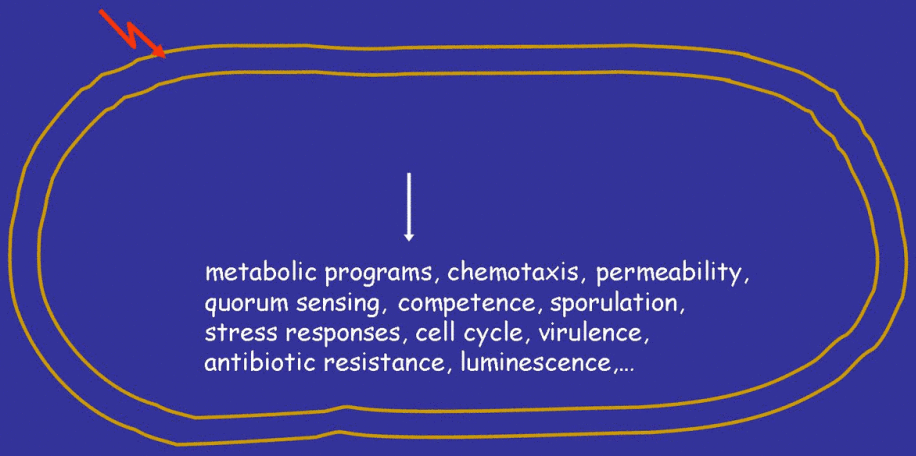
Perturbing, imaging, modeling, and evolving cell signaling circuits in bacteria

Two-Component Signaling

light, pheromones, metals, phosphate, nitrogen, e- acceptors, sugars,
amino acids, peptides, osmolarity, antibiotics, misfolded proteins, plant wounds …



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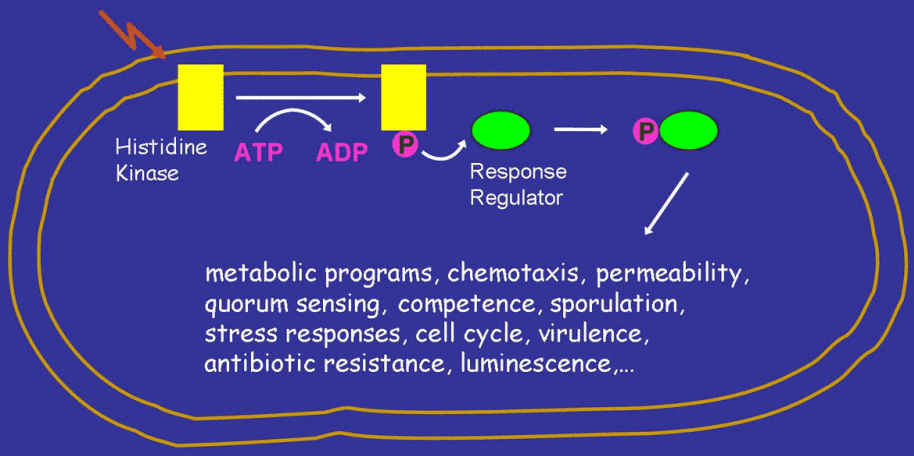

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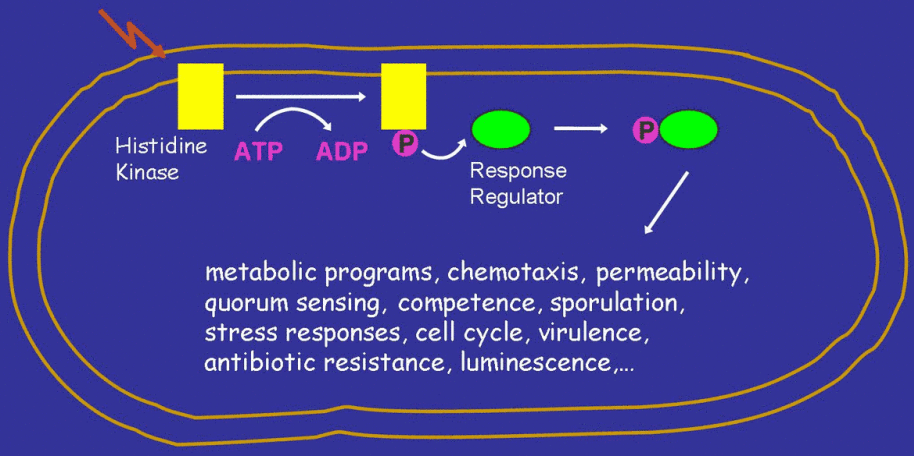
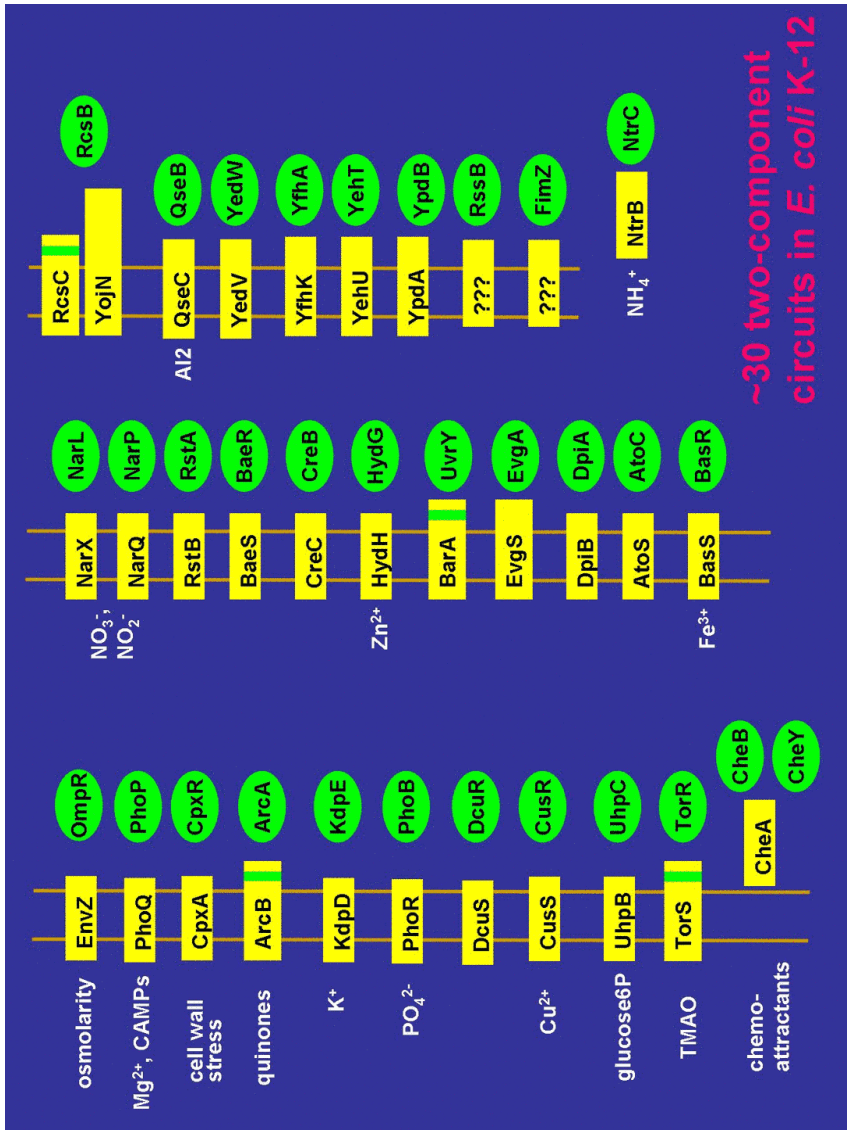
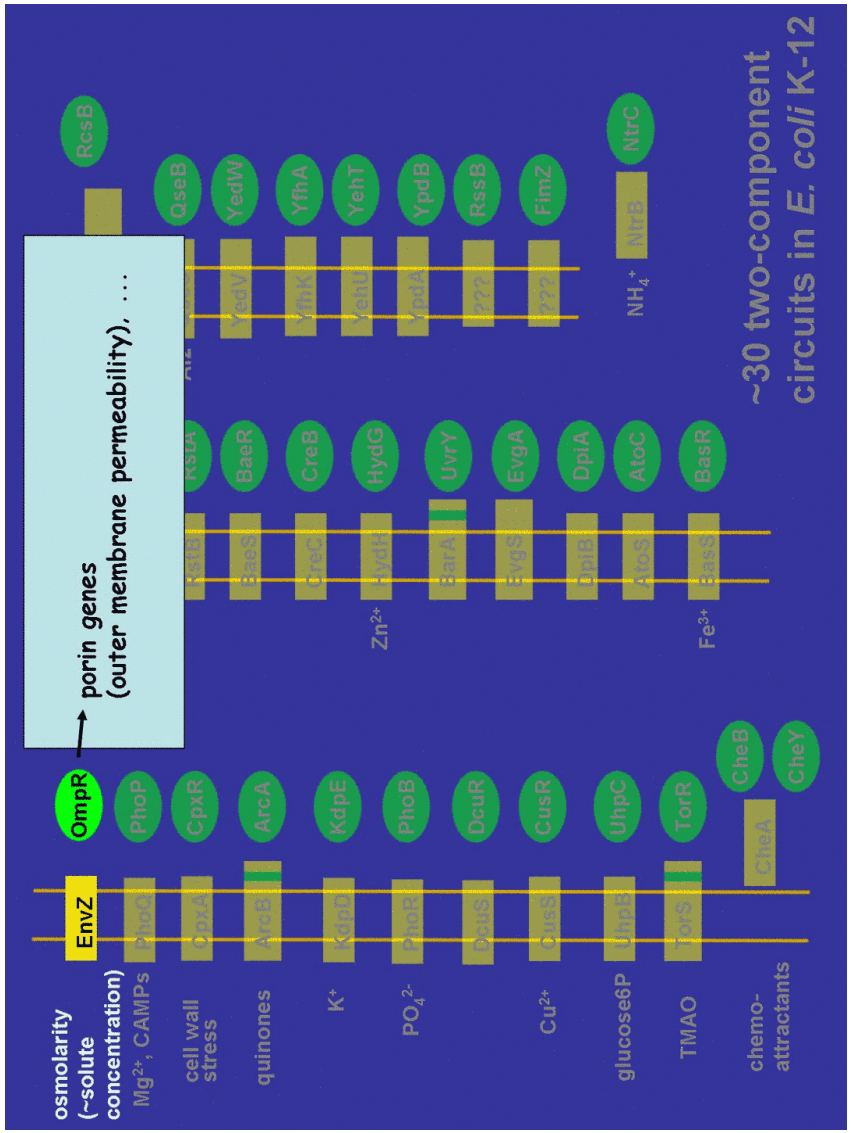
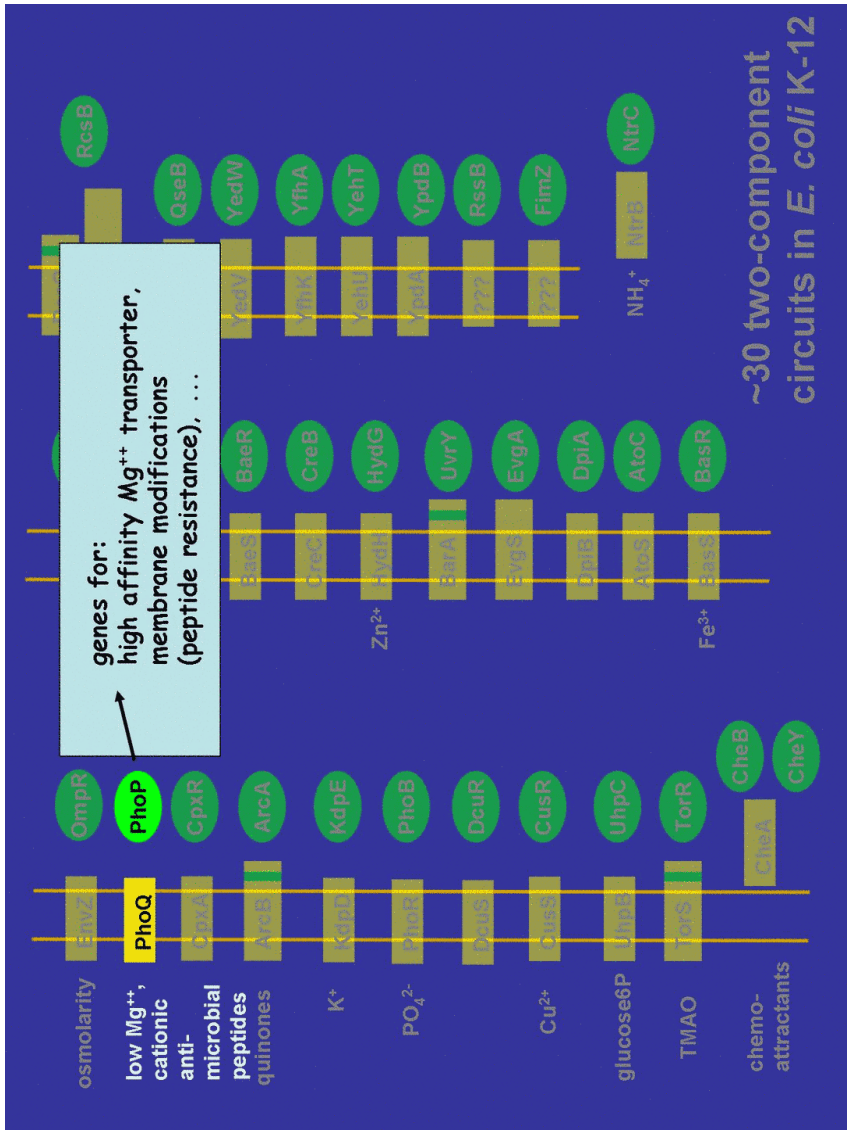
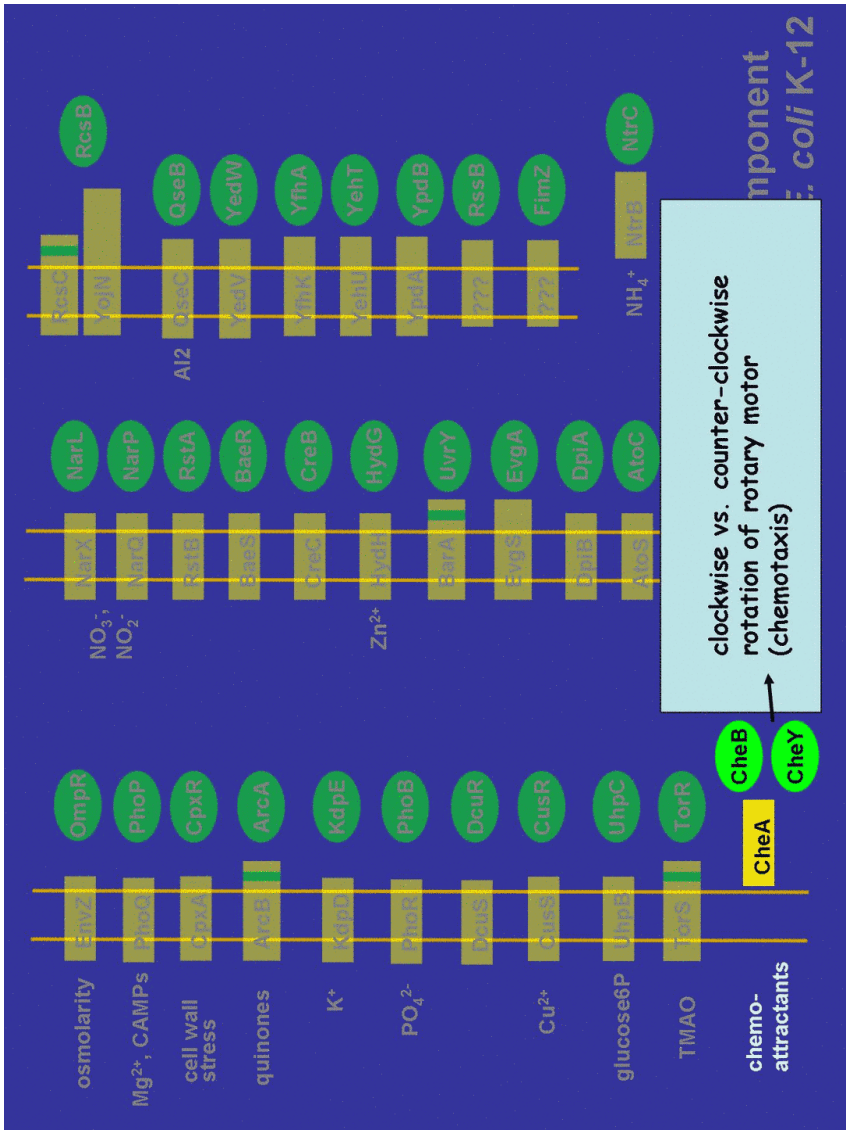


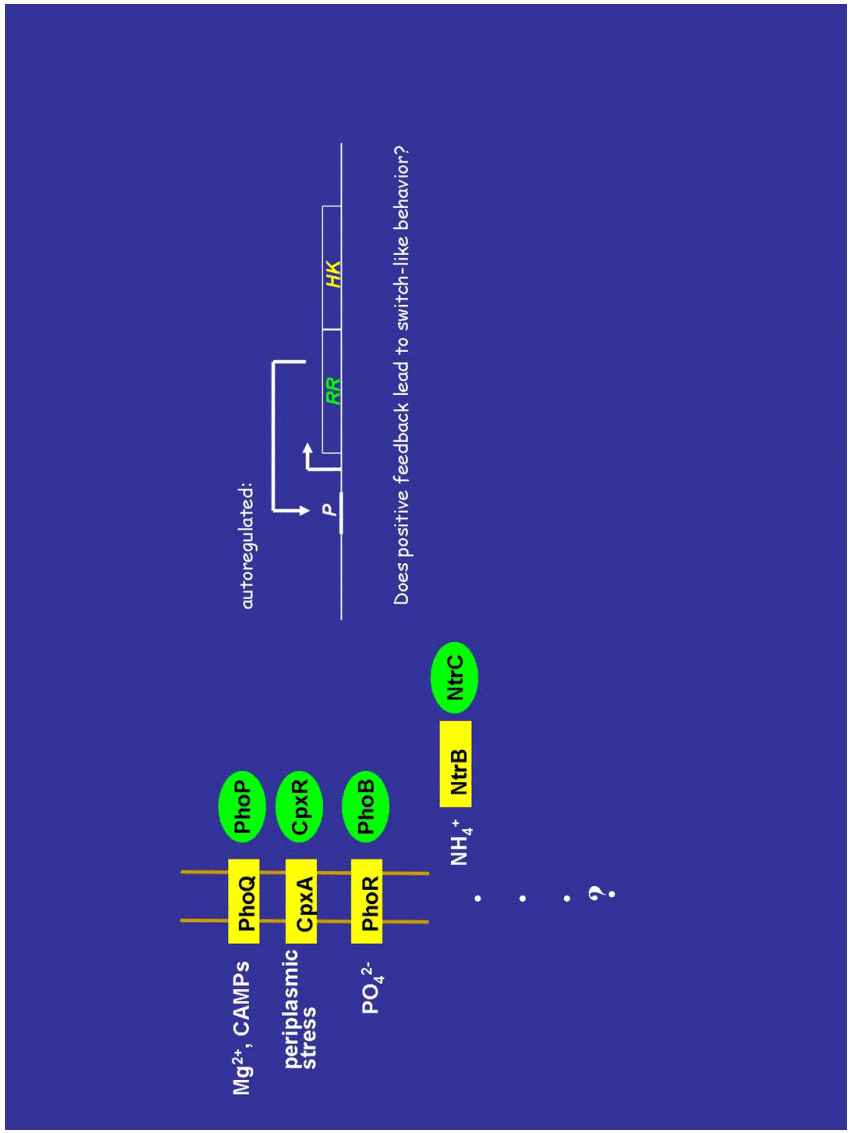
- Important for microbial physiology, pathogenesis, …
- Relatively simple and tractable class of systems for studying biological circuits, many examples

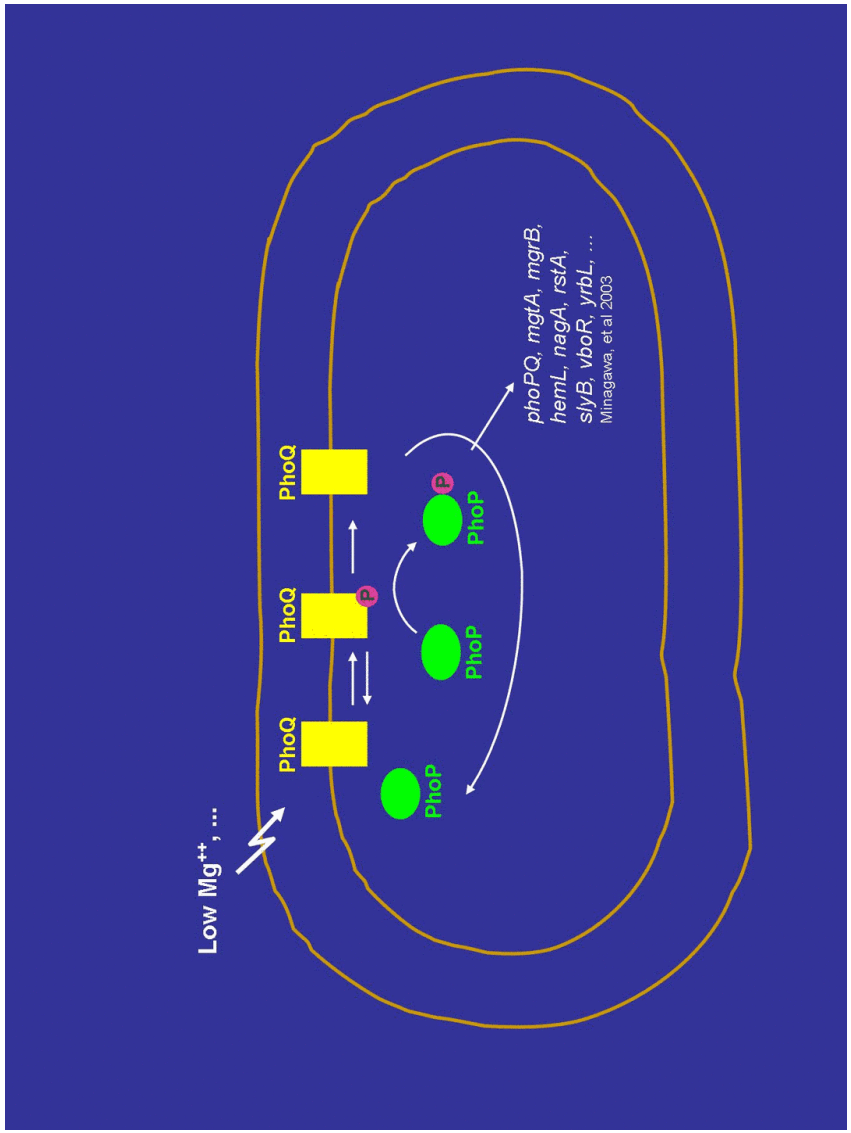

~30 two-component circuits in E. coli K-12
osmolarity
Mg2+, CAMPs
cell wall stress
quinones
K+
PO4 2-
Cu2+
glucose6P
TMAO
chemo-attractants
EnvZ
PhoQ
CpxA
ArcB
KdpD
PhoR
DcuS
CusS
UhpB
TorS
CheA
OmpR
PhoP
CpxR
ArcA
KdpE
PhoB
DcuR
CusR
UhpC
TorR
CheB
CheY
NO3-, NO2-
NarX
NarQ
NarL
NarP
Zn2+
RstA
RstB
BaeR
BaeS
CreB
CreC
HydG
HydH
Fe3+
UvrY
BarA
EvgA
EvgS
DpiA
DpiB
AtoC
AtoS
BasR
BasS
AI2
RcsC
YojN
RcsB
QseB
QseC
YedW
YedV
YfhA
YfhK
YehT
YehU
YpdB
YpdA
RssB
???
FimZ
???
NH4+
NtrC
NtrB


~30 two-component circuits in E. coli K-12
porin genes
(outer membrane permeability), ...
osmolarity
(~solute concentration)
Mg2+, CAMPs
cell wall stress
quinones
K+
PO4 2-
Cu2+
glucose6P
TMAO
chemo-attractants
EnvZ
OmpR
RcsB
NH4+
NtrC
NtrB

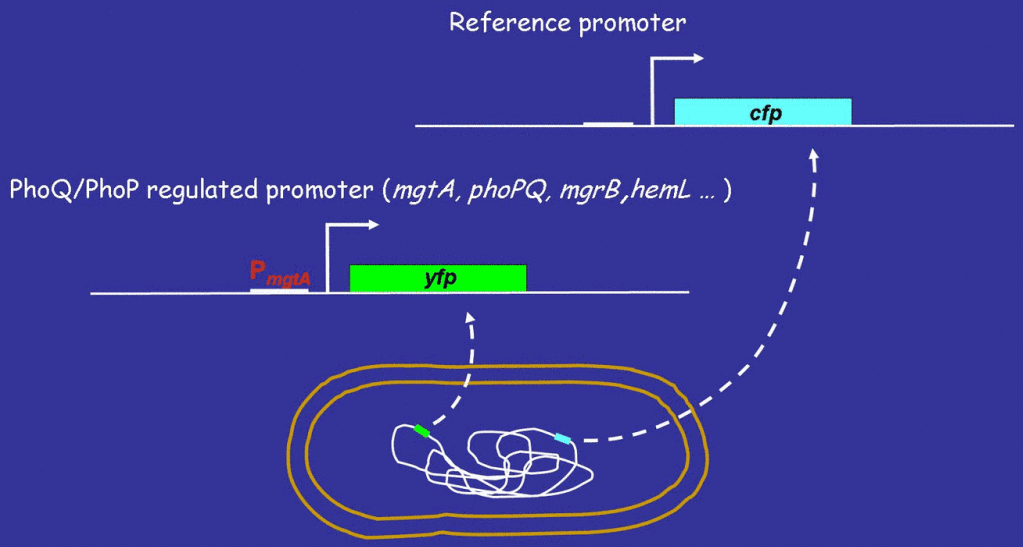

~30 two-component circuits in E. coli K-12
genes for:
high affinity Mg++ transporter,
membrane modifications
(peptide resistance), ...
osmolarity
low Mg++, cationic anti-microbial peptides
quinones
K+
PO4 2-
Cu2+
glucose6P
TMAO
chemo-attractants
EnvZ
PhoQ
CpxA
ArcB
KdpD
PhoR
DcuS
CusS
UhpB
TorS
CheA
OmpR
PhoP
CpxR
ArcA
KdpE
PhoB
DcuR
CusR
UhpC
TorR
CheB
CheY
RcsB
QseB
YedW
YfhA
YehT
YpdB
RssB
FimZ
NtrC
NtrB
BaeR
CreB
HydG
UvrY
EvgA
DpiA
AtoC
BasR
BaeS
CreC
HydS
BarA
EvgS
DpiB
AtoS
BasS
Zn2+
Fe3+
NH4+
???

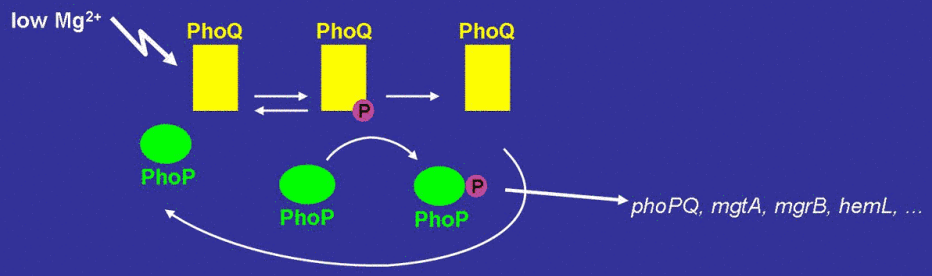

nponent
E. coli K-12
clockwise vs. counter-clockwise
rotation of rotary motor
(chemotaxis)
osmolarity
Mg2+, CAMPs
cell wall stress
quinones
K+
PO4 2-
Cu2+
glucose6P
TMAO
chemo-attractants
EnvZ
PhoQ
CpxA
ArcB
KdpD
PhoR
DcuS
CusS
UhpB
TorS
CheA
OmpR
PhoP
CpxR
ArcA
KdpE
PhoB
DcuR
CusR
UhpC
TorR
CheB
CheY
RcsB
QseB
YedW
YfhA
YehT
YpdB
RssB
FimZ
NtrC
NtrB
NarL
NarP
RstA
BaeR
CreB
HydG
UvrY
EvgA
DpiA
AtoC
NarX
NarQ
RstB
BaeS
CreC
HydS
BarA
EvgS
DpiB
AtoS
NO3-, NO2-
Zn2+
AI2
NH4+
???

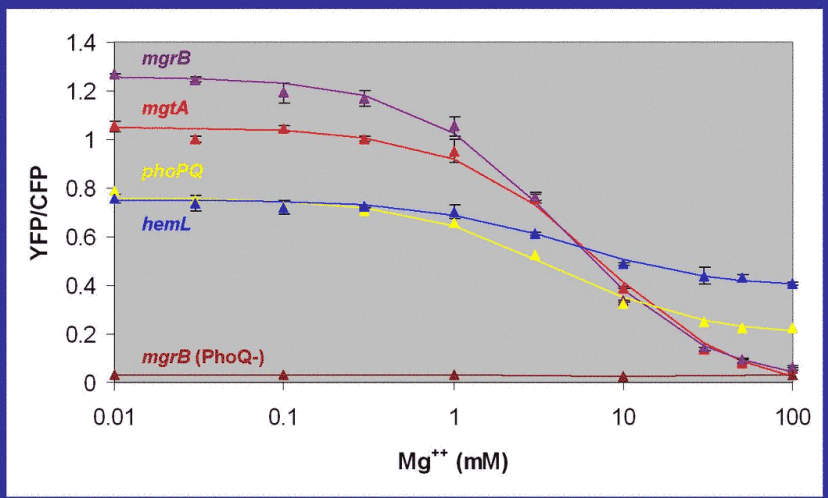

Mg2+, CAMPs
periplasmic stress
PO4 2-
PhoQ
CpxA
PhoR
PhoP
CpxR
PhoB
NH4 +
NtrB
NtrC
. . . ?
autoregulated:
P
RR
HK
Does positive feedback lead to switch-like behavior?

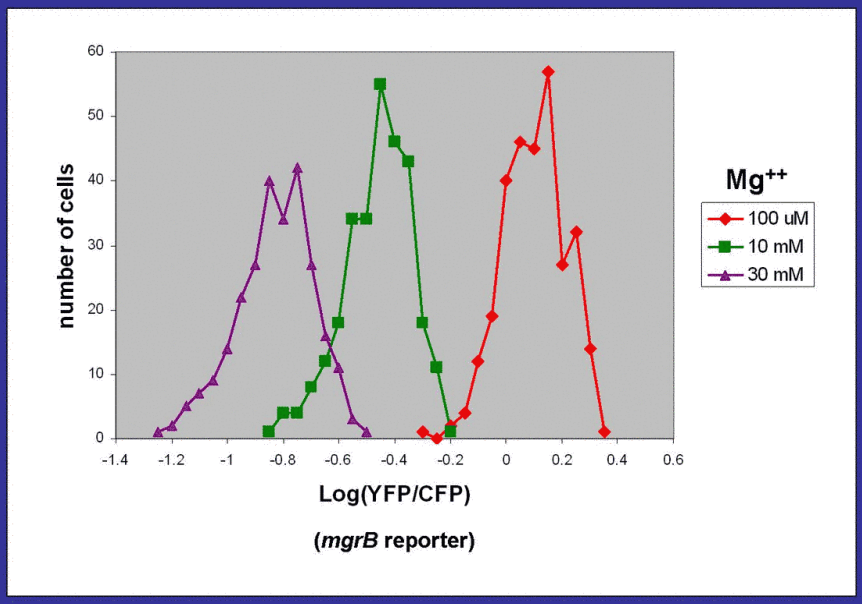

Low Mg++, ...
PhoQ
PhoQ
PhoQ
P
P
PhoP
PhoP
PhoP
phoPQ, mgtA, mgrB,
hemL, nagA, rstA,
slyB, vboR, yrbL, ...
Minagawa, et al 2003

Reporter genes for yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP) integrated in the chromosome:

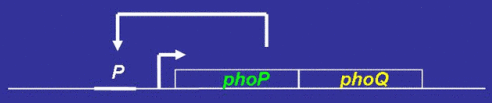




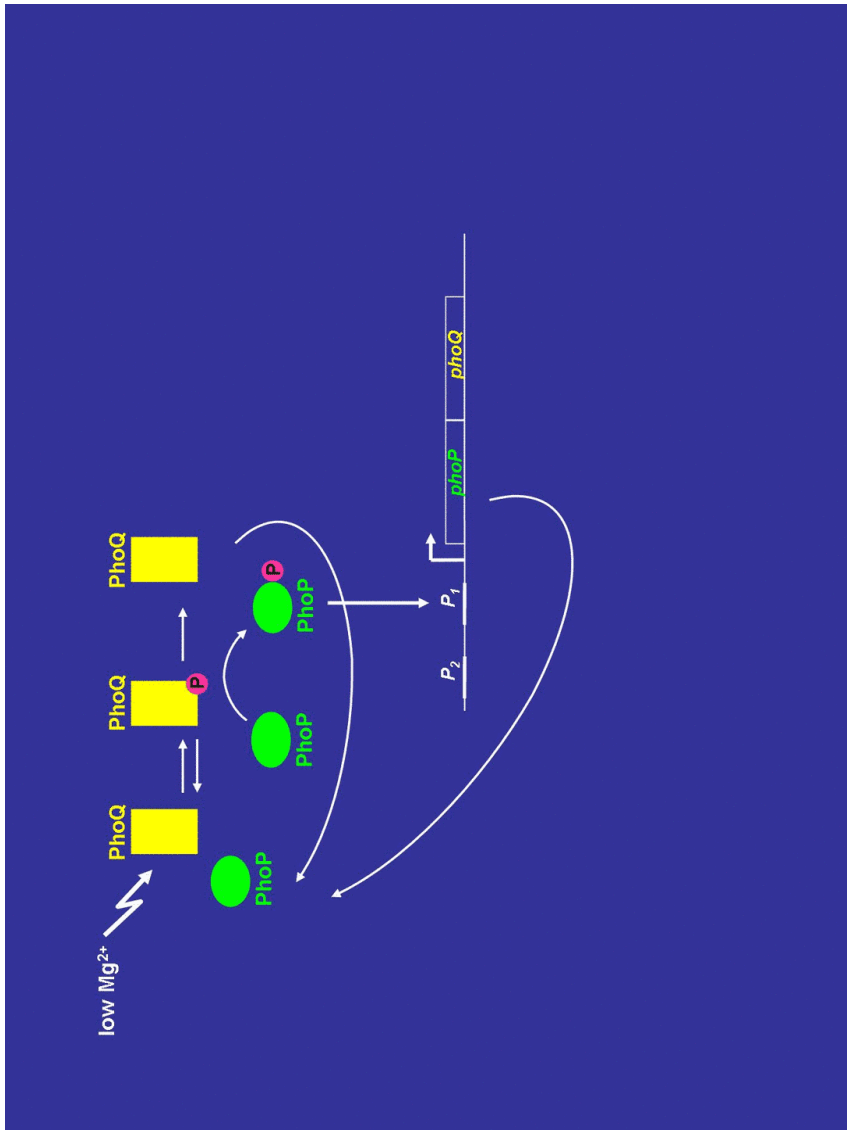


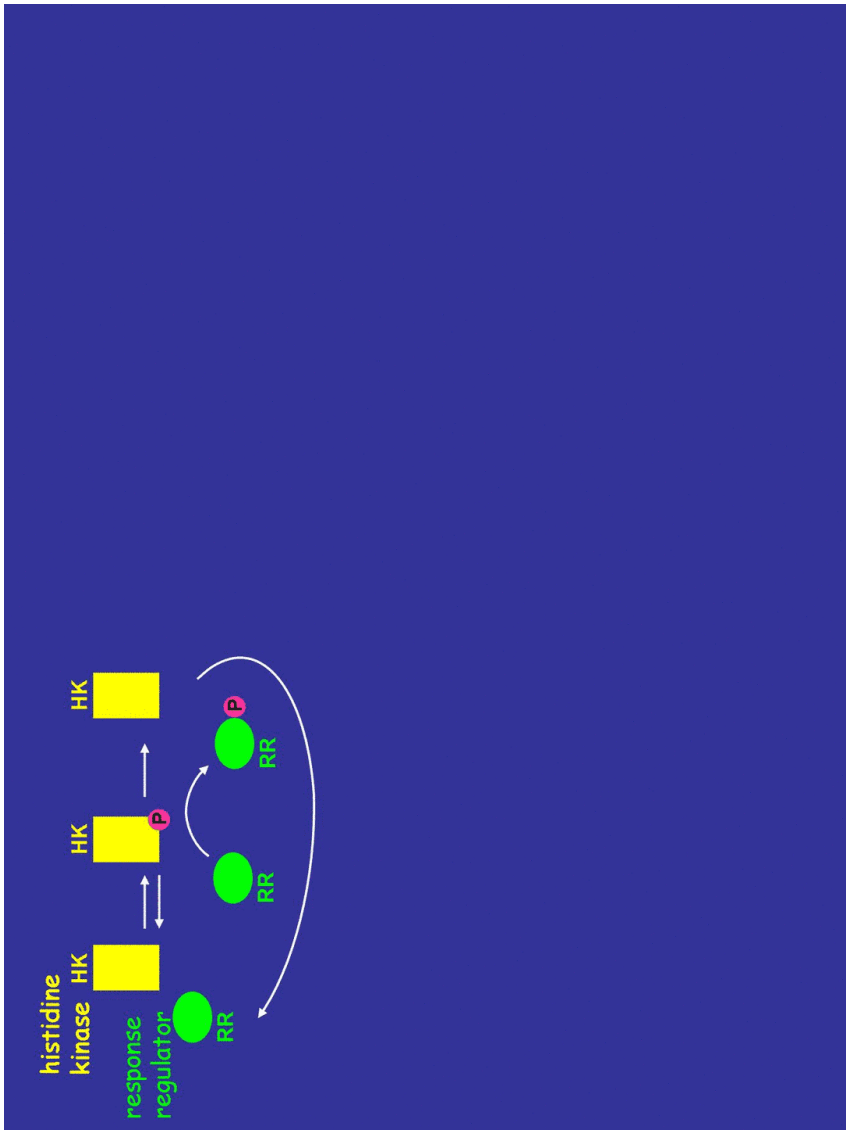


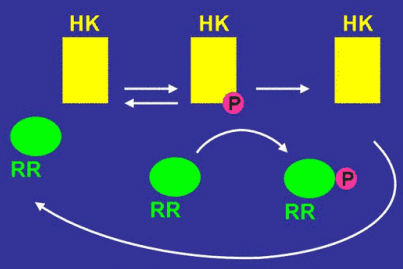
The PhoQ/PhoP circuit exhibits continuous (not switch-like or digital) control in single cells.

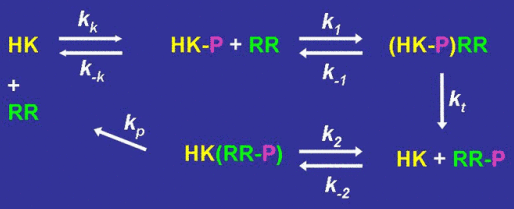


How does the circuit maintain a stable graded output?


PhoQ
PhoQ
PhoQ
P
P
PhoP
PhoP
PhoP
low Mg2+
P1
P2
phoP
phoQ


HK
HK
HK
P
P
RR
RR
RR
histidine kinase
response regulator



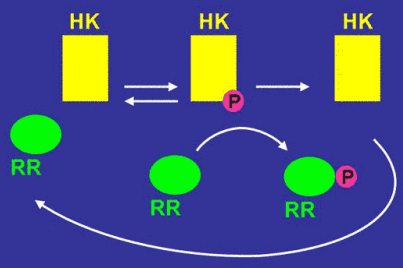


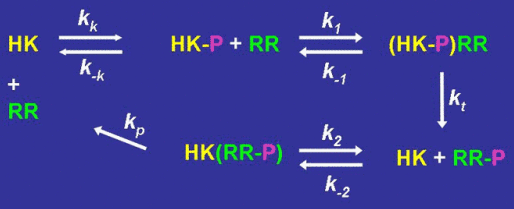
characteristic concentrations:

$C_p = (k_k / k_p)(k_p + k_{-2})/k_2$, $C_t = (k_{-k} / k_t)(k_t + k_{-1})/k_1$

C_p depends on the strength of the input signal

two conservation laws: $[HK]_{total}$, $[RR]_{total}$

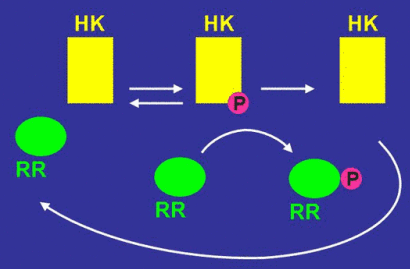


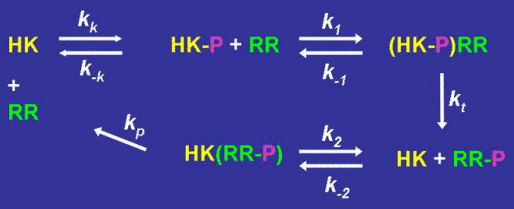


$C_p = (k_k / k_p)(k_p + k_{-2})/k_2$, $C_t = (k_{-k} / k_t)(k_t + k_{-1})/k_1$

1) $$[RR\text{-}P] = \frac{C_p\,[RR]}{C_t + [RR]}$$

output is bounded: $[RR\text{-}P] \le C_p$



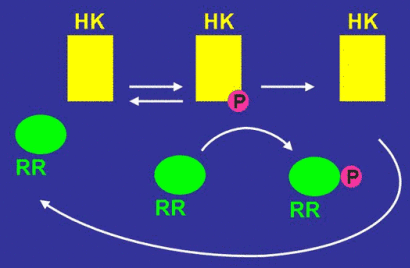


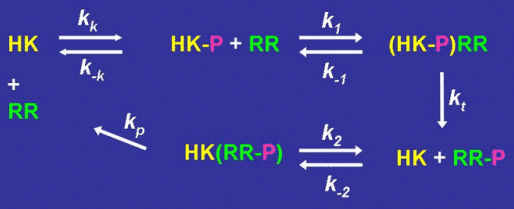
$C_p = (k_k / k_p)(k_p + k_{-2})/k_2$, $C_t = (k_{-k} / k_t)(k_t + k_{-1})/k_1$

1) $[RR\text{-}P] = \dfrac{C_p\,[RR]}{C_t + [RR]}$

2) $[RR]_{total} >> [HK]_{total} \Rightarrow [RR]_{total} \approx [RR] + [RR\text{-}P]$

$\therefore$ [RR-P] ~ independent of $[HK]_{total}$





$C_p = (k_k / k_p)(k_p + k_{-2})/k_2$, $C_t = (k_{-k} / k_t)(k_t + k_{-1})/k_1$

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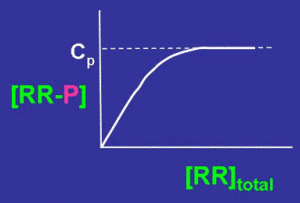
2) $[RR]_{total} >> [HK]_{total} \Rightarrow [RR]_{total} \approx [RR] + [RR\text{-}P]$

$\therefore$ [RR-P] ~ independent of $[HK]_{total}$

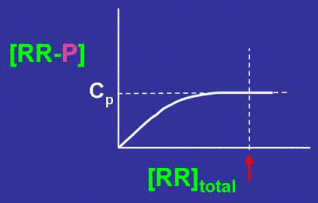
3) $[RR]_{total} >> C_p + C_t \Rightarrow [RR\text{-}P] \approx C_p$
(the output saturates)



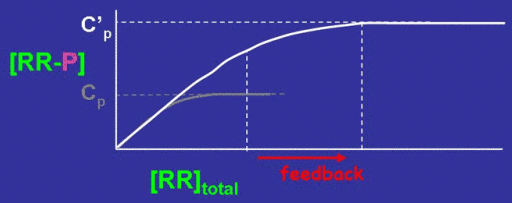
At saturation, the system is insensitive to variations in $[HK]_{total}$ and $[RR]_{total}$

Stable amplification from positive feedback.

Low to intermediate signal:
unstimulated promoter pushes system into saturation ⇒ feedback does nothing.



High signal ⇒ C_p increases to C'_p



Stable amplification from positive feedback.

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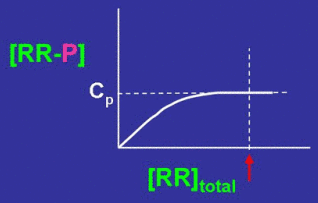
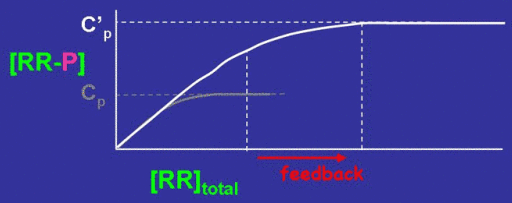
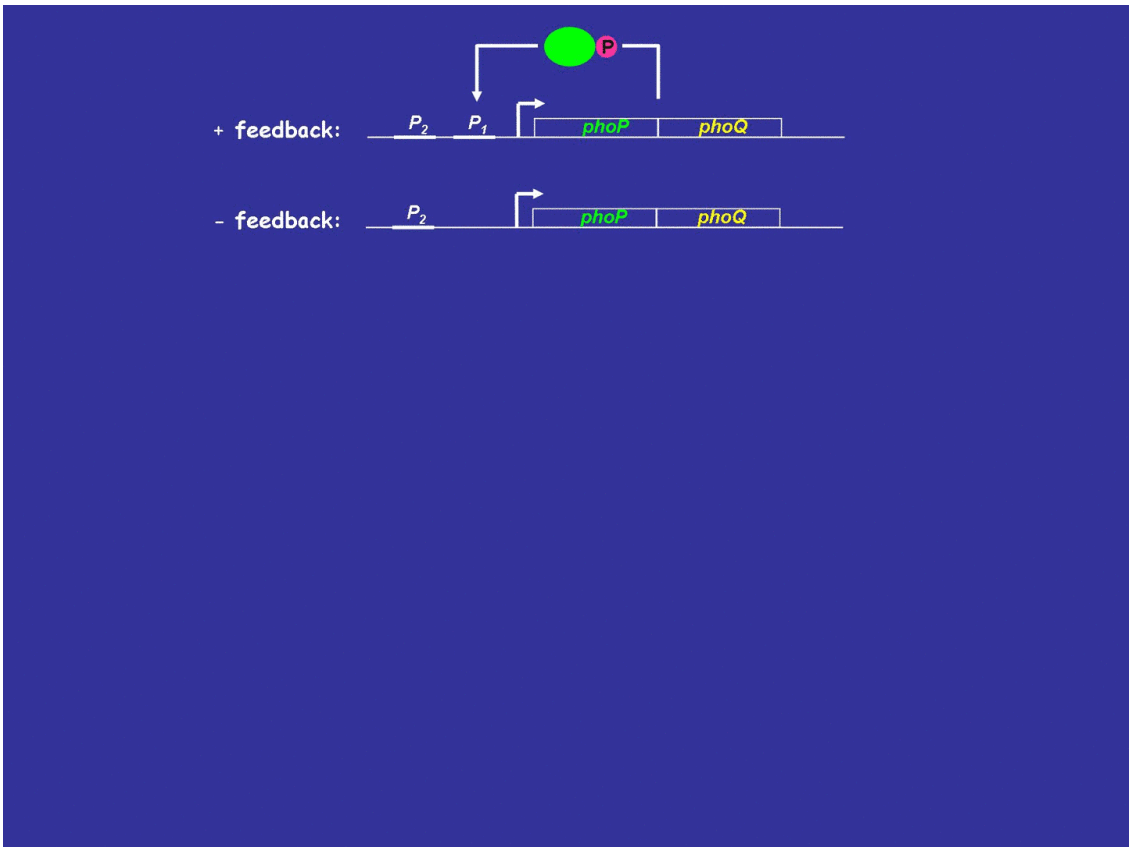


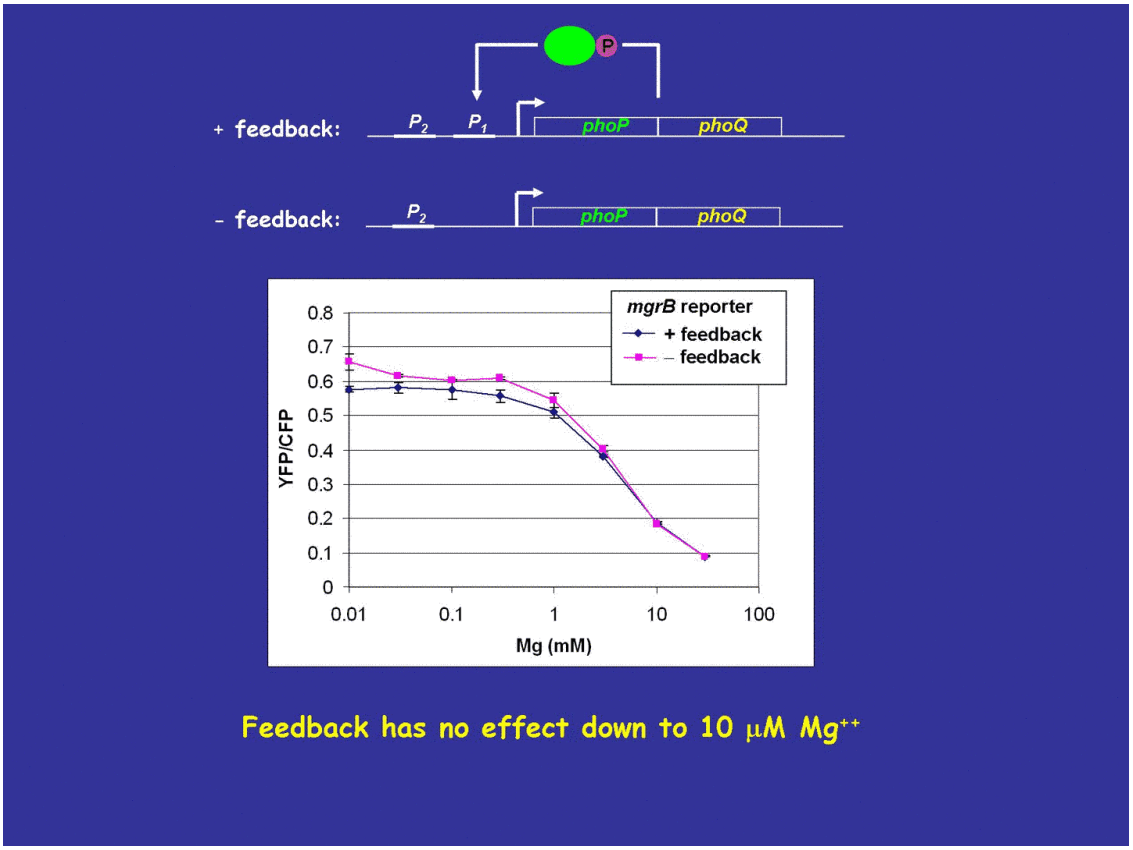
High signal ⇒ C_p increases to C'_p



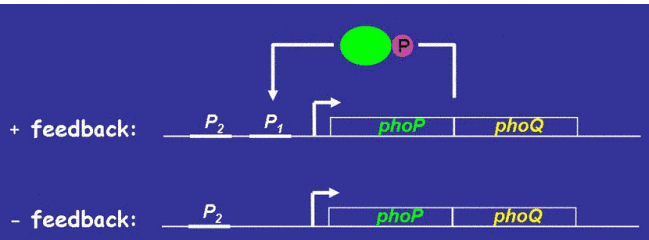
Predictions:

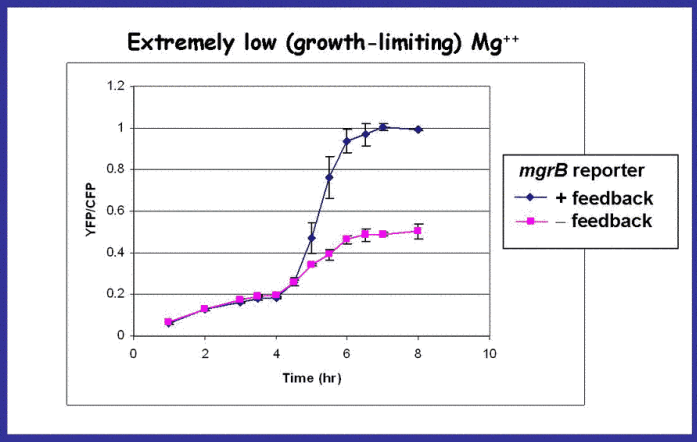
- The output is a graded function of applied stimulus (graded not switch-like behavior).
- Autoregulation is unimportant at low to intermediate stimulus.
- At high stimulus, autoregulation amplifies the output.
- At high stimulus, amplification is independent of $[HK]_{total}$.
- The output is a saturating function of $[RR]_{total}$.



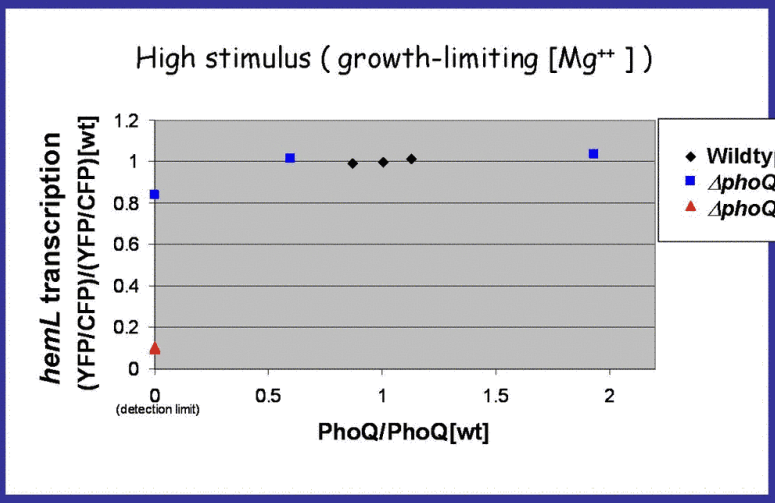


Feedback has no effect down to 10 μM Mg^{++}

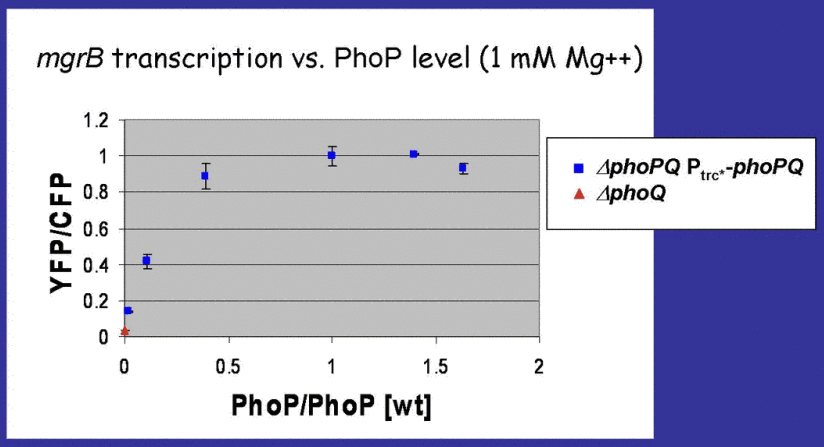




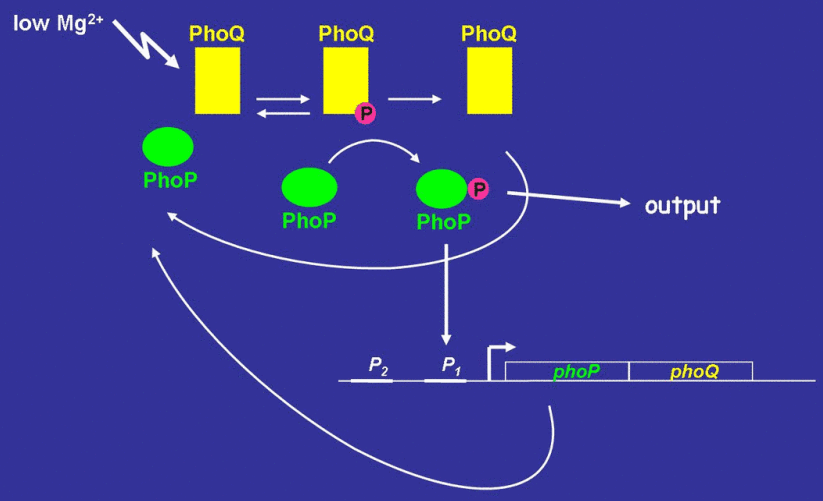
Feedback amplifies the signal at very high stimulus



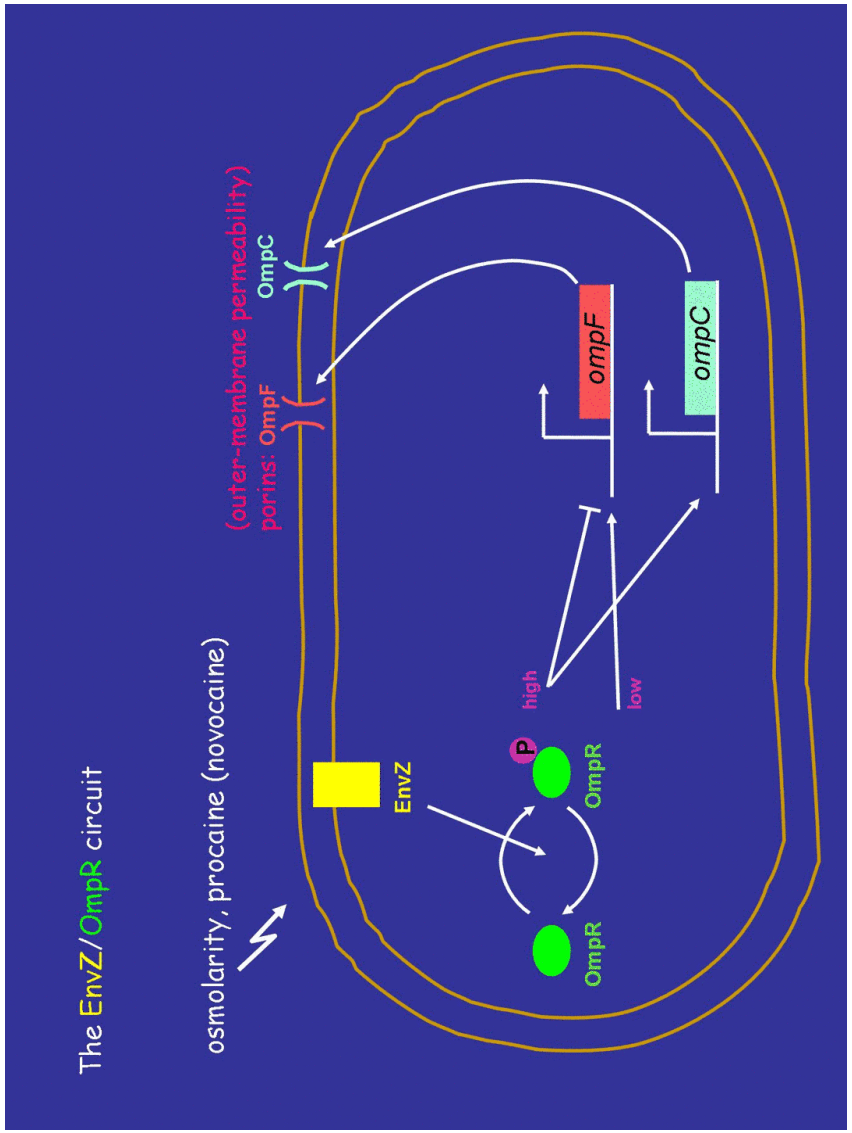
Output does not depend on $[PhoQ]_{total}$

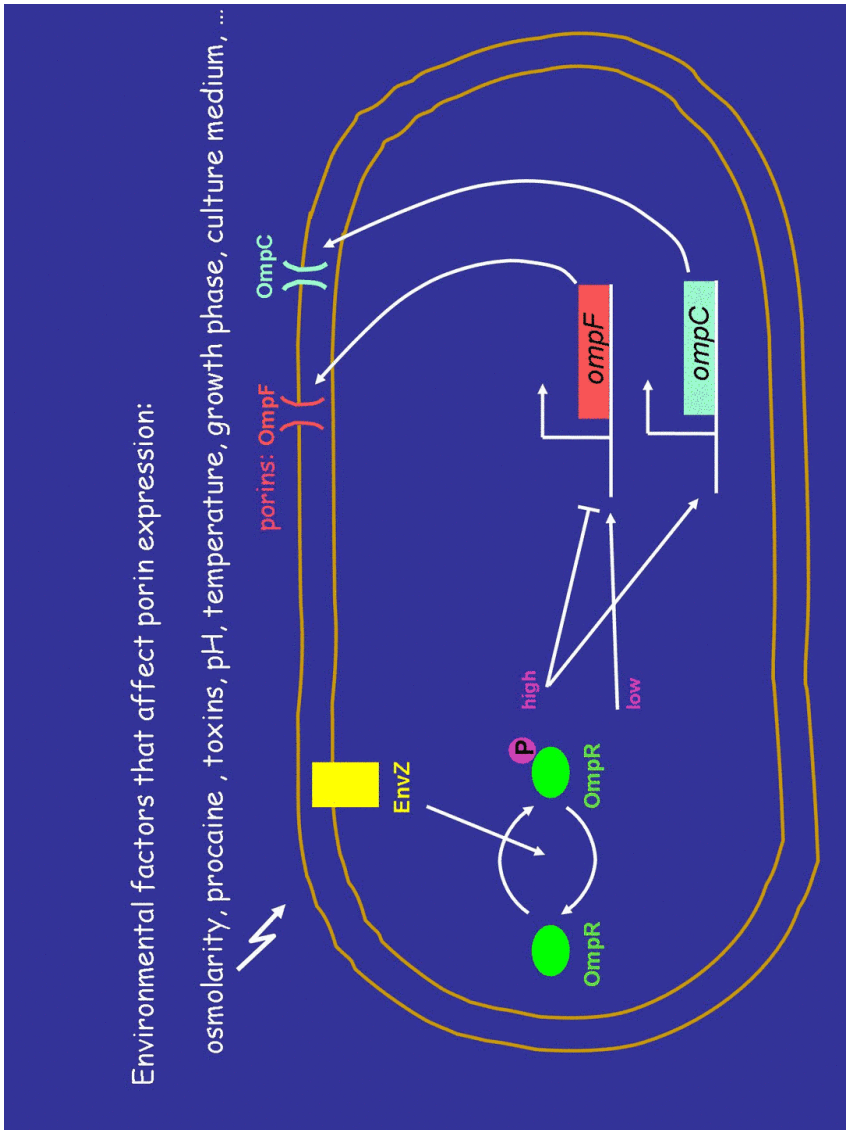


Output is a saturating function of $[PhoP]_{total}$



Bifunctional sensor kinase (phosphorylation cycle)
+ positive feedback ⇒ stable amplifier

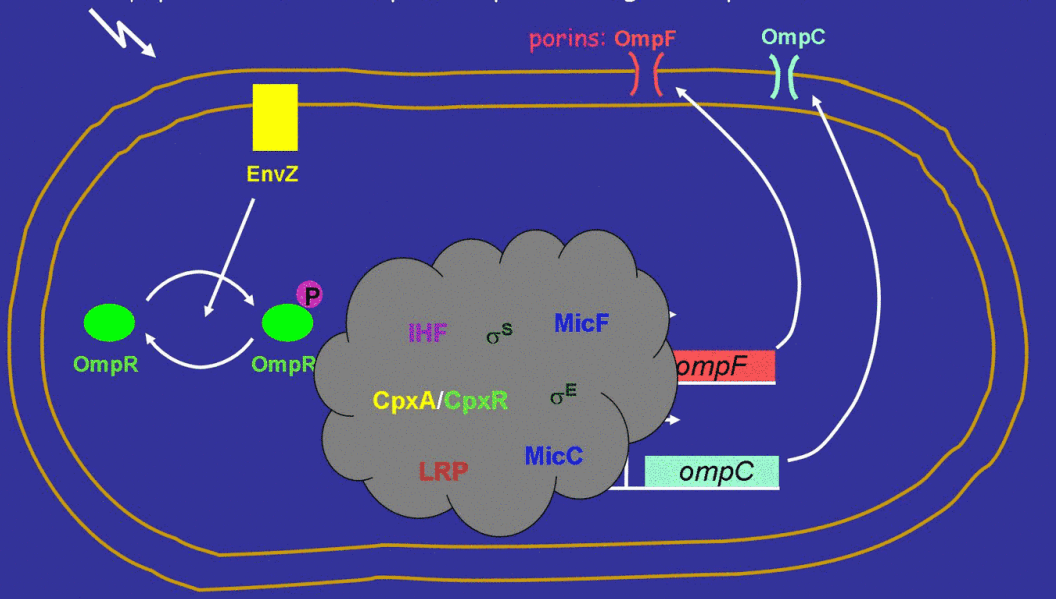

The EnvZ/OmpR circuit
osmolarity, procaine (novocaine)
(outer-membrane permeability)
porins: OmpF
OmpC
EnvZ
P
OmpR
OmpR
high
low
ompF
ompC


Environmental factors that affect porin expression:
osmolarity, procaine , toxins, pH, temperature, growth phase, culture medium, ...
porins: OmpF
OmpC
EnvZ
P
OmpR
OmpR
high
low
ompF
ompC

How to follow earlier steps in EnvZ/OmpR signaling?

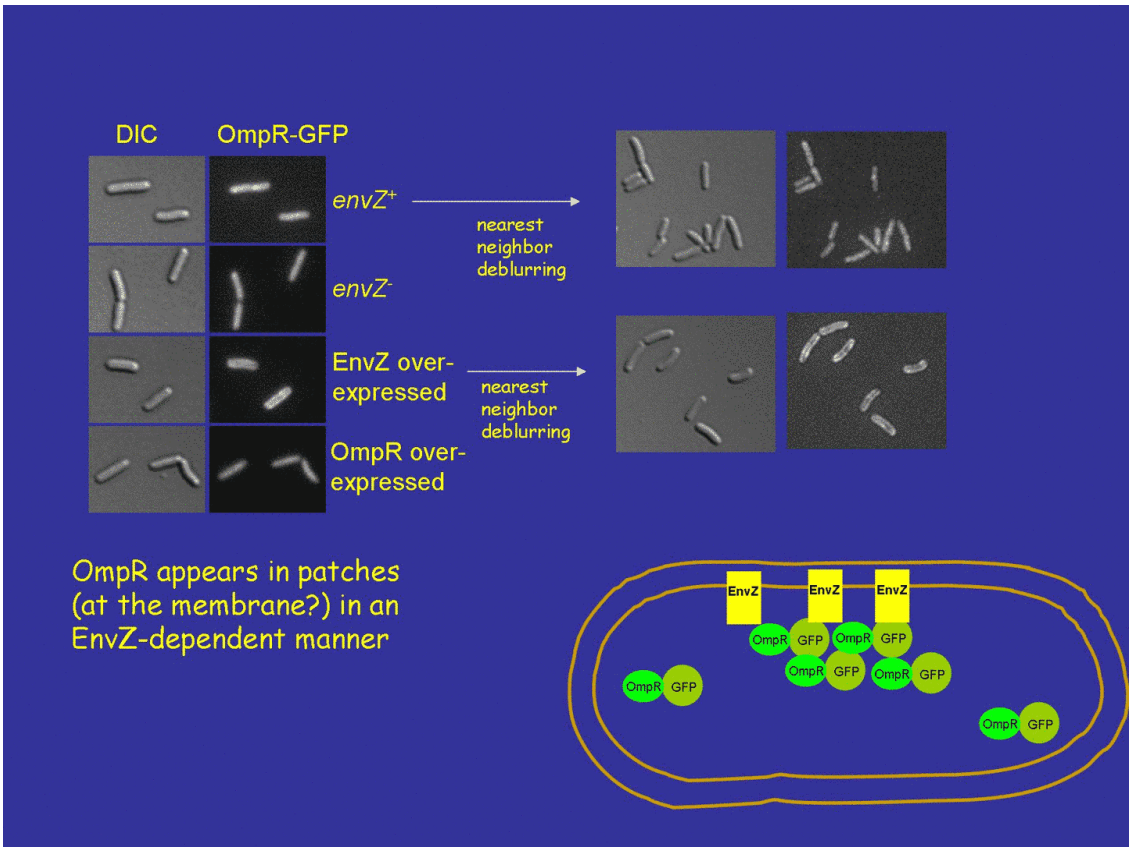
Environmental factors that affect porin expression:

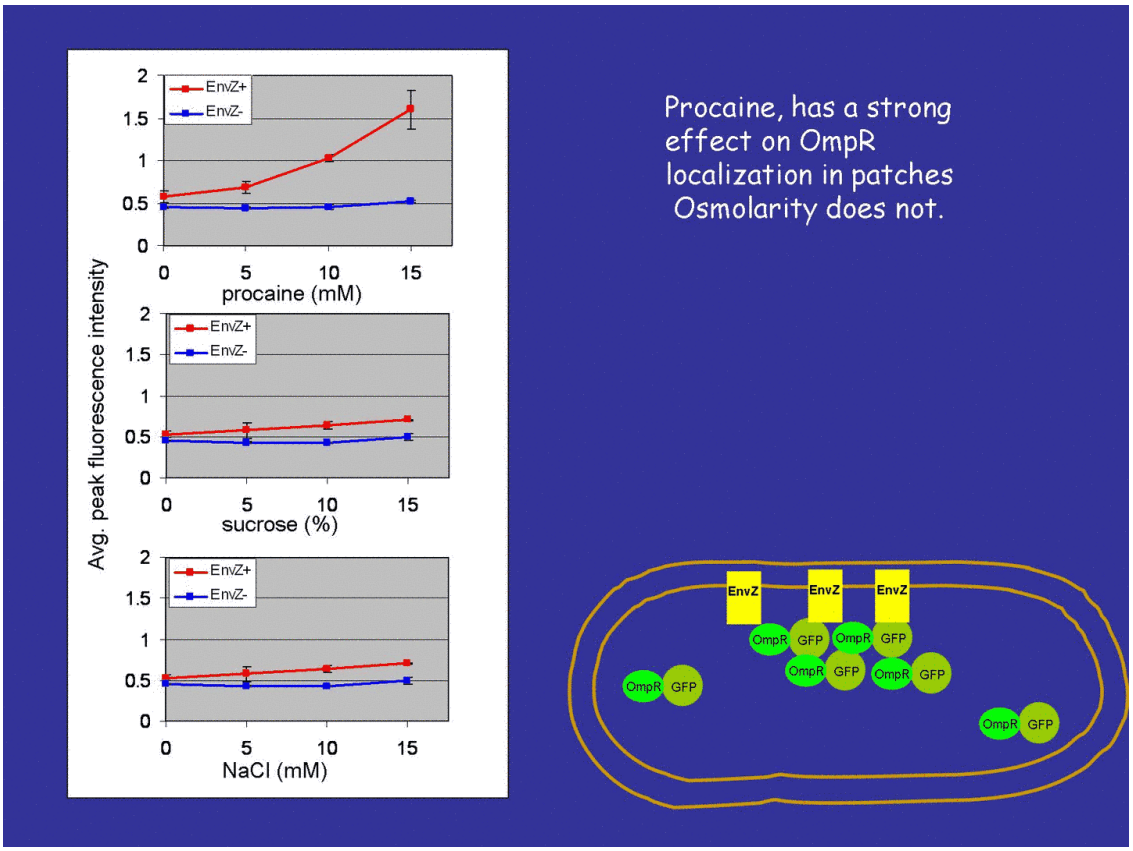
osmolarity, procaine , toxins, pH, temperature, growth phase, culture medium, ...

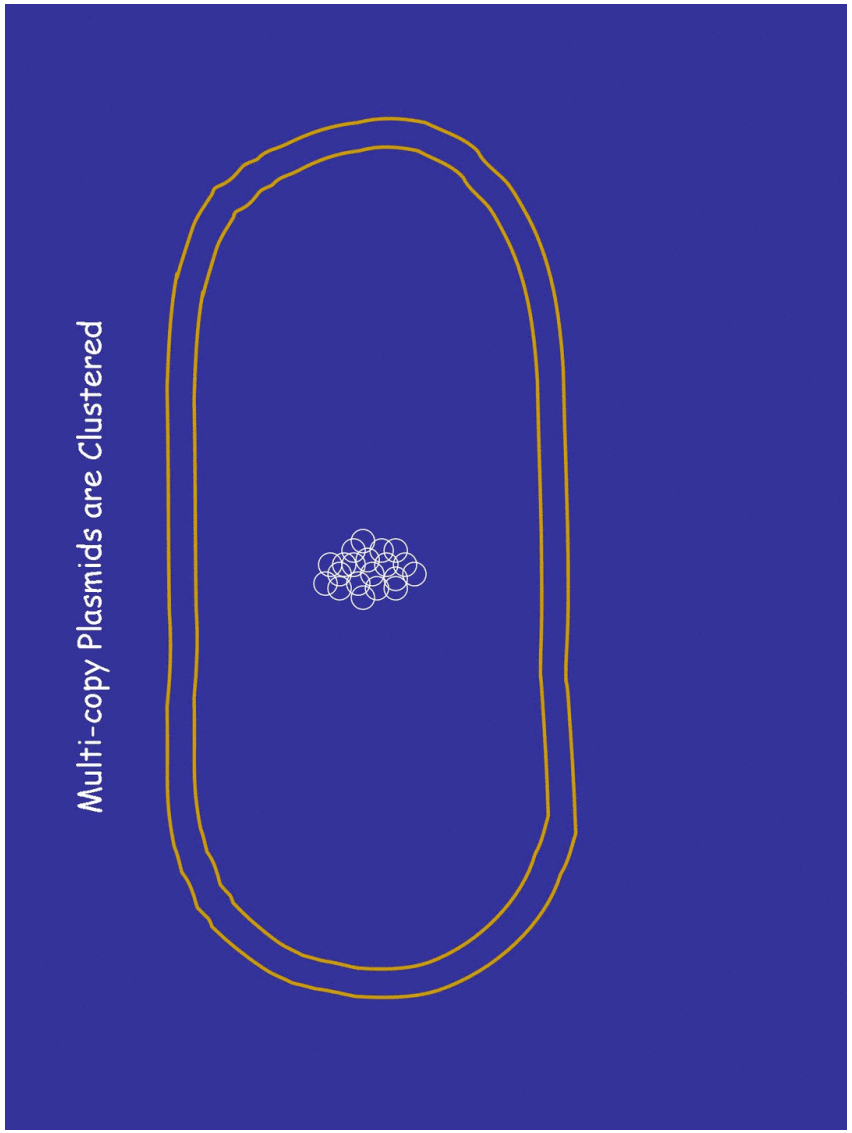


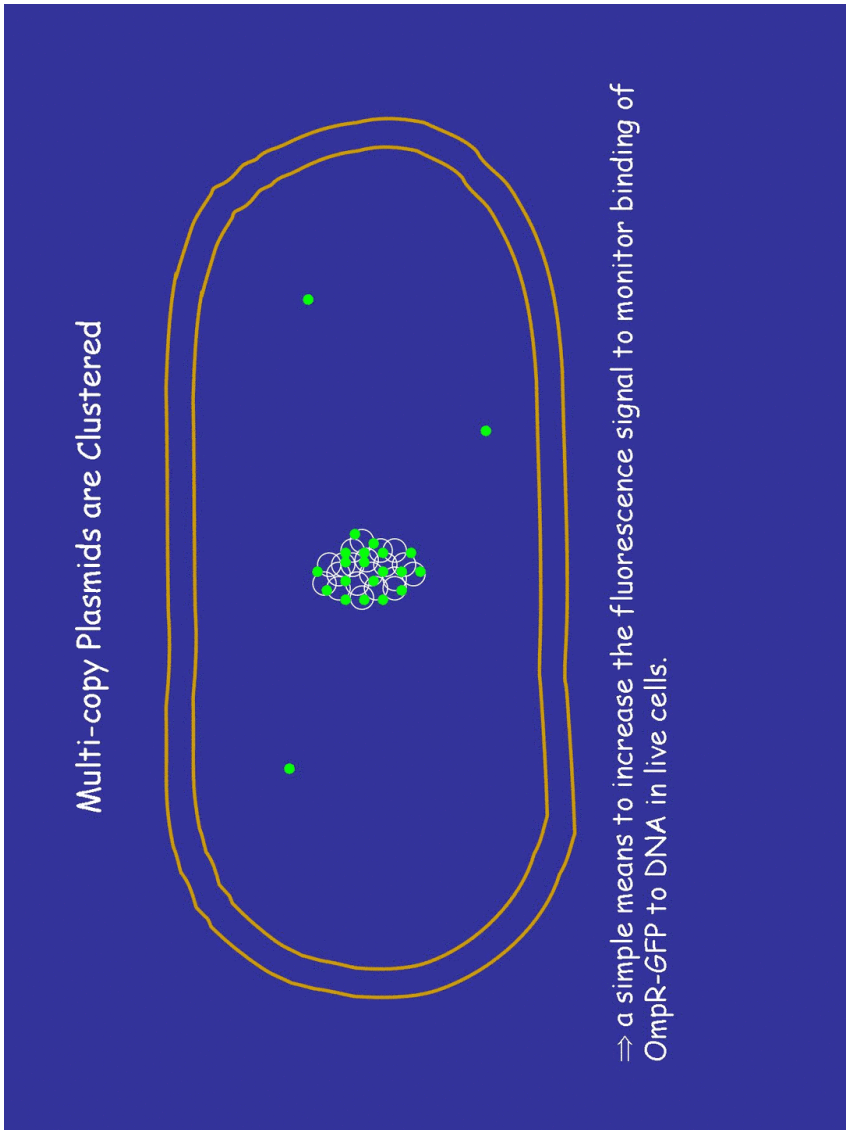
Fusion of green fluorescent protein (GFP) to OmpR

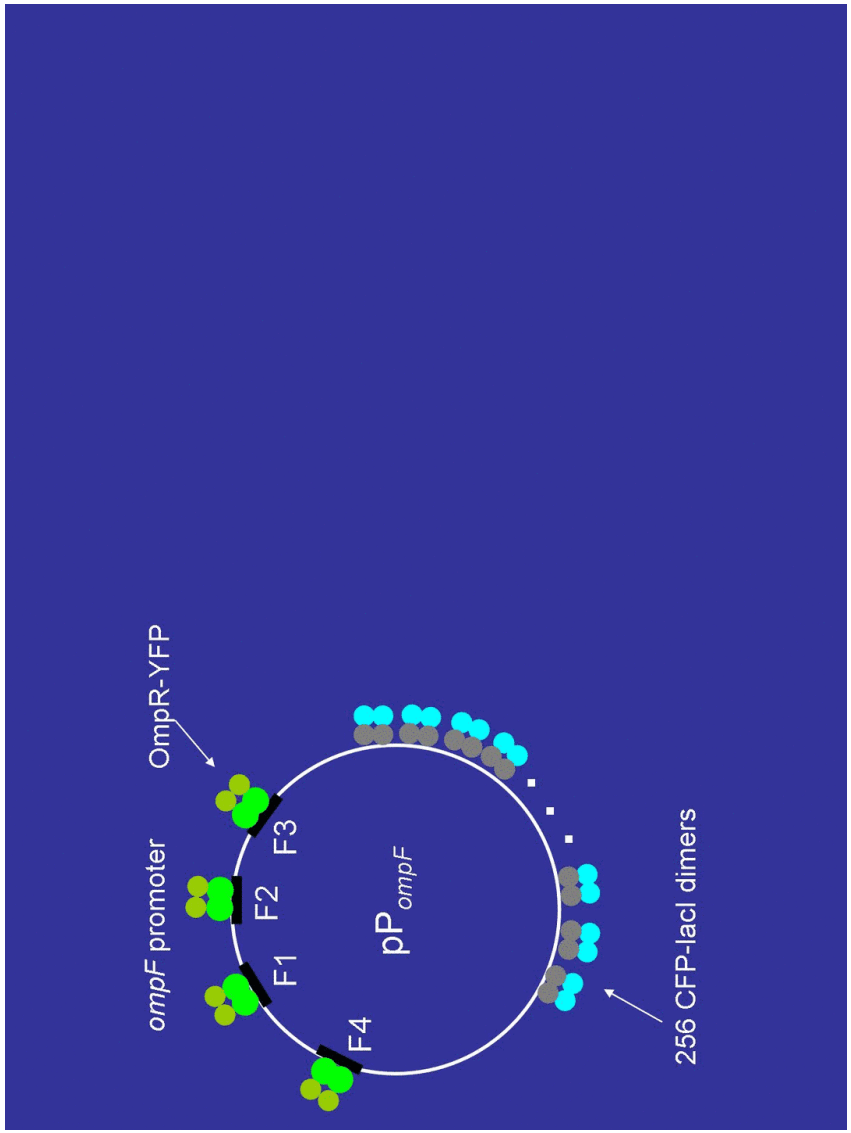
Image cellular location of OmpR:
association of OmpR with EnvZ
binding of OmpR to DNA.

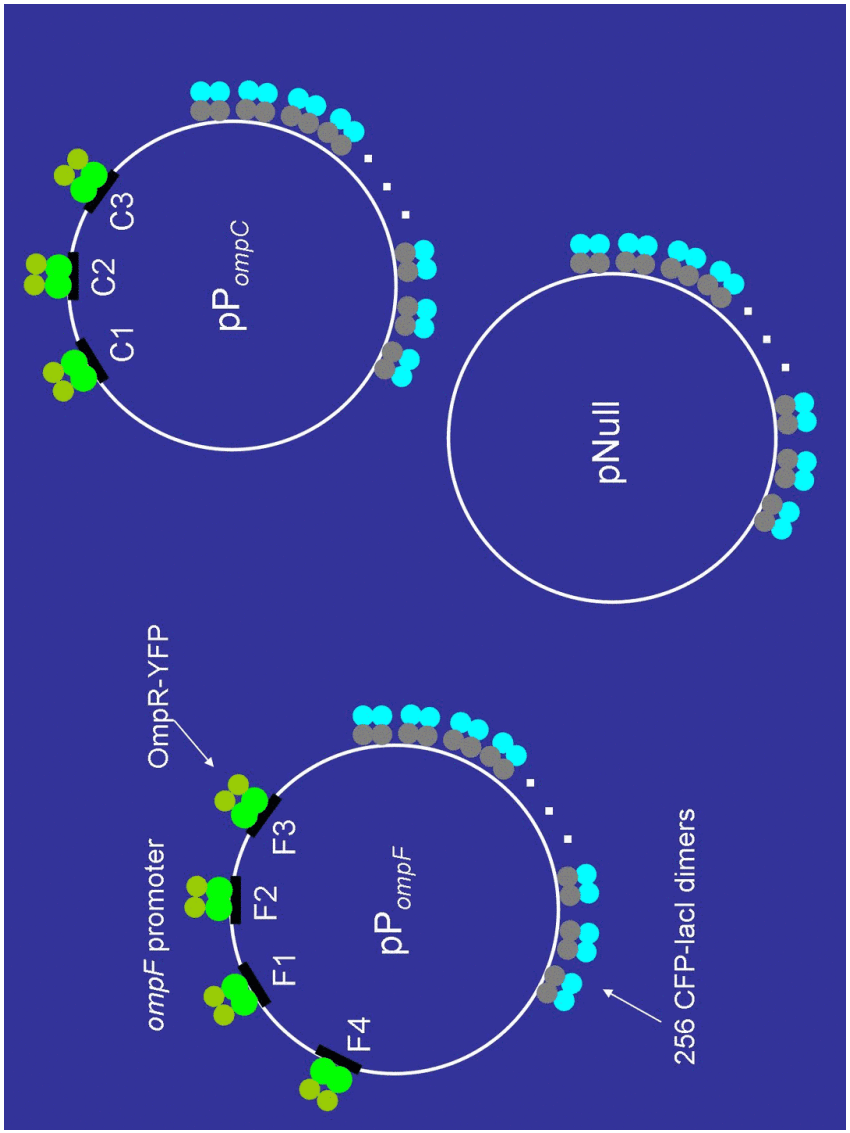

DIC
OmpR-GFP
envZ+
envZ-
EnvZ over-expressed
OmpR over-expressed
nearest neighbor deblurring
nearest neighbor deblurring
OmpR appears in patches (at the membrane?) in an EnvZ-dependent manner
EnvZ
OmpR
GFP

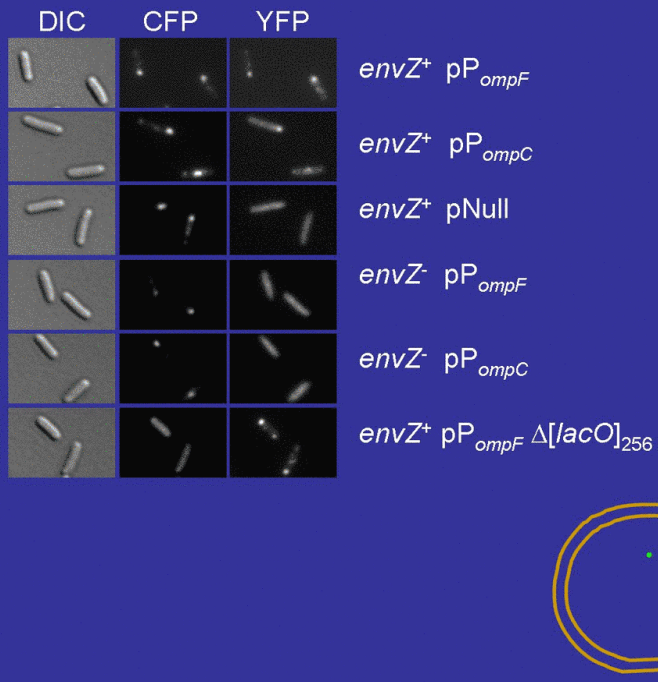

EnvZ+
EnvZ-
Avg. peak fluorescence intensity
procaine (mM)
sucrose (%)
NaCl (mM)
Procaine, has a strong effect on OmpR localization in patches
Osmolarity does not.
EnvZ
OmpR
GFP

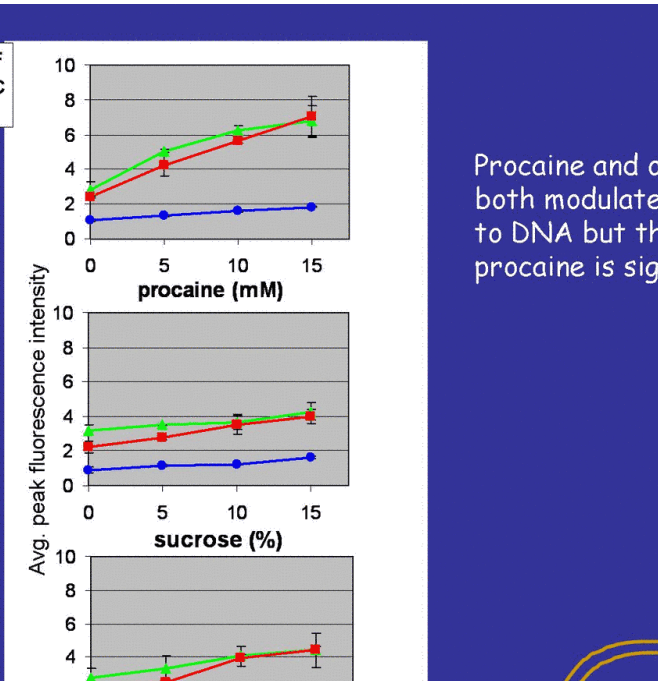

Multi-copy Plasmids are Clustered

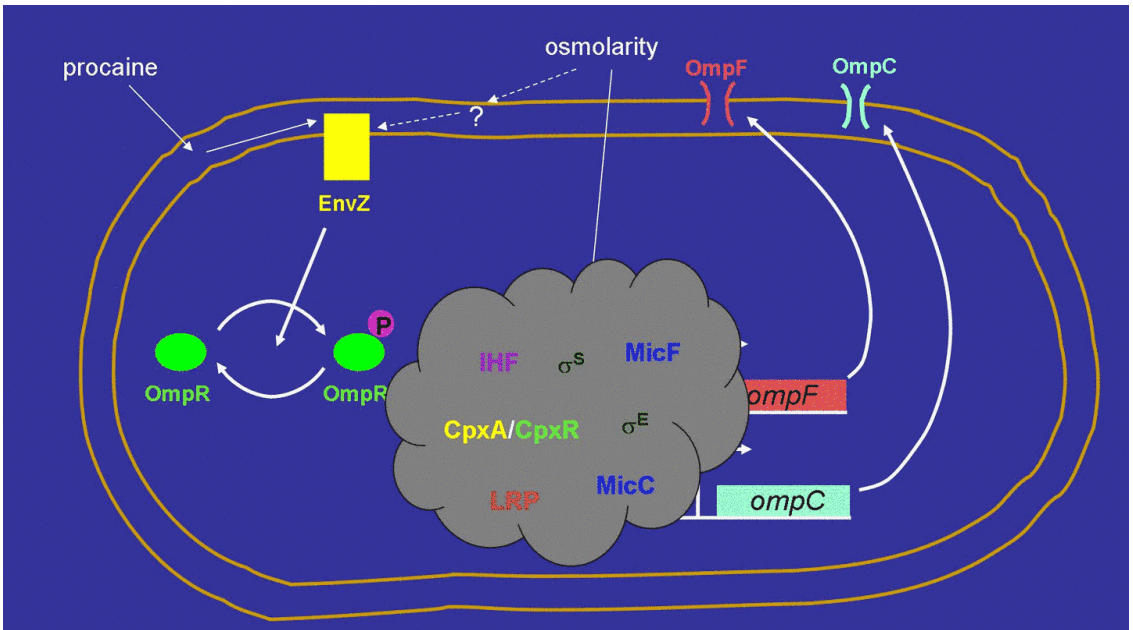

Multi-copy Plasmids are Clustered
⇒ a simple means to increase the fluorescence signal to monitor binding of OmpR-GFP to DNA in live cells.


OmpR-YFP
ompF promoter
F1
F2
F3
F4
pP ompF
256 CFP-lacI dimers

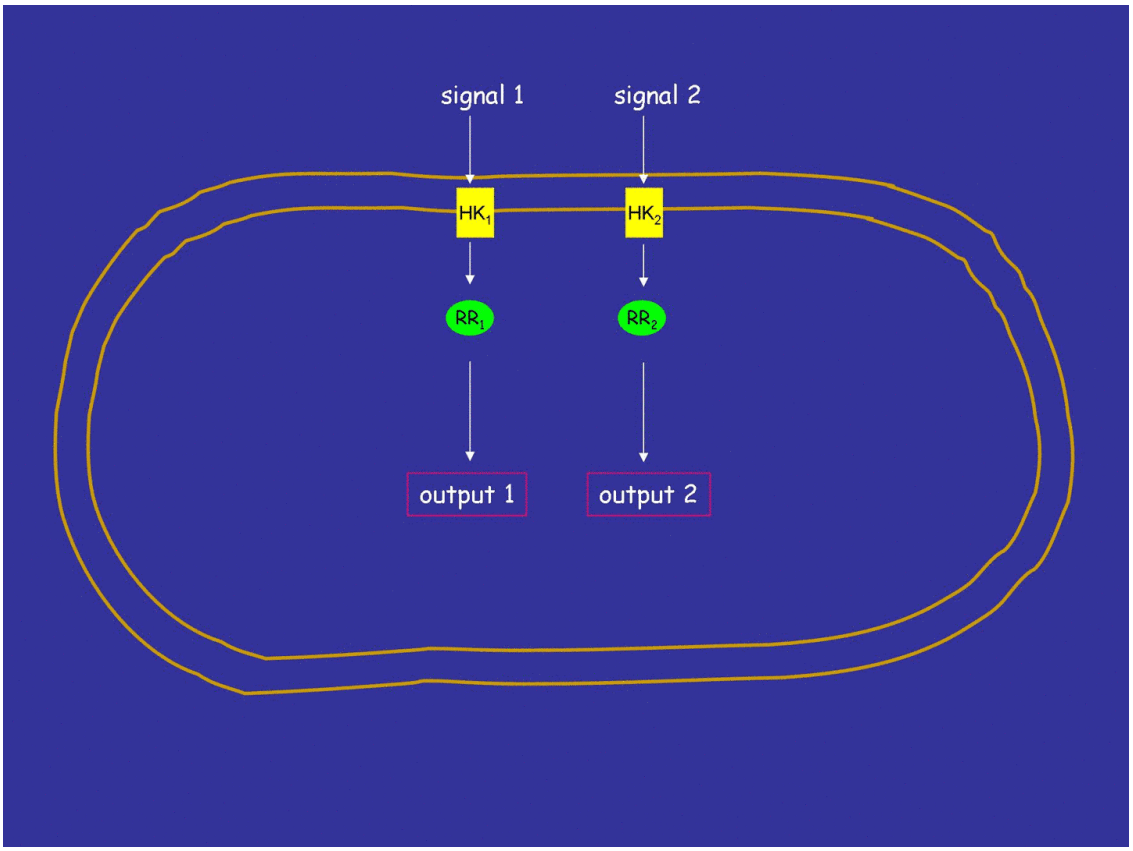

C1
C2
C3
pP ompC
pNull
OmpR-YFP
ompF promoter
F1
F2
F3
F4
pP ompF
256 CFP-lacI dimers

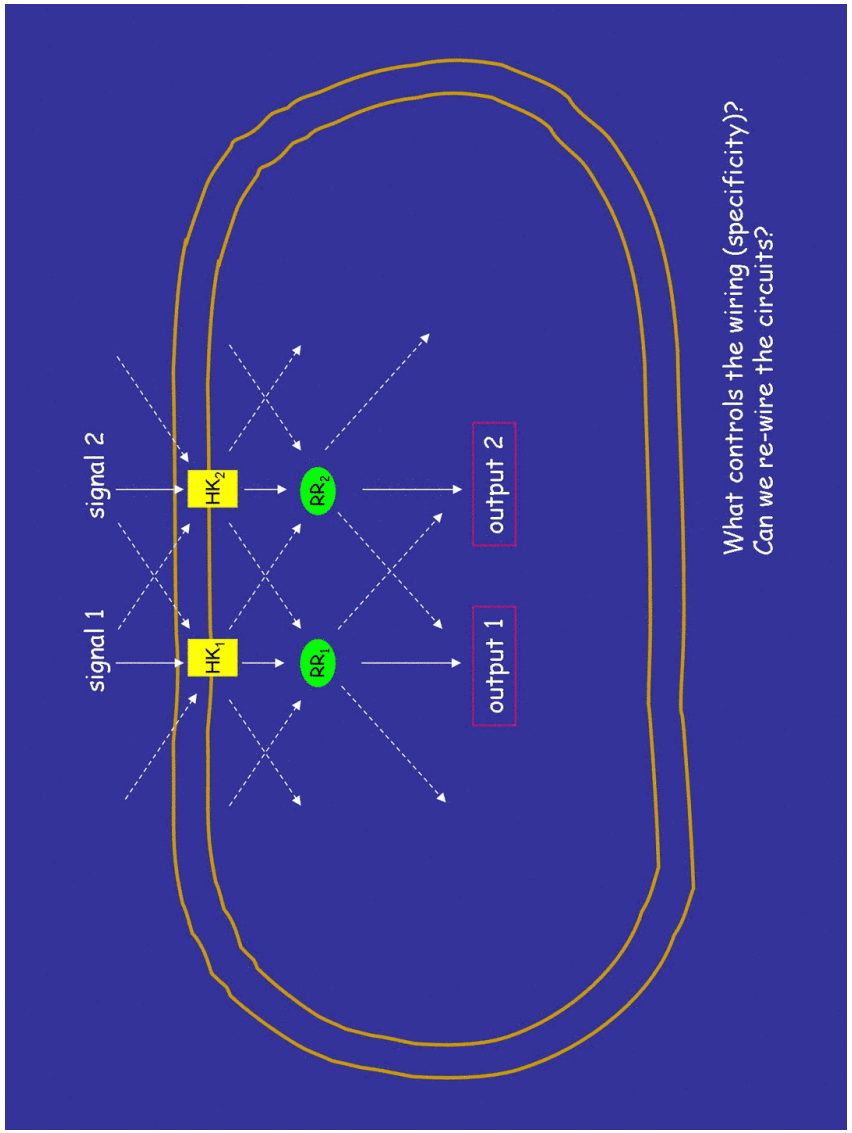

DIC
CFP
YFP
envZ+ pP ompF
envZ+ pP ompC
envZ+ pNull
envZ- pP ompF
envZ- pP ompC
envZ+ pP ompF Δ[lacO]256


pPompF
pPompC
pNull
Avg. peak fluorescence intensity
procaine (mM)
sucrose (%)
NaCl (mM)
Procaine and osmolarity both modulate OmpR-GFP binding to DNA but the effect of procaine is significantly stronger.



- New method for visualizing protein - DNA binding in vivo (requires a functional GFP fusion).
- Why does OmpR-GFP show EnvZ-dependent localization?
- Does osmolarity signal through EnvZ at all?
- What is the stimulus for EnvZ?




signal 2
signal 1
HK_2
HK_1
RR_2
RR_1
output 2
output 1
What controls the wiring (specificity)?
Can we re-wire the circuits?