones and mate?

2009 Asian Control Conference (ASCC), 26 August 2009

# Example 1: Chemotaxis




Phillips, Kondev, Theriot (2008)

**Basic mechanism**

- When ligand (nutrient) is present, CheY protein is inactive and motor turns counter clockwise (run)
- When ligand is not present, CheY activated, binds to motor protein to give clockwise motion (tumble)
- Result: move toward nutrients (on average)
- Circuitry adapts to baseline stimulation level

**References**


- Barkai and Leibler, *Nature*, 1997
- Rao, Kirby and Arkin, *PLoS Biology*, 2004


http://www.genomics.princeton.edu/ryulab

# Control Systems operation

**Actuation**

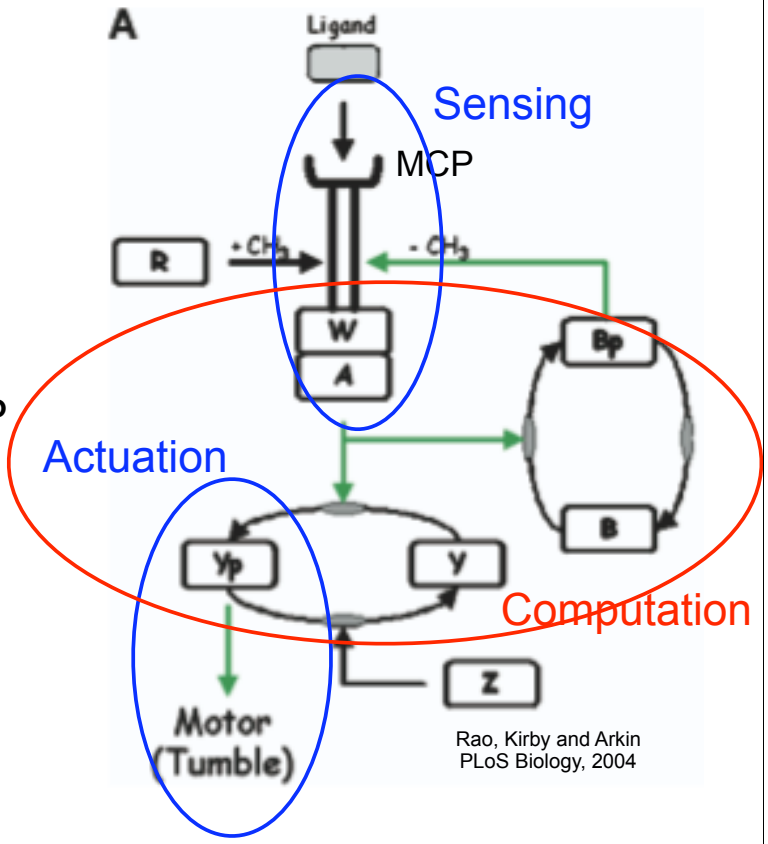
- Phosphorylated CheY (Yp) binds to motor (M) and increases the likelihood of tumbles (vs runs)

**Sensing**

- Ligand (L) binds to receptor complex (MCP:W:A)
  - MCP is membrane bound receptor
  - ChW (W) and CheA (A) form complex w/ MCP

**Computation**

- CheA phosphorylates (Ap) and transfers phosphate group to CheY ("kinase")
  - CheA activity depends on methylation of receptor complex: more methylation => more activity
- CheR (R) methylates receptor complex
- CheBp (Bp) demethylates receptor complex
  - Amount of CheBp is affected by amount of active CheA (Ap) - negative feedback loop
- Additional effects: CheZ, motor binding (M:Yp), ...



# Dynamics: Forward Information Processing

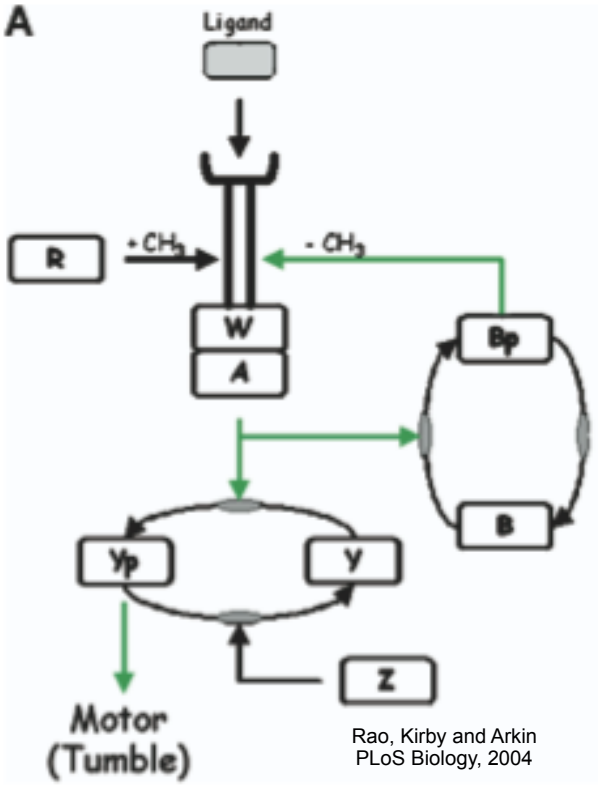
**Motor dynamics controlled by receptor complex *T***

- Complex can be active or inactive, depending on both methylation and whether ligand is bound

$$T^A = \sum_{i=0}^{4} \alpha_i(L) T_i, \qquad T^I = \sum_{i=0}^{4} (1 - \alpha_i(L)) T_i$$

$$\alpha_i(L) = \frac{a_i L}{K_L + L} + \frac{a_i^0 K_L}{K_L + L}$$

- $T_i$ = concentration of receptor complex with $i$ methylation sites occupied ($i \in \{0, 1, 2, 3, 4\}$)
- $\alpha_i(L)$ = probability that receptor complex with $i$ methylation sites occupied is active; $L$ = ligand concentration



Rao, Kirby and Arkin
PLoS Biology, 2004

**Receptor complex drives CheY and CheB via Ap**

- Use standard mass action to keep track of species:

$$\frac{dA_p}{dt} = 50 T^A A - 100 A_p Y - 30 A_p B,$$

$$\frac{d[MY_p]}{dt} = 5 MY_p - 19 [MY_p],$$

[MYp] = M:Yp

$$\frac{dY_p}{dt} = 100 A_p Y - 0.1 Y_p - 5 MY_p + 19 [MY_p] - 30 Y_p,$$

$$\frac{dB_p}{dt} = 30 A_p B - B_p.$$

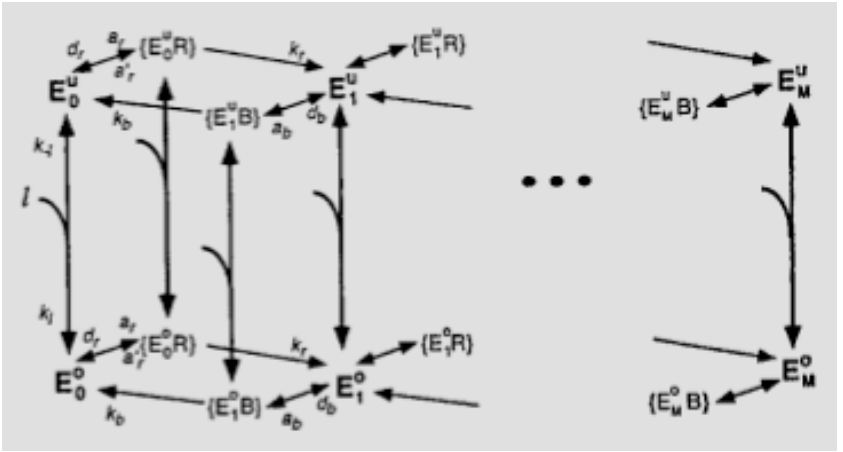
# Adaptation via Methylation

**Keep track of occupied receptors w/ different numbers of methylated sites**

- $E_i^u$ = i methylated sites, no ligand
- $E_i^o$ = i methylated sites, w/ ligand
- Transitions follow standard mass action kinetics
- Notational switch: $T_i = E_i^u + E_i^o$
- Use $\alpha_i(L)$ to capture aggregate effect of ligand binding

$$\alpha_i(L) = \frac{a_i L}{K_L + L} + \frac{a_i^0 K_L}{K_L + L}$$

- Additionally assume Michaelis-Menten kinetics for CheR, CheB actions

$$\frac{dT_0}{dt} = -r_R(1-\alpha_0(L))T_0 + r_B\alpha_1(L)T_1,$$

$$\frac{dT_1}{dt} = -r_R(1-\alpha_1(L))T_1 + r_B\alpha_2(L)T_2 + r_R(1-\alpha_0(L))T_0 - r_B\alpha_1(L)T_1,$$

$$\frac{dT_2}{dt} = -r_R(1-\alpha_2(L))T_2 + r_B\alpha_3(L)T_3 + r_R(1-\alpha_1(L))T_1 - r_B\alpha_2(L)T_2,$$

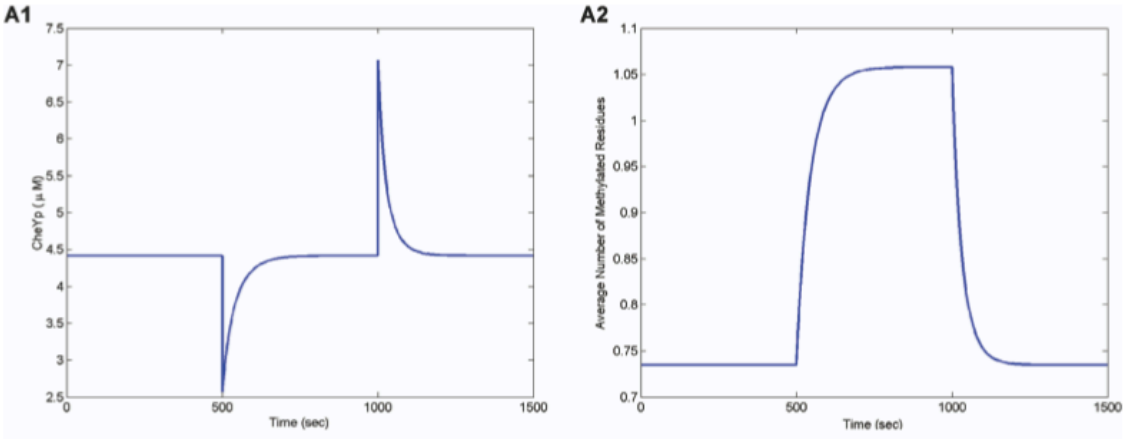
$$r_B = \frac{k_b B_p}{K_B + T^A}$$

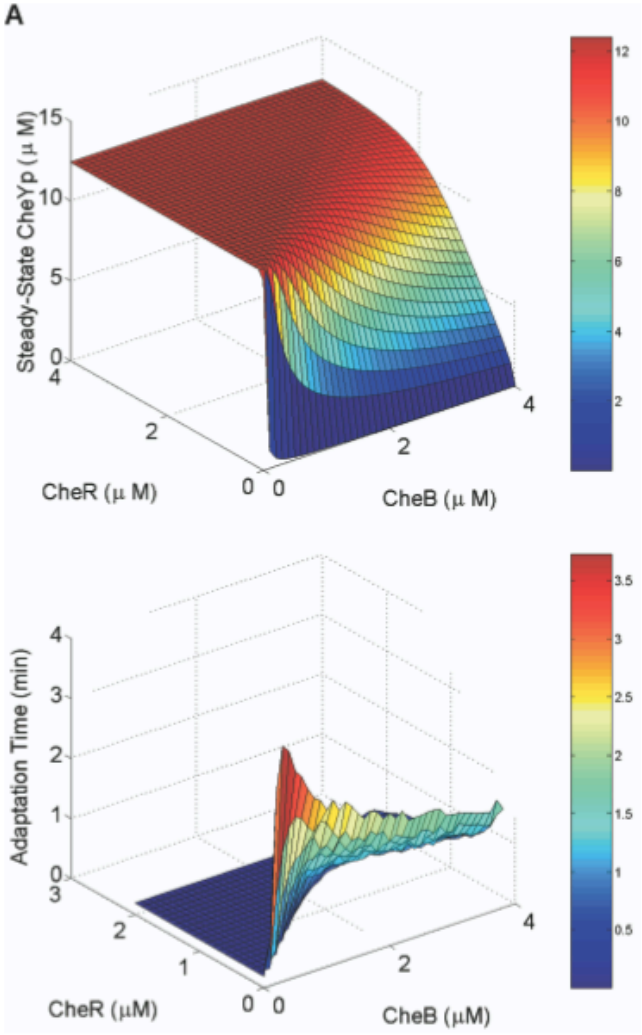
$$r_R = \frac{k_r R}{K_R + T^I}.$$

# Simulations and Analysis

**Model-based analysis to study robustness properties**

- Deterministic ODE; 9 states, 19 parameters
- Simulations (below) show adaptive response
  - Pulse ligand concentration up and down
  - CheYp maintains constant value after transient
  - Average number of methylated receptors serves as integrator to keep track of disturbance level
- Parametric studies show the effects of CheR/CheB
  - Adaptation is relatively robust, but adaptation time can vary significantly



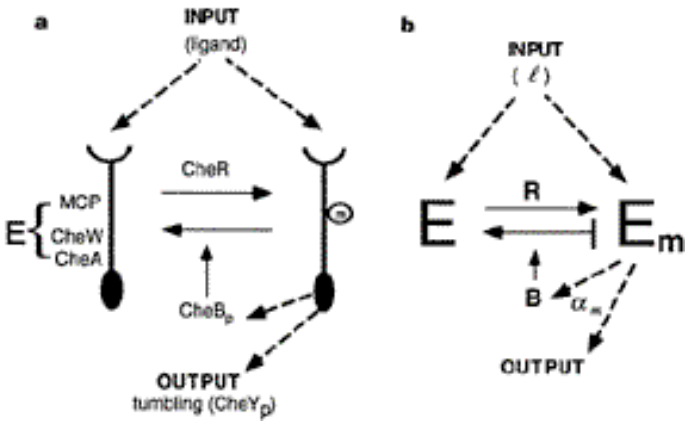



# Reduced-Order Modeling (Barkai and Leibler)

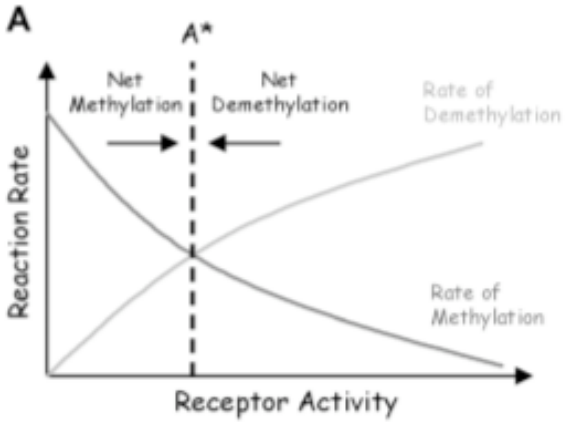
**Construct reduced order model to explore adaptation mechanism**

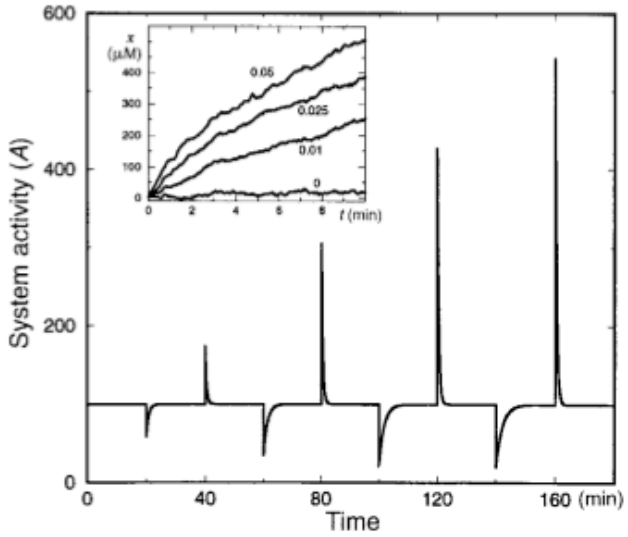
- Model entire receptor complex as a single complex (E) that can be modified (Em) by enzymes R and B
- Can model the resulting amount of receptor methylation using a single ODE:

$$\frac{dm}{dt} = \frac{k_R R T^I}{K_R + T^I} - \frac{k_B B_p T^A}{K_B + T^A}$$

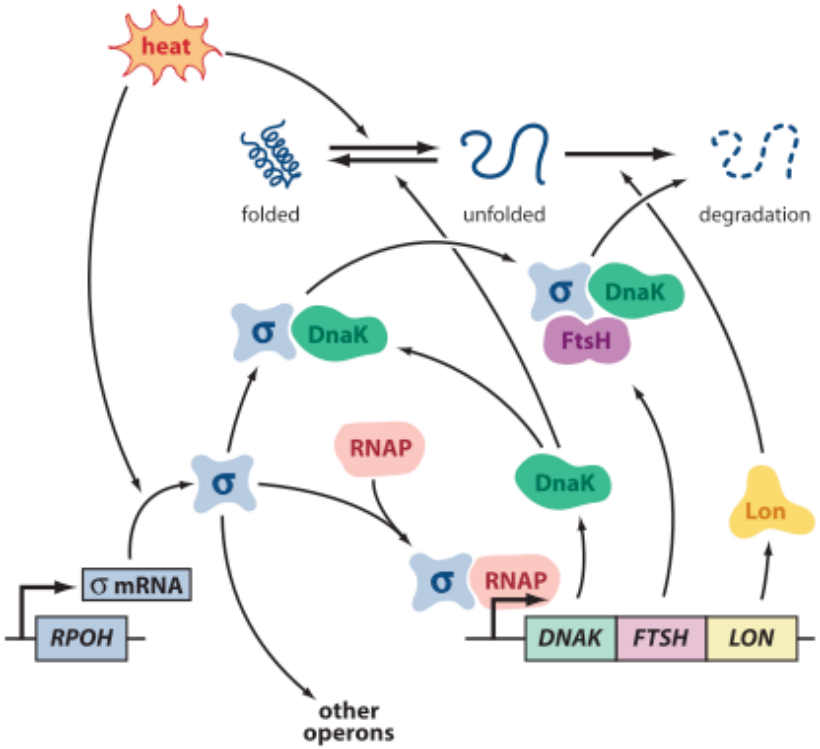



- Inactive complex increase rate of methylation, active complexes decrease rate of methylation => get balance based on activity level



# Example #2: Heat Shock Response




El-Samad et al, 2005

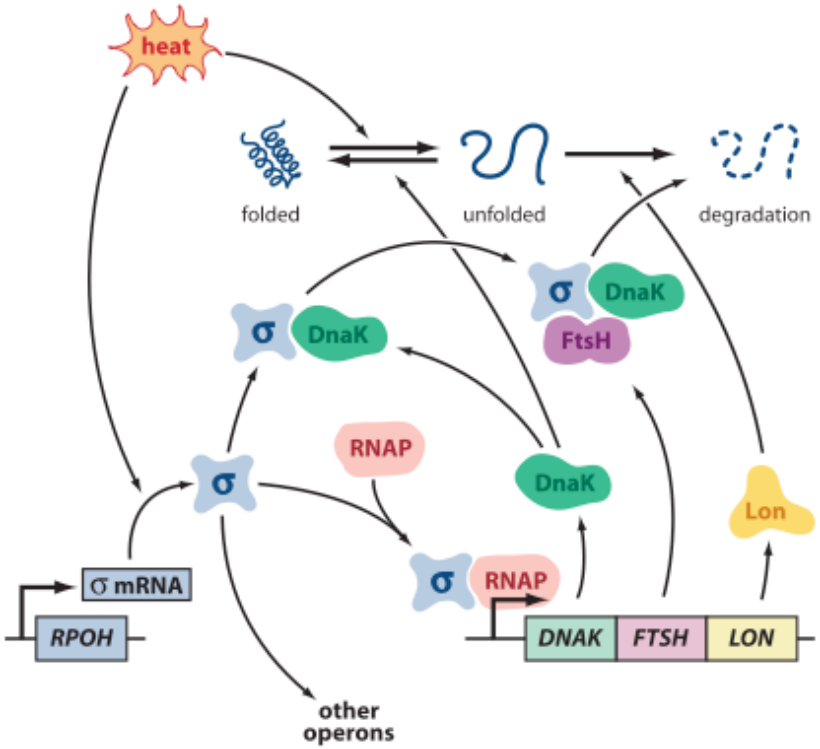
**Heat Shock (HS) Response**

- Heat causes proteins to become unfolded and lose function
- Cell responds in two ways
  - Creates "chaperone" proteins that refold denatured proteins
  - Creates "proteases" that degrade non-functional proteins
- Circuitry (right) contains a number of additional feedback loops that appear to play some role

**References**


- H. El-Samad, Kurata, Doyle, Gross, Khammash, "Surviving heat shock: Control strategies for robustness and performance". PNAS, 2005.

# Heat Shock Response Components




El-Samad et al, 2005

## Sigma factors

- In bacteria, RNAP requires "sigma factors" to bind to DNA
- $\sigma^{70}$ is sigma factor for standard proteins
- $\sigma^{32}$ is sigma factor for HS proteins

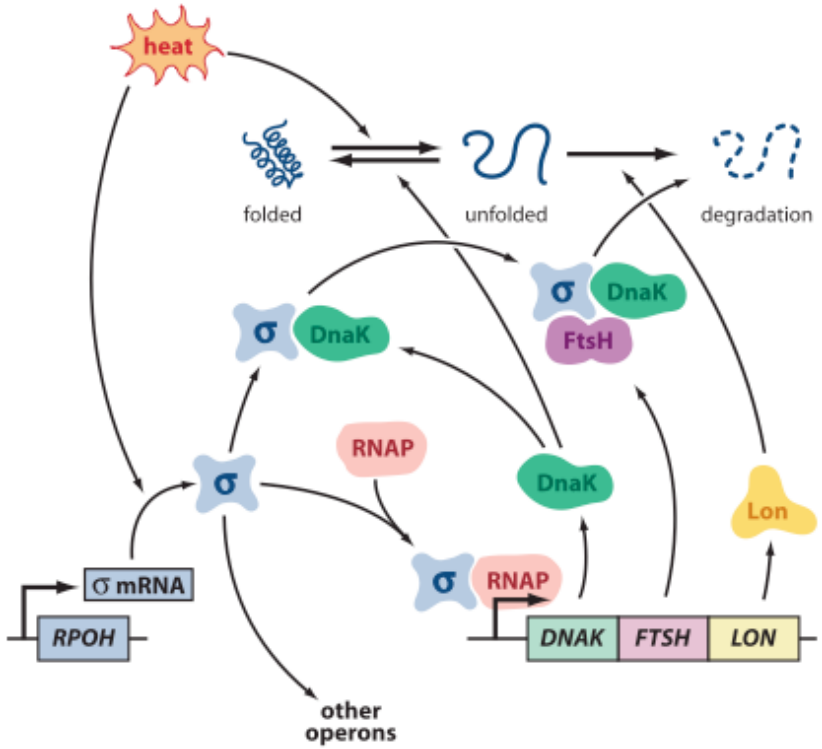
## Post-translational regulation

- $\sigma^{32}$ mRNA is always present in cell, but folds so that ribosome can't translate
- Heat unfolds $\sigma^{32}$ & activates translation

## Heat shock proteins

- DnaK = chaperone protein + sequesters $\sigma^{32}$ (providing negative feedback)
- FtsH = degrades bound σ32, providing finer control
- Lon = protease (degrades unfolded proteins)

# Heat Shock Modeling




El-Samad et al, 2005

$$
\begin{aligned}
d[\mathrm{mDnaK}]/dt &= k_{\mathrm{tr}1}[\sigma^{32} : \mathrm{RNAP}] - \alpha_m[\mathrm{mDnaK}] \\
d[\mathrm{DnaK_t}]/dt &= k_{\mathrm{tl}}[\mathrm{mDnaK}] - \alpha_p[\mathrm{DnaK_t}] \\
d[\mathrm{mFtsH}]/dt &= k_{\mathrm{tr}2}[\sigma^{32} : \mathrm{RNAP}] - \alpha_m[\mathrm{mFtsH}] \\
d[\mathrm{FtsH_t}]/dt &= k_{\mathrm{tl}}[\mathrm{mFtsH}] - \alpha_p[\mathrm{FtsH_t}] \\
d[\mathrm{mLon}]/dt &= k_{\mathrm{tr}3}[\sigma^{32} : \mathrm{RNAP}] - \alpha_m[\mathrm{mLon}] \\
d[\mathrm{Lon_t}]/dt &= k_{\mathrm{tl}}[\mathrm{mLon}] - \alpha_p[\mathrm{Lon_t}] \\
d[\mathrm{m}\sigma^{32}]/dt &= k_{\mathrm{tr}4}[\sigma^{32} : \mathrm{RNAP}] - \alpha_m[\mathrm{m}\sigma^{32}] \\
d[\sigma_t^{32}]/dt &= k_{\mathrm{tl}}\eta(T)[\mathrm{m}\sigma^{32}] - \alpha_p[\mathrm{m}\sigma_{\mathrm{f}}^{32}] \\
&\quad - \alpha_{\mathrm{FtsH}}[\sigma^{32}\text{:DnaK:FtsH}] \\
&\quad - \alpha_{\mathrm{Lon}}(T)[\sigma^{32}\text{:DnaK:FtsH}] \\
d[\mathrm{P_{fold}}] &= k_{\mathrm{fold}}[\mathrm{P_{unfold}}\text{:DnaK}] - K(T)[\mathrm{P_{fold}}]
\end{aligned}
$$

## Algebraic binding equations

- Model remaining reactions assuming they reach steady state quickly

$$
\begin{aligned}
[\sigma^{32}\text{:DnaK}] &= k_1[\sigma_f^{32}] \cdot [\mathrm{DnaK_f}] \\
[\sigma^{32}\text{:DnaK:FtsH}] &= k_2[\sigma^{32}\text{:DnaK}] \cdot [\mathrm{FtsH}]
\end{aligned}
$$

## Mass balance equations

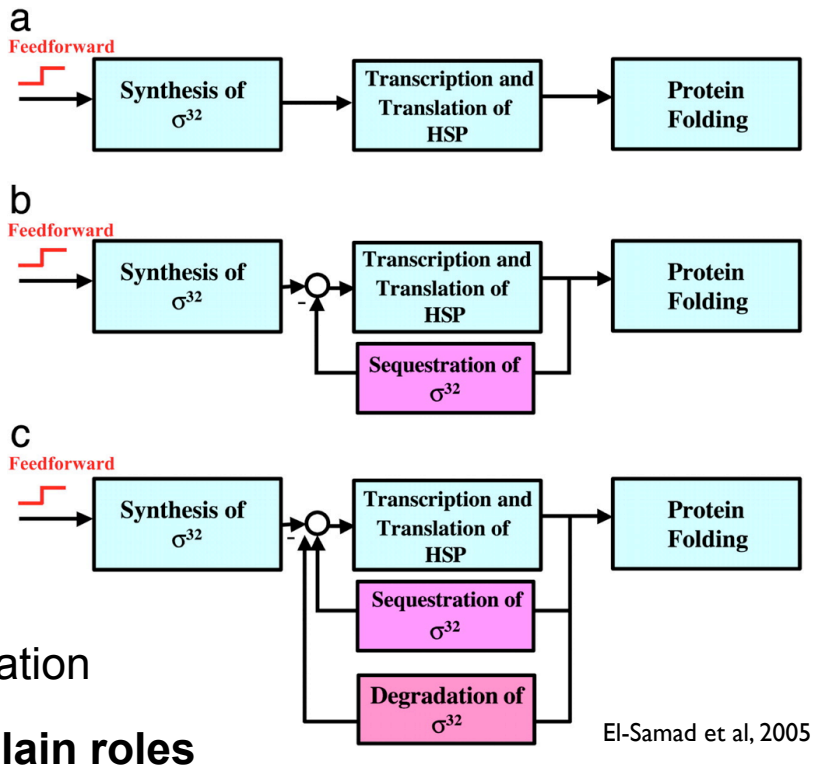
- Total protein concentration = sum of concentrations of all compounds containing that protein

$$[\mathrm{FtsH_t}] = [\mathrm{FtsH_f}] + [\sigma^{32} : DnaK : FtsH]$$

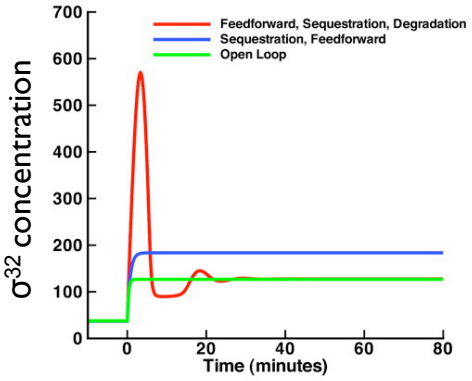
# Role of Feedback

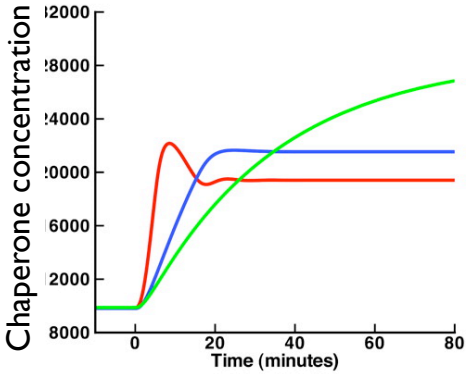
**Question: what is the role of the additional feedback in heat shock circuit?**

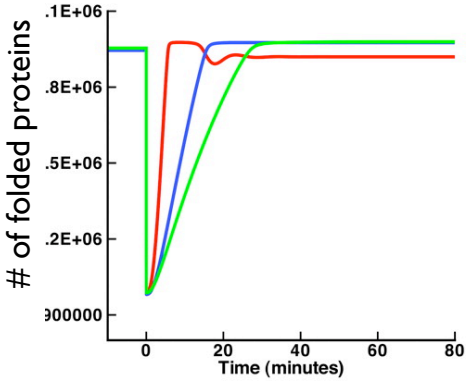
- Option a: feedforward circuit, w/ tuned params
- Option b: neg fbk via sequestration
- Option c: add'l neg fbk via degredation




El-Samad et al, 2005



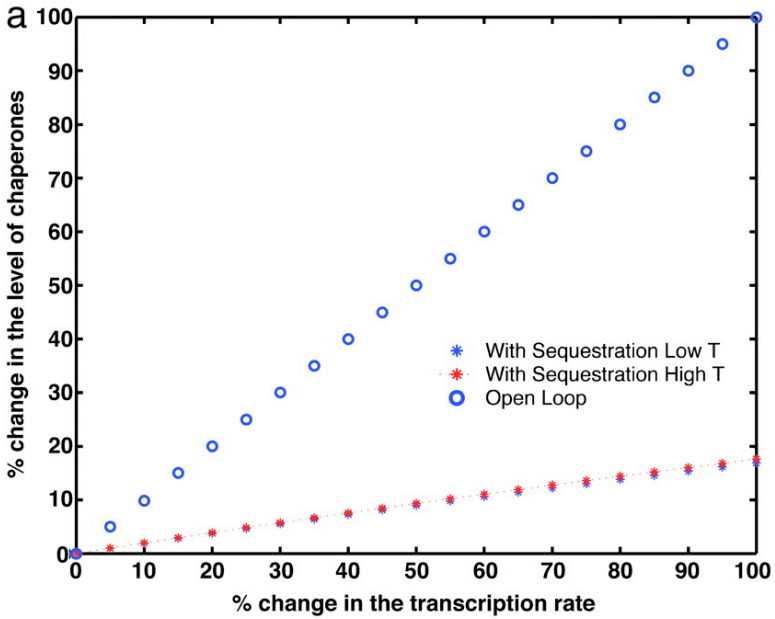

**Simulations help explain roles**

- Use step input of heat and examine how system responses
- $\sigma^{32}$ able to act more quickly with neg fbk to tune response
- Chaperone concentrations have much faster rise time
- Net result: very fast disturbance rejection in folded proteins

# Additional Analyses

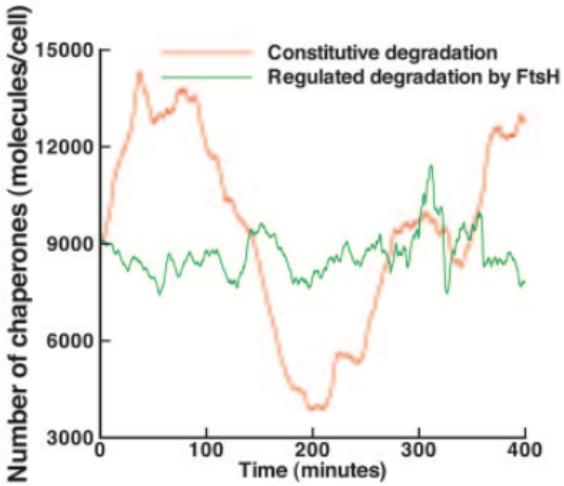
## System robustness

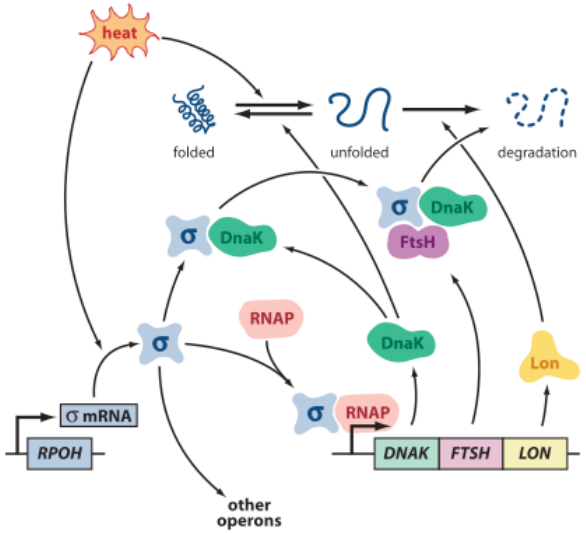
- Feedback provides additional robustness with respect to changes in parameters
- Figure: modification of transcription rate (global parameter) on level of chaperones
- With feedback, much less sensitivity



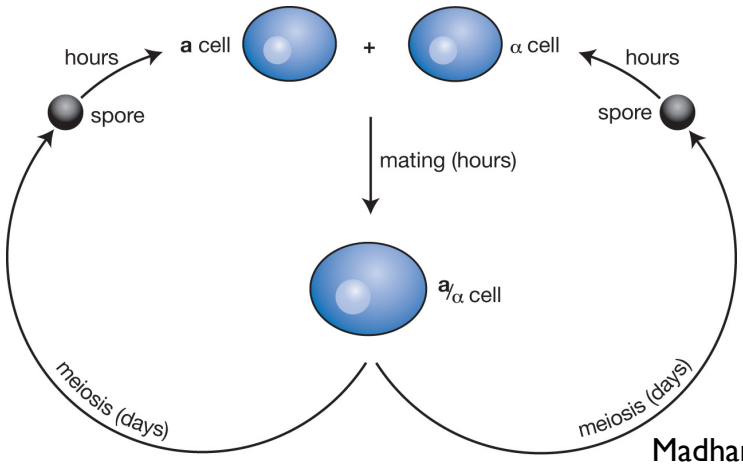

## Noise response

- Stochastic simulation (SSA) of system
- Degradation by FtsH shows less noisy response in chaperone count



# Example #3: Yeast Pheromone Mating Response




Madhani, 2006

http://www.youtube.com/watch?v=dcNEfUnEt_g

**References**


- H. Madhani, *From a to α.* CSHL Press, 2006
- Kofahl and Klipp, *Yeast,* 2004
- Bardwell, *Peptides,* 2005


**Yeast cells exist in four basic phenotypes:**

- Haploid a: contains a single set of chromosomes with MATa locus
- Haploid α: contains a single set of chromosomes with MATα locus
- Diploid a/α: contains two copies of each chromosome
- Spore: under stress, diploid cell can form spores of types a and α; these become haploid cells when environment improves
- Haploid and diploid cells are both capable of cell division (cell type is preserved)

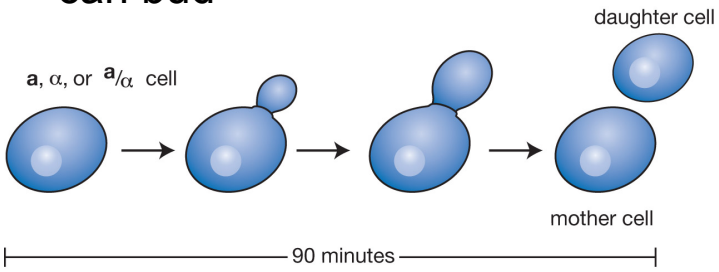
**Yeast cells "mate" via "shmooing"**

- Cells of type a detect pheromone secreted by α cells and extend shmoo
- Cells of type α do the same the converse
- Shmoos join and form diploid cell type
- Haploid cells that mate stop dividing (and die if shmooing doesn't succeed)

# Yeast Phenotypes

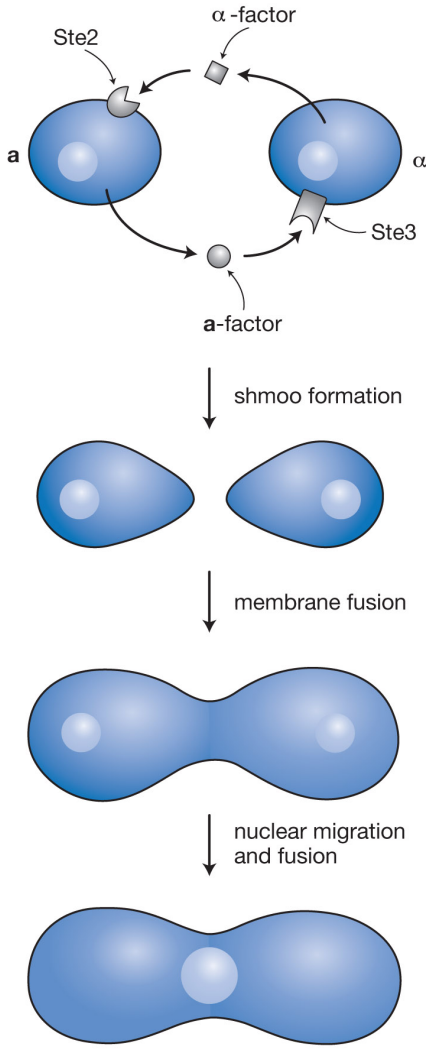
## Budding

- Primary mechanism for cell replication (mitosis)
- Both haploid and diploid cells can bud



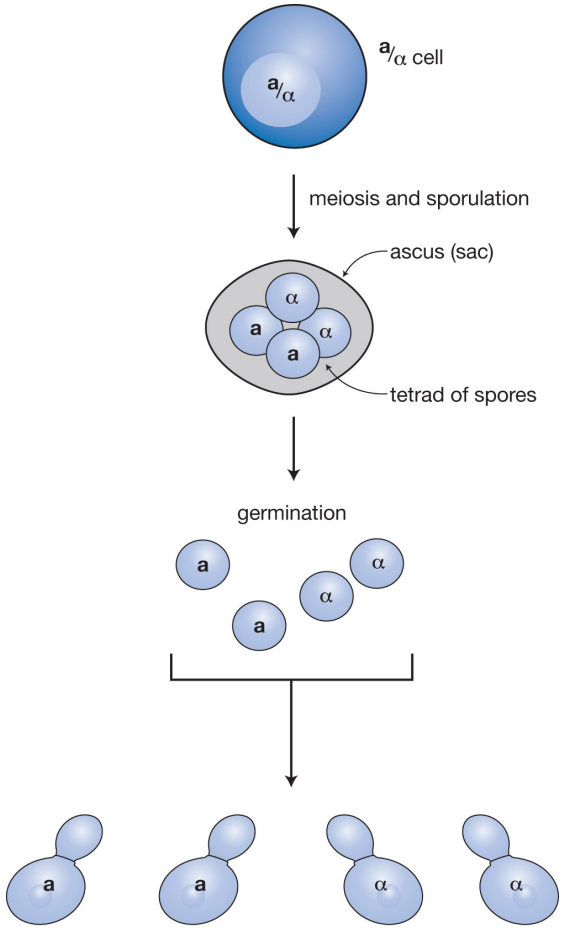

## Mating response

- Haploid cells detect pheromones from complementary type and send “schmoos” toward other cell
- Diploid cell results



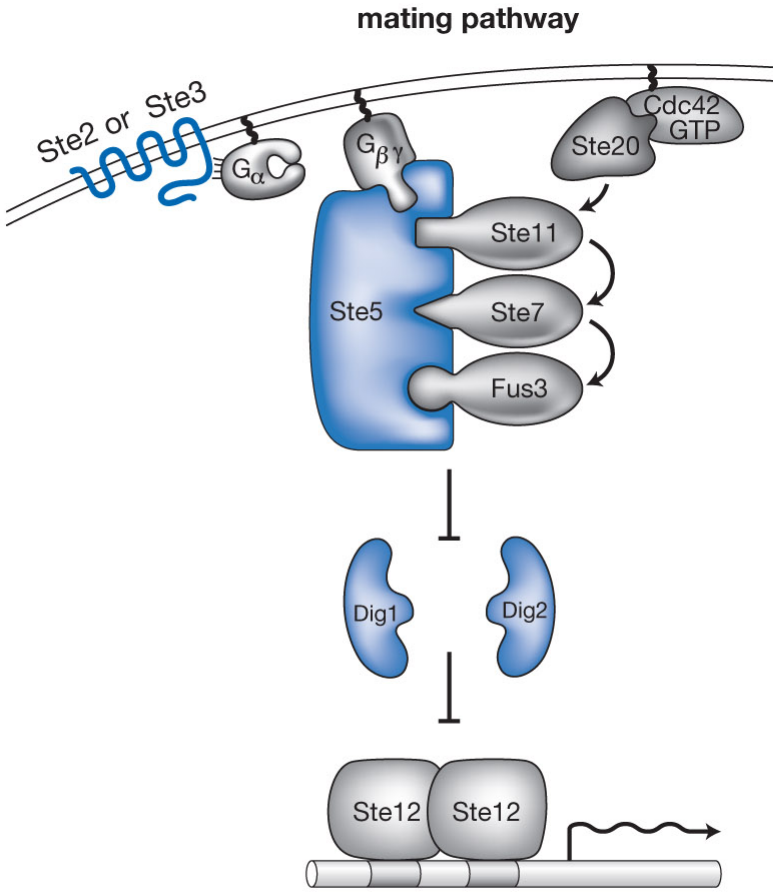

## Sporulation

- Diploid cells can form spores under environmental stress
- Spores grow when environment stabilizes




Madhani, 2006

# Mating Response: Phenomenological Description




Madhani, 2006

**Sensor: G-protein**

- Ste2/Ste3 protein binds in cell membrane
- Pheromone from opposite cell type causes conformational change
- G-protein is used to dock scaffold protein

**Computation: MAP kinase + double repression**

- Cdc42/Ste20 bound to membrane
- Sequence of phosphorylations occurs between proteins linked to scaffold
- Fus3 breaks free when active, causes activation (via double repression with Dig)

**Actuation: transcriptional regulation**

- Ste12 binds to DNA as transcriptional co-factor; Dig1/Dig2 bind to Ste12 and repress gene expression
- Downstream genes encode for proteins required to arrest cell cycle and form shmoo

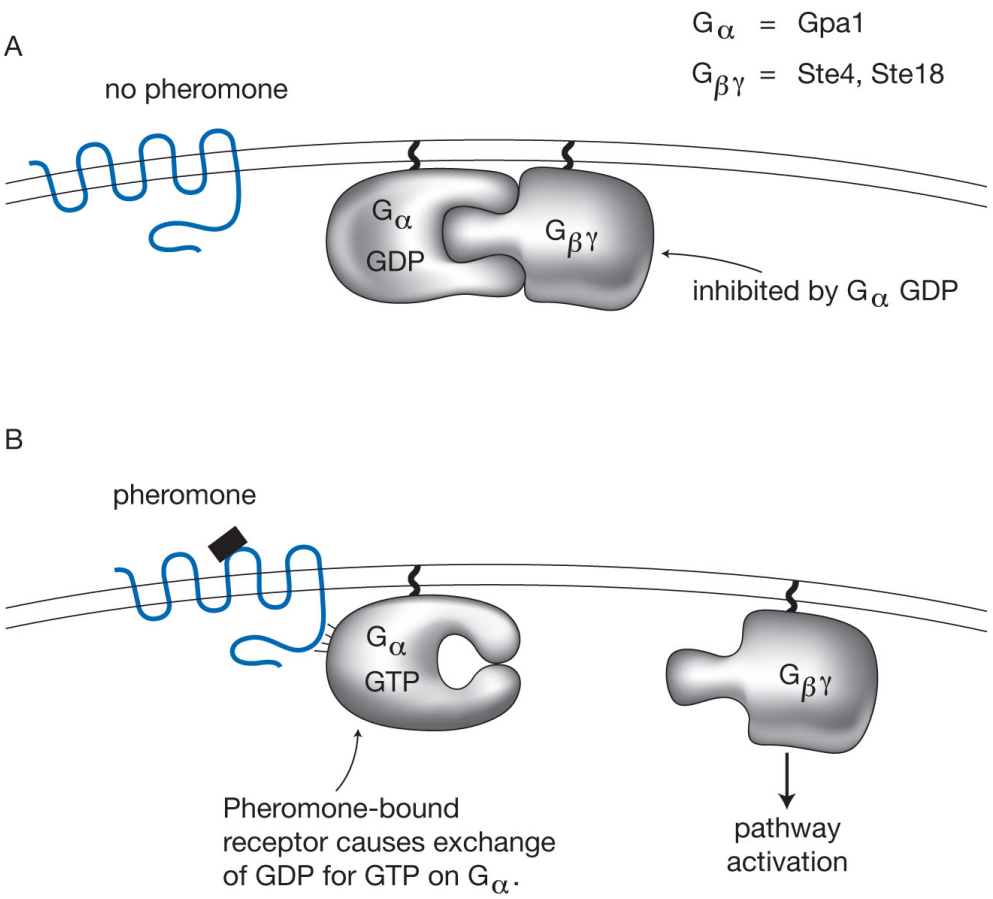
# G-protein signal transduction

**Common mechanism for signal transduction in eukaryotes**

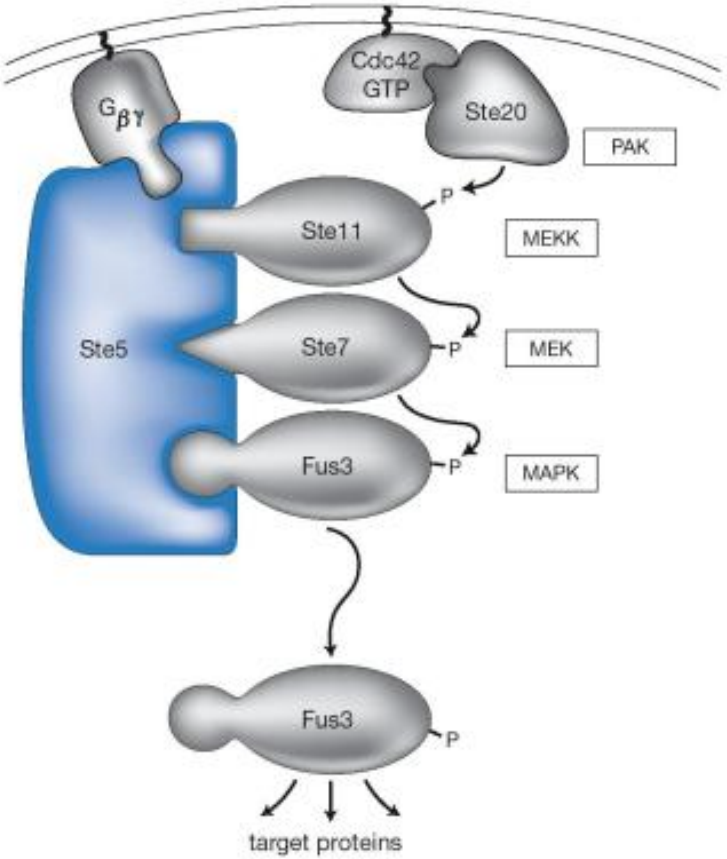
- Membrane-linked protein with seven membrane crossings
- Protein complex with α, β and γ units (specific proteins change)
- α unit binds to GDP

**Signal triggers release of subunits**

- Pheromone binds to receptor & causes conformational change
- Active receptor protein causes exchange of GTP for GDP on α unit
- GTP causes conformational change $G_{\beta\gamma}$ separate from $G_{\alpha}$
- Individual subunits now available to interact with other proteins



# MAP kinase cascade




Madhani, 2006

Add definitions of PAK, MEKK, MEK, MAPK

**Common mechanism for signal propogation**

- Found in many eukaryotes including yeast
- Proteins vary, but function is preserved
- MAP = mitogen activated protein (from originally discovered function - external signal that causes mitosis)

**Sequence of phosphorylation reactions**

- Ste20 is activated by binding to membrane protein
- Activated Ste20 phosphorylates Ste 11, causing Ste 11 to become active
- Activated Ste 11 phosphorylates Ste 7
- Activated Ste 7 phosphorylates Fus3
- Activated Fus3 undocks and activates proteins

**Why a cascade: not completely known**

- In some sytems (eg, w/out scaffold) there may be an amplification factor

# Modeling the Pheromone Response

**Each component can be modeled using basic mechanisms described earlier**

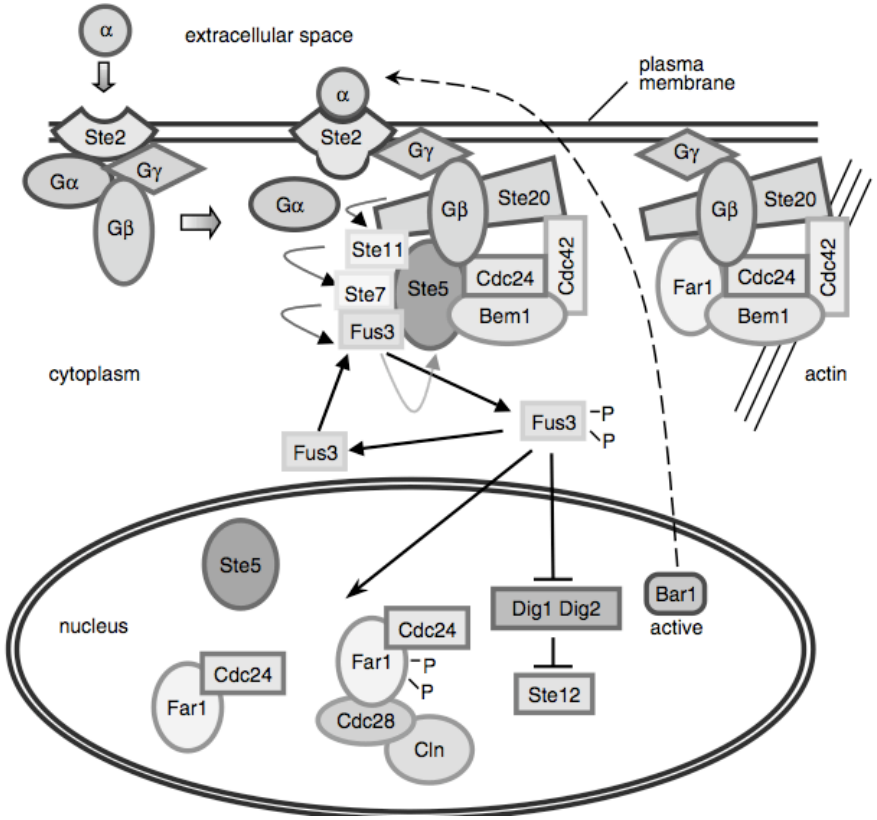



**Table 1.** Equations governing the dynamics of th pheromone pathway (mathematical model)

Ordinary differential equations

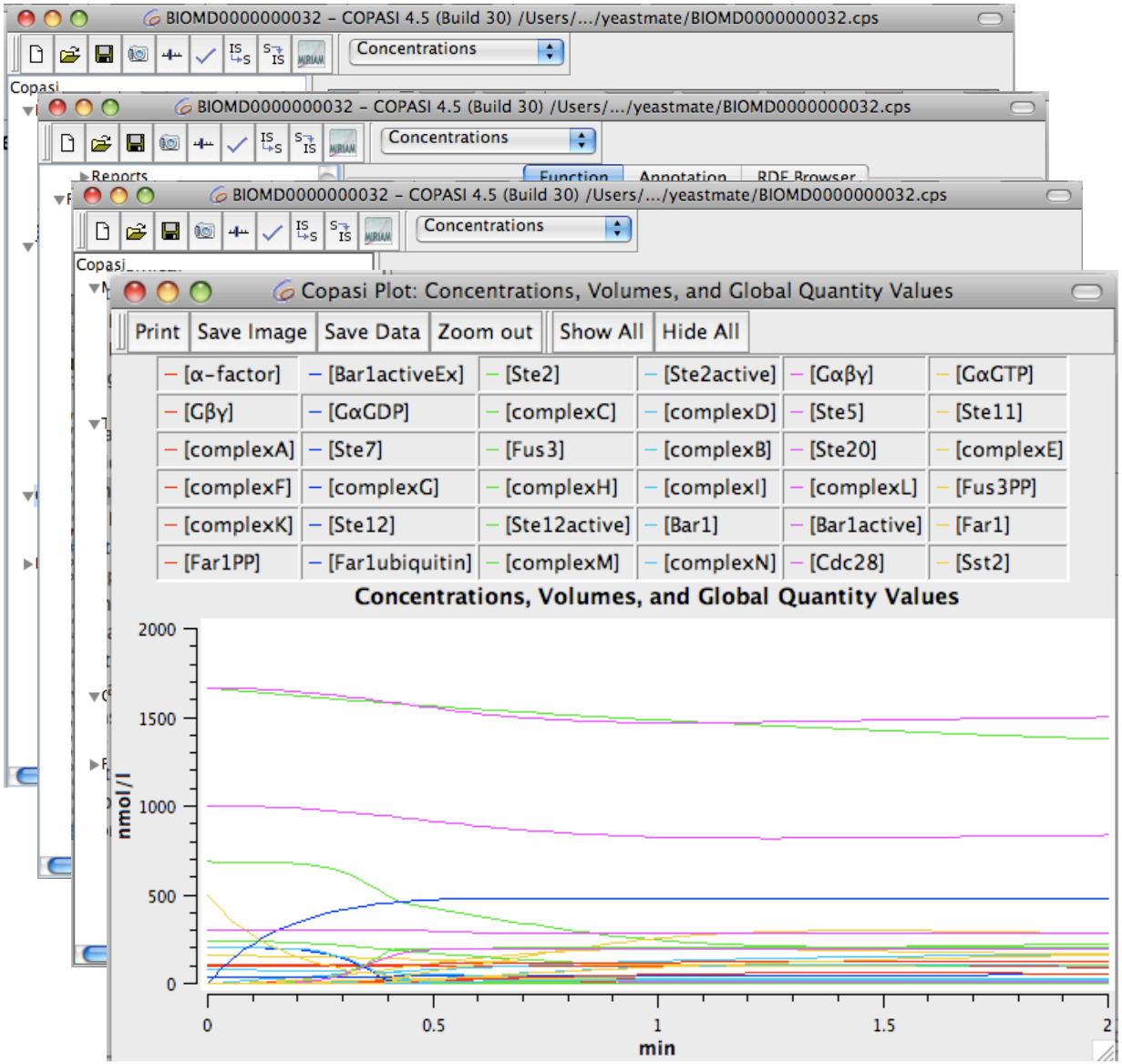
$$\frac{d\alpha}{dt} = -v_1$$
$$\frac{dSte2}{dt} = -v_2 + v_3 - v_5$$
$$\frac{dSte2_{active}}{dt} = v_2 - v_3 - v_4$$
$$\frac{dSst2_{active}}{dt} = v_{46} - v_{47}$$
$$\frac{dG\alpha\beta\gamma}{dt} = -v_6 + v_9$$
$$\frac{dG\alpha GTP}{dt} = v_6 - v_7 - v_8$$
$$\frac{dG\alpha GDP}{dt} = v_7 + v_8 - v_9$$
$$\frac{dG\beta\gamma}{dt} = v_6 - v_9 - v_{10} + v_{11} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32} - v_{42} + v_{43}$$
$$\frac{dSte5}{dt} = -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$
$$\frac{dSte11}{dt} = -v_{12} + v_{13} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$
$$\frac{dSte7}{dt} = -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$
$$\frac{dFus3}{dt} = -v_{14} + v_{15} + v_{17} + v_{21} + v_{23} + v_{25} + v_{27} - v_{29} + v_{30} + v_{33}$$
$$\frac{dSte20}{dt} = -v_{18} + v_{19} + v_{21} + v_{23} + v_{25} + v_{27} + v_{32}$$
$$\frac{dA}{dt} = v_{12} - v_{13} - v_{16}$$
$$\frac{dB}{dt} = v_{14} - v_{15} - v_{16}$$
$$\frac{dC}{dt} = -v_{10} + v_{11} + v_{16} - v_{17}$$
$$\frac{dD}{dt} = v_{10} - v_{11} - v_{18} + v_{19}$$
$$\frac{dE}{dt} = v_{18} - v_{19} - v_{20} - v_{21}$$
$$\frac{dF}{dt} = v_{20} - v_{22} - v_{23}$$
$$\frac{dG}{dt} = v_{22} - v_{24} - v_{25}$$
$$\frac{dH}{dt} = v_{24} - v_{26} - v_{27}$$
$$\frac{dI}{dt} = v_{26} - v_{28} + v_{31}$$
$$\frac{dK}{dt} = v_{28} - v_{29} + v_{30} - v_{32}$$
$$\frac{dL}{dt} = v_{29} - v_{30} - v_{31}$$
$$\frac{dFus3PP}{dt} = v_{28} - v_{33} - v_{34} + v_{35}$$
$$\frac{dSte12}{dt} = -v_{34} + v_{35}$$
$$\frac{dSte12_{active}}{dt} = v_{34} - v_{35}$$
$$\frac{dBar1}{dt} = -v_{36} + v_{37}$$
$$\frac{dBar1_{active}}{dt} = v_{36} - v_{37} - v_{38}$$
$$\frac{dFar1}{dt} = -v_{39} + v_{40} - v_{41}$$
$$\frac{dFar1PP}{dt} = v_{39} - v_{40} - v_{42} + v_{43} + v_{44} - v_{45}$$
$$\frac{dM}{dt} = v_{42} - v_{43}$$
$$\frac{dFar1_{Ubiquitin}}{dt} = v_{41}$$
$$\frac{dCdc28}{dt} = v_{44} - v_{45}$$

Rate equations

$$v_1 = \alpha[t] \cdot Bar1_{active}[t] \cdot k_1$$
$$v_2 = Ste2[t] \cdot \alpha[t] \cdot k_2$$
$$v_3 = Ste2_{active}[t] \cdot k_3$$
$$v_4 = Ste2_{active}[t] \cdot k_4$$
$$v_5 = Ste2[t] \cdot k_5$$
$$v_6 = Ste2_{active}[t] \cdot G\alpha\beta\gamma[t] \cdot k_6$$
$$v_7 = G\alpha GTP[t] \cdot k_7$$
$$v_8 = G\alpha GTP[t] \cdot Sst2_{active}[t] \cdot k_8$$
$$v_9 = G\alpha GDP[t] \cdot G\beta\gamma[t] \cdot k_9$$
$$v_{10} = G\beta\gamma[t] \cdot C[t] \cdot k_{10}$$
$$v_{11} = D[t] \cdot k_{11}$$
$$v_{12} = Ste5[t] \cdot Ste11[t] \cdot k_{12}$$
$$v_{13} = A[t] \cdot k_{13}$$
$$v_{14} = Ste7[t] \cdot Fus3[t] \cdot k_{14}$$
$$v_{15} = B[t] \cdot k_{15}$$
$$v_{16} = A[t] \cdot B[t] \cdot k_{16}$$
$$v_{17} = C[t] \cdot k_{17}$$
$$v_{18} = D[t] \cdot Ste20[t] \cdot k_{18}$$
$$v_{19} = E[t] \cdot k_{19}$$
$$v_{20} = E[t] \cdot k_{20}$$
$$v_{21} = E[t] \cdot k_{21}$$
$$v_{22} = F[t] \cdot k_{22}$$
$$v_{23} = F[t] \cdot k_{23}$$
$$v_{24} = G[t] \cdot k_{24}$$
$$v_{25} = G[t] \cdot k_{25}$$
$$v_{26} = H[t] \cdot k_{26}$$
$$v_{27} = H[t] \cdot k_{27}$$
$$v_{28} = I[t] \cdot k_{28}$$
$$v_{29} = L[t] \cdot Fus3[t] \cdot k_{29}$$
$$v_{30} = K[t] \cdot k_{30}$$
$$v_{31} = K[t] \cdot k_{31}$$
$$v_{32} = L[t] \cdot k_{32}$$
$$v_{33} = Fus3PP[t] \cdot k_{33}$$
$$v_{34} = Ste12[t] \cdot Fus3PP[t] \cdot k_{34}$$
$$v_{35} = Ste12_{active}[t] \cdot k_{35}$$
$$v_{36} = Ste12_{active}[t] \cdot Bar1[t] \cdot k_{36}$$
$$v_{37} = Bar1_{active}[t] \cdot k_{37}$$
$$v_{38} = Bar1_{active}[t] \cdot k_{38}$$
$$v_{39} = Far1[t] \cdot \frac{Fus3PP[t]^2}{\quad} \cdot k_{39}$$

- B. Kofahl and E. Klipp, Modelling the dynamics of the yeast pheromone pathway. *Yeast,* 21(10):831-850, 2004

# Interlude: SBML and Associated Software




**Building models**

- Supports arbitrary rate expressions
- Allows multiple compartments (eg, cytoplasm, nucleus, ...)

**Many standard reactions**

- Has built in reactions for Michaelis-Menten, Hill functions, etc

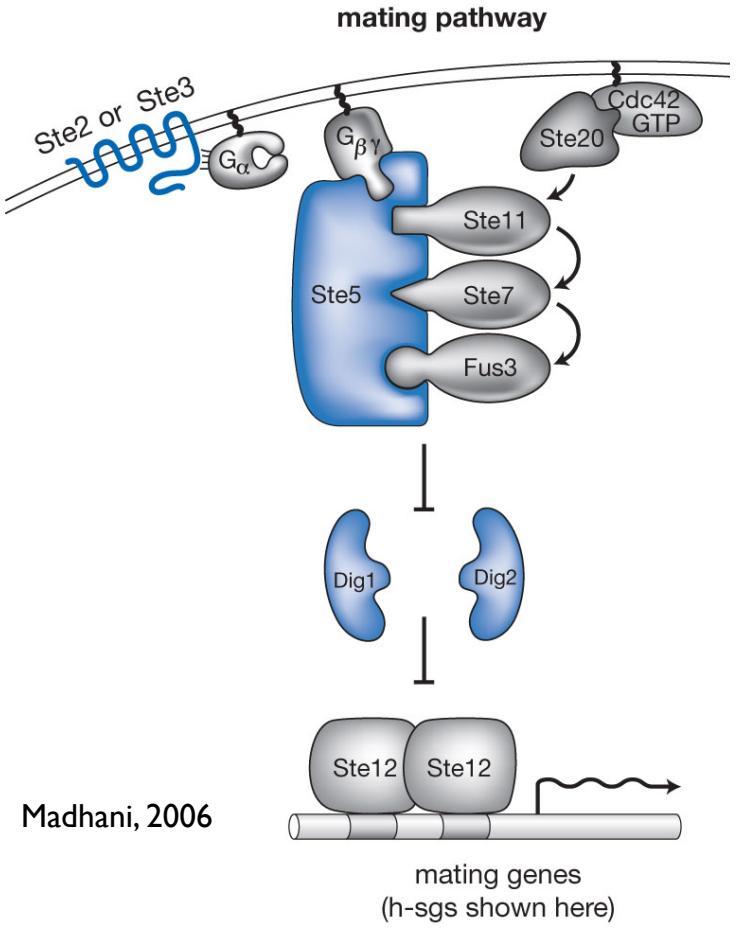
**Analysis capabilities**

- Simulate either deterministically or stochastically (SSA)
- Also supports sensitivity analysis, parameter estimation, etc

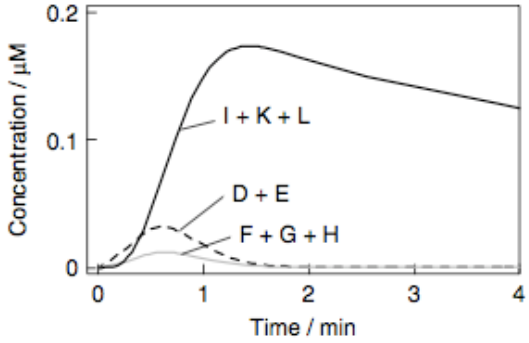
**Output**

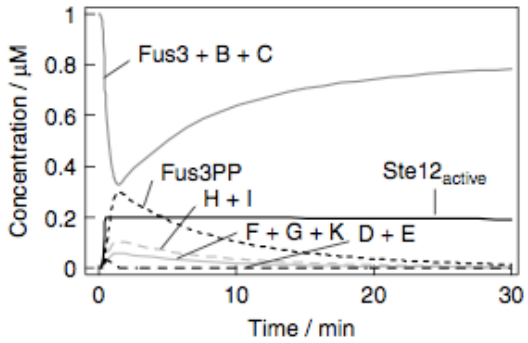
- Interactive selection

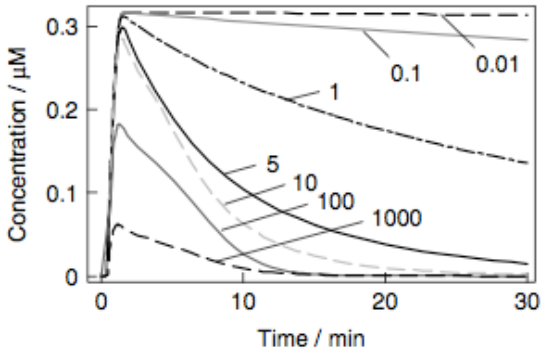
# Sample Simulation Results




Madhani, 2006




## MAPK activity

- D+E = unphos-phorylated complex
- F+G+H = partially phosphorylated
- I+J+K = active complex

## Free/bound Fus3

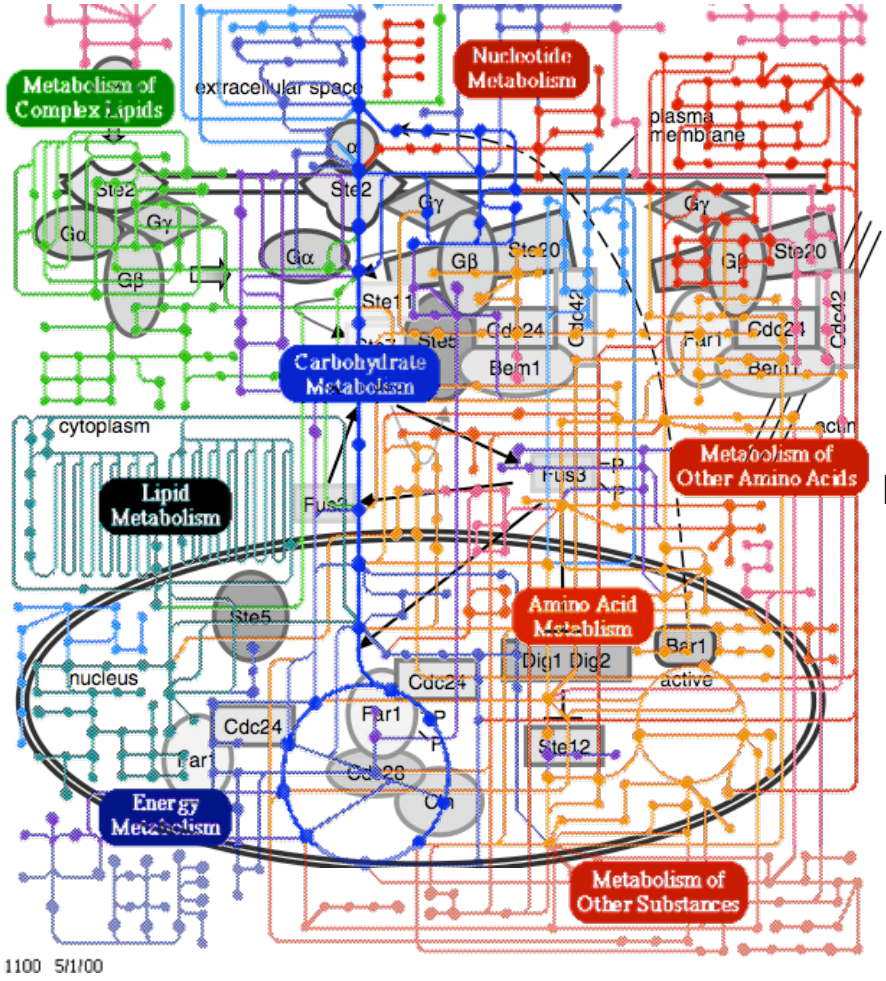
- Fus3 + B + C = free Fus3
- Fus3PP = activated Fus3 (binds to Dig)

## Parametric changes

- Effect of increasing scaffold degradation on Fus3PP

Kofahl and Klipp, 2004

# Some Open Questions



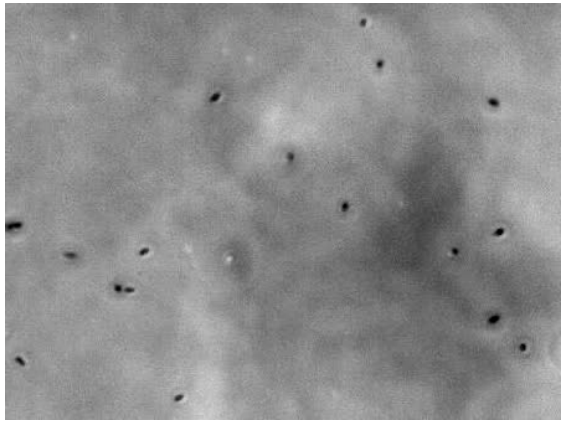

**What's different about biological systems**

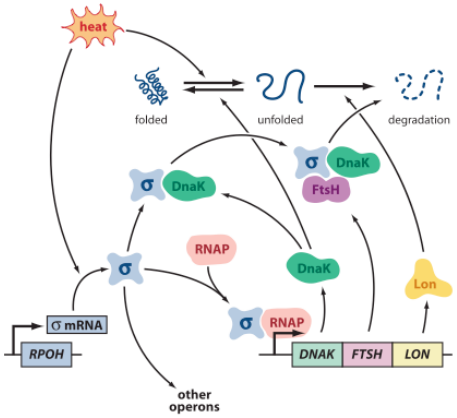
- *Complexity* - biological systems are *much* more complicated than engineered systems
- *Communications* - signal representations are very different (spikes, proteins, etc)
- *Uncertainty* - very large uncertainty in components; don't match current tools
- *Evolvability* - mutation, selection, etc

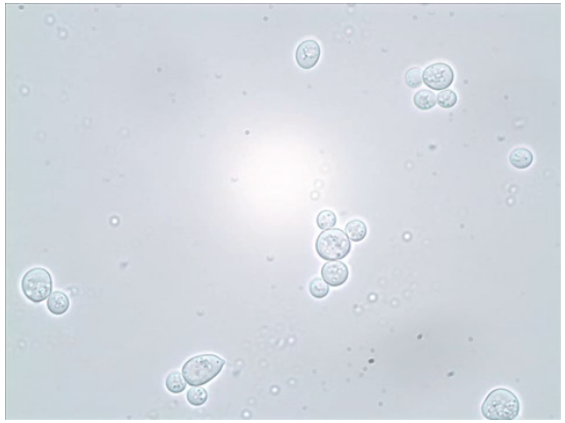
**Potential application areas for control tools**

- *System ID* - what are the appropriate component abstractions and models?
- *Analysis* - what are key biological feedback mechanisms that lead to robust behavior?
- *Design* - how to we (re-)design biological systems to provided desired function?
- *Fundamental limits* - what are the limits of performance and robustness for a given biological network topology?

# Summary





**Chemotaxis**

- Regulate the rate of runs versus tumbles to move along increasing gradients
- Methylation provides adaptation (integral feedback) to constant biases

**Heat shock**

- Turn on refolding machinery when proteins begin to denature due to heat
- Feedback mechanisms: sequestration, degradation, post-transcriptional modifications

**Yeast mating response**

- Detect presence of opposite cell type and generate a shmoo for possible mating
- Molecular machinery: G-proteins, phosphorylation, MAP kinases