Dynamics of post-translational genetic regulation in enteric bacteria

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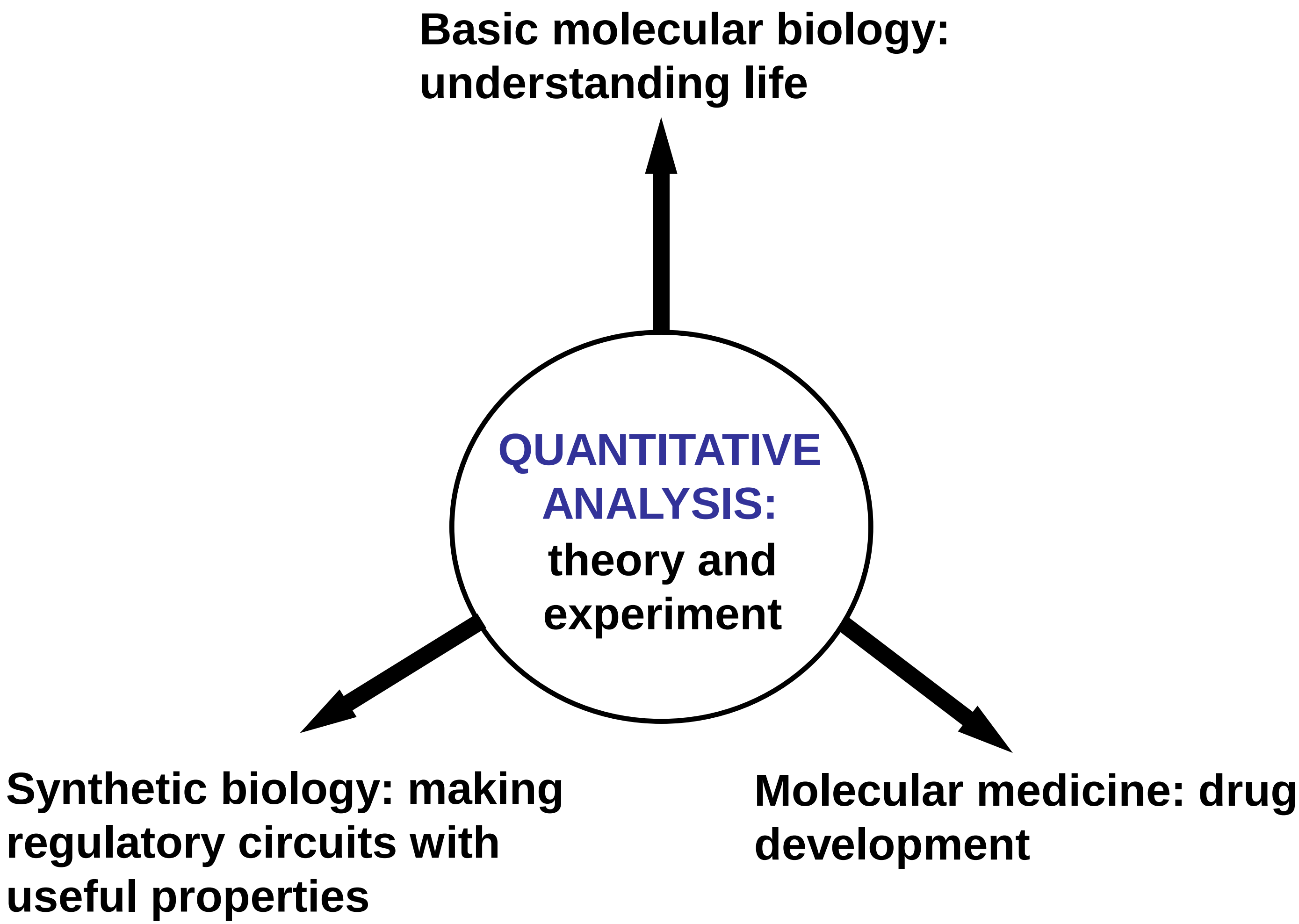
Basic molecular biology: understanding life
QUANTITATIVE ANALYSIS:
theory and experiment
Synthetic biology: making regulatory circuits with useful properties
Molecular medicine: drug development

This talk

Mathematical modeling

Genotype → phenotype

This talk

Mathematical modeling

Genotype → phenotype

Signal integration systems: structure → function

This talk

Mathematical modeling

Genotype → phenotype

Signal integration systems: structure → function

Bacterial antibiotic resistance mechanisms

Main questions

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1. What are the rules that govern the dynamics of genetic circuits involving protein–protein interactions?

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2. What are the functional consequences of structural diversity in genetic circuits of closely related bacterial species?

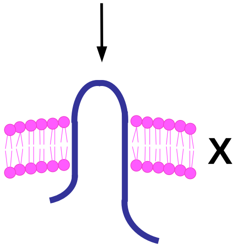
Extracellular signals modulate gene expression

Signal **Metal ions, small molecules, pH, etc.**

Extracellular signals modulate gene expression

Signal — **Metal ions, small molecules, pH, etc.**

Sensor

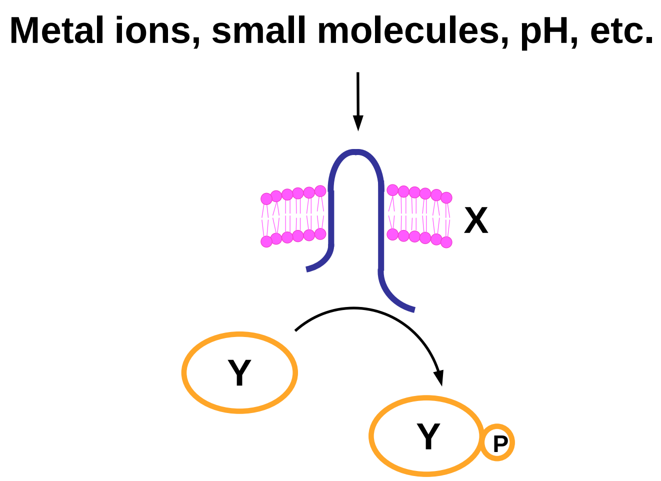


Extracellular signals modulate gene expression

Signal

Sensor

Regulatory circuit



Extracellular signals modulate gene expression

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Metal ions, small molecules, pH, etc.

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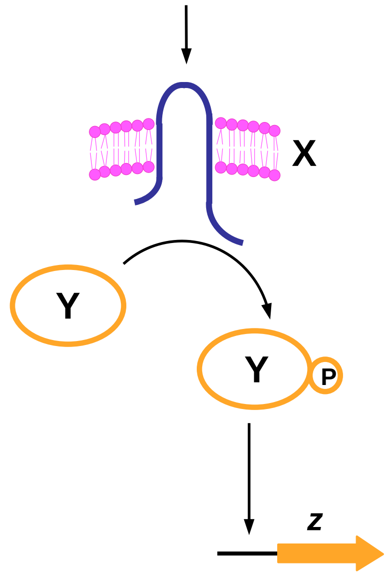
Regulatory circuit

Effectors



Extracellular signals modulate gene expression

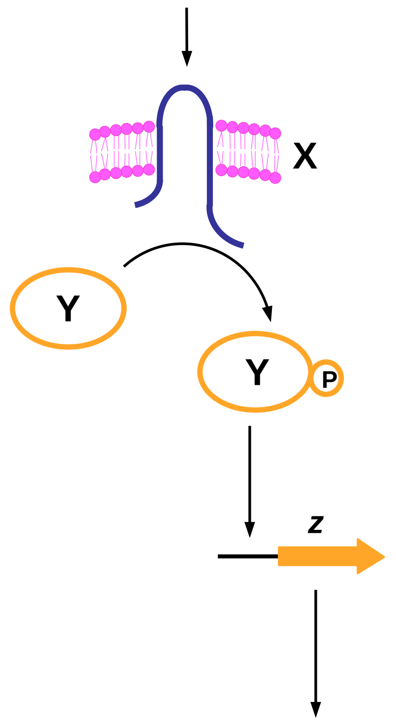
Signal — **Metal ions, small molecules, pH, etc.**

Sensor

Regulatory circuit

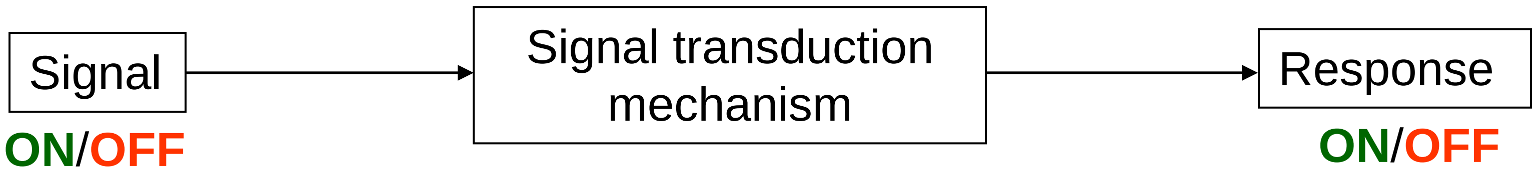
Effectors

Response — **Physiological reaction**



Qualitative vs. quantitative analysis of signal transduction

1. Qualitative analysis



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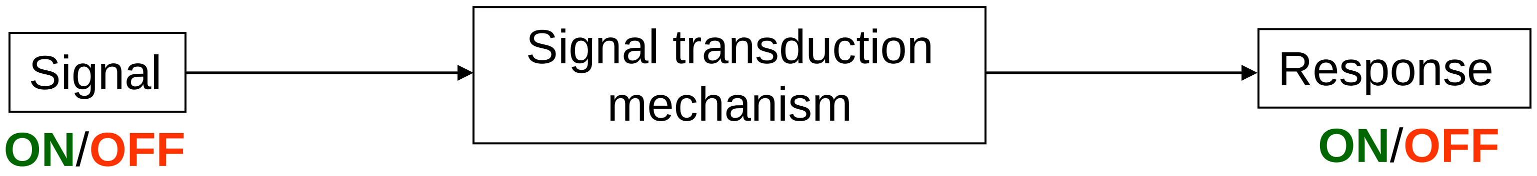
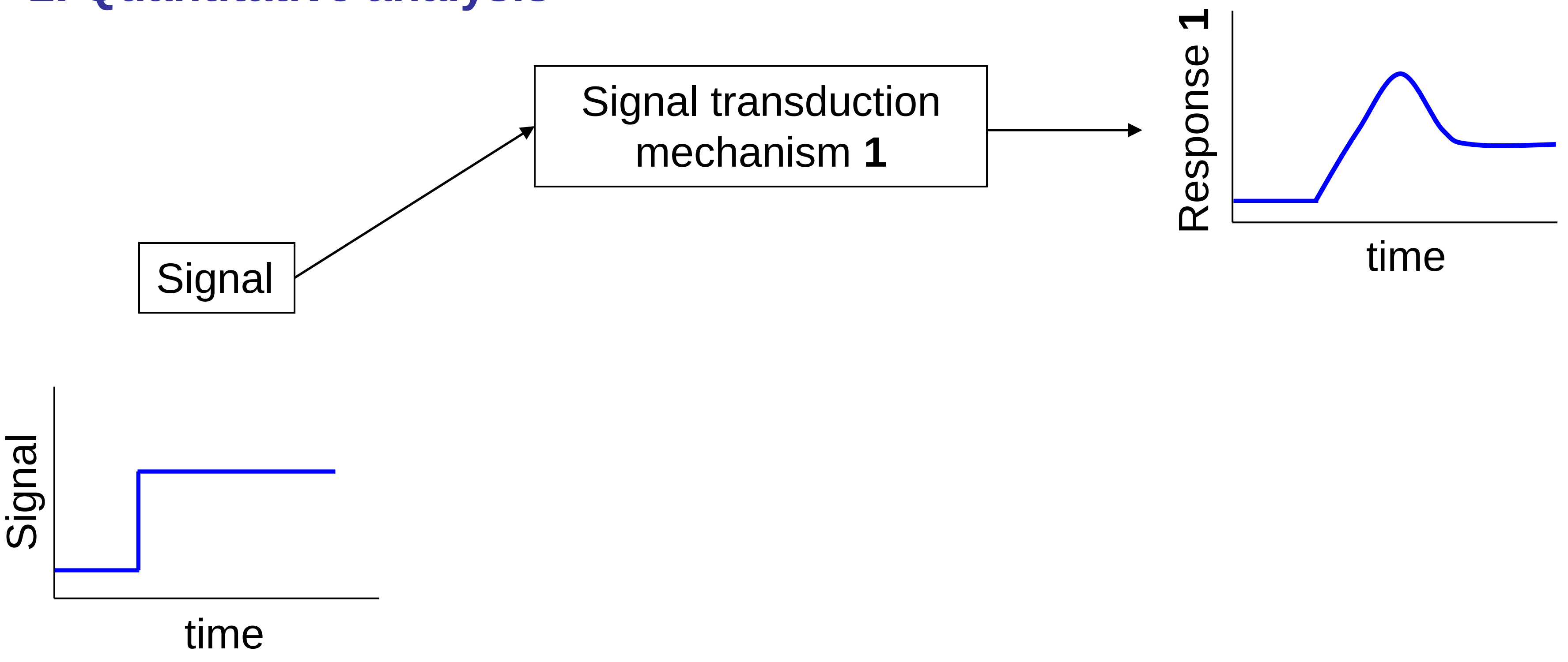


2. Quantitative analysis



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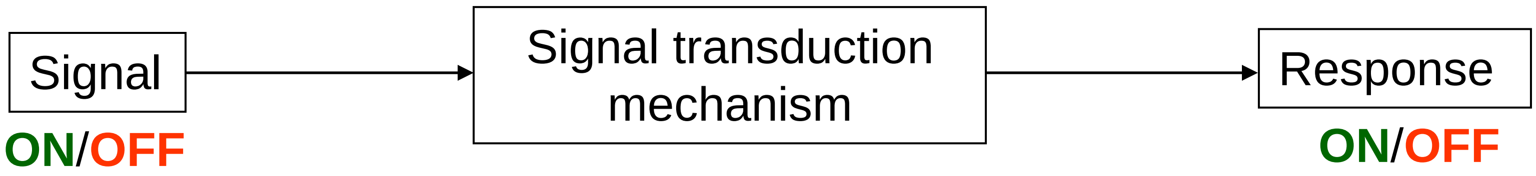

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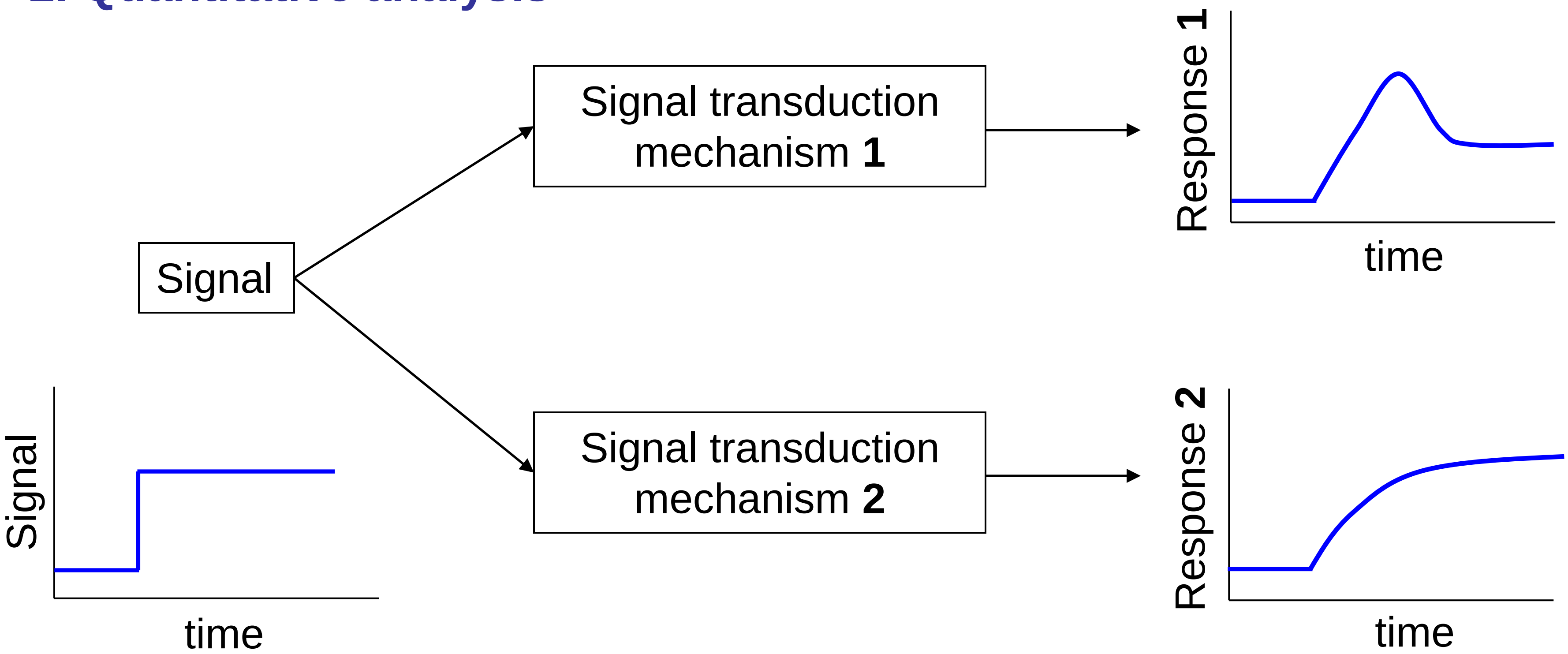


Mathematical modeling of signal transduction mechanisms

Experiment

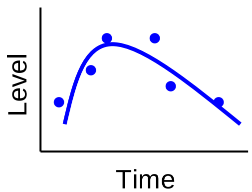
Conceptual model

A ⇄ B

Mathematical model

$$\frac{dX}{dt} = S\nu(X(t))$$

Model verification



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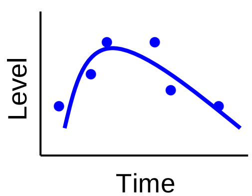
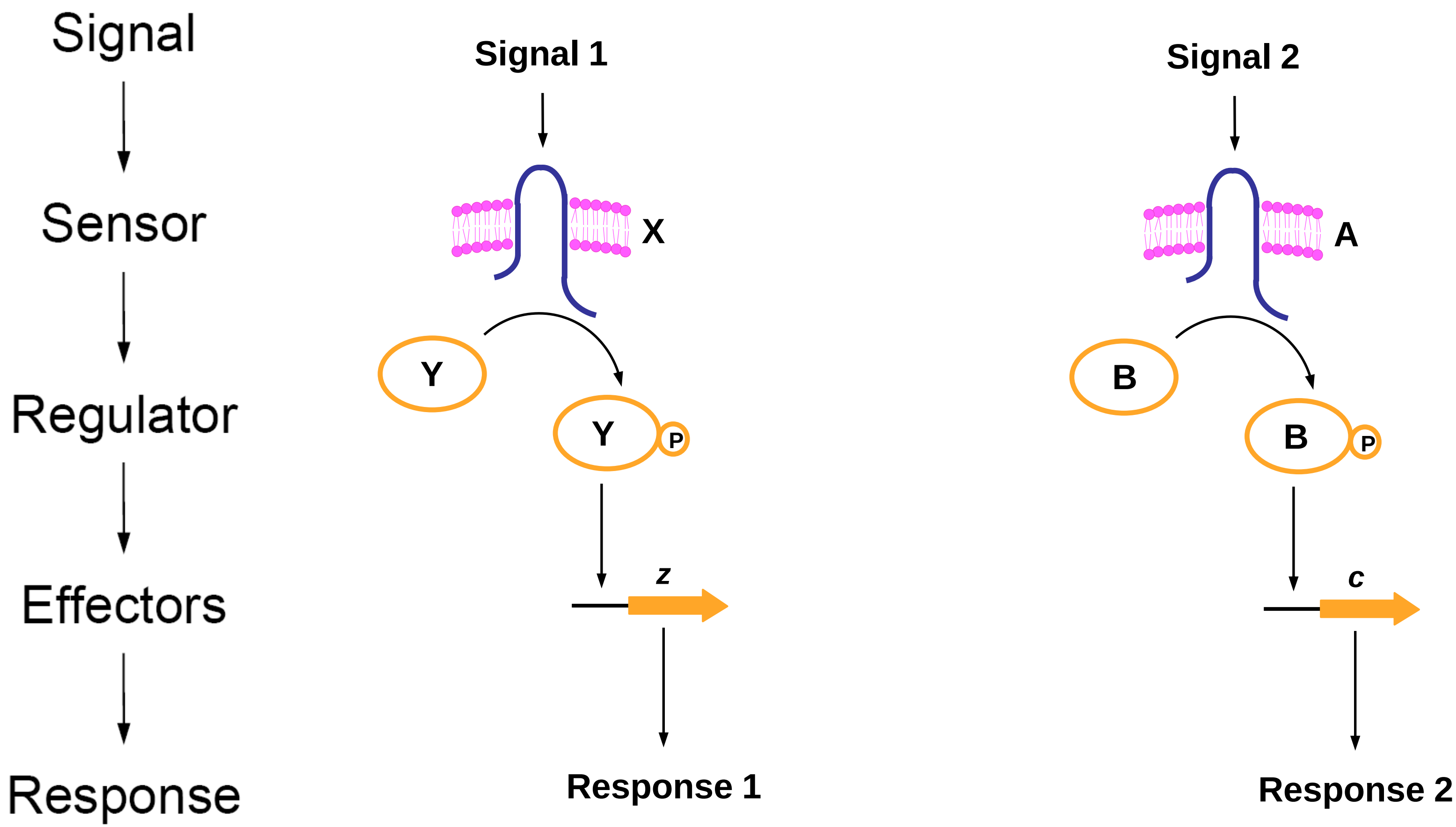


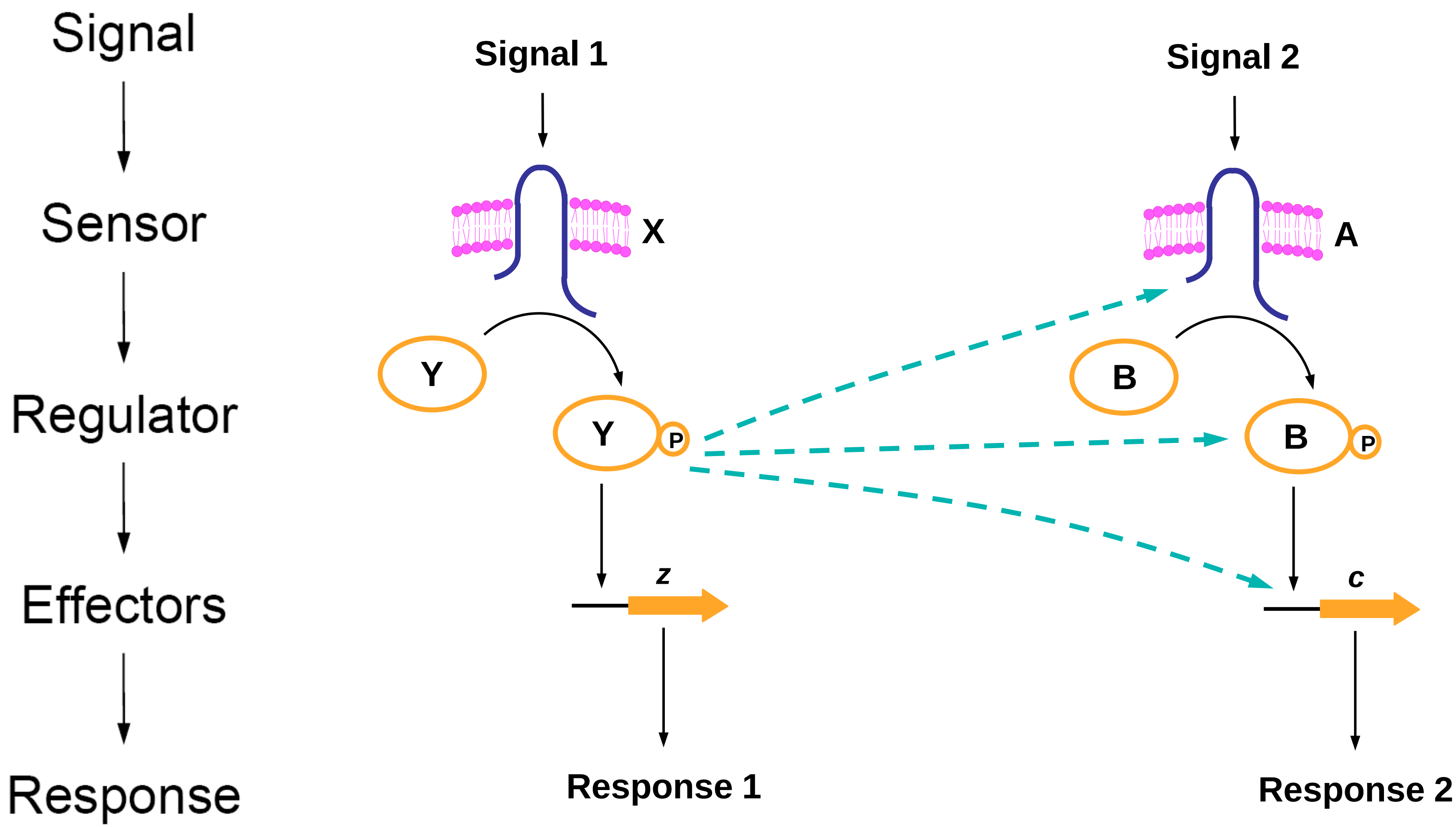
Advantages of modeling

- **Explanation and prediction of quantitative effects**
- **General results difficult to obtain experimentally**
- **Means to modify system behavior in desirable ways**

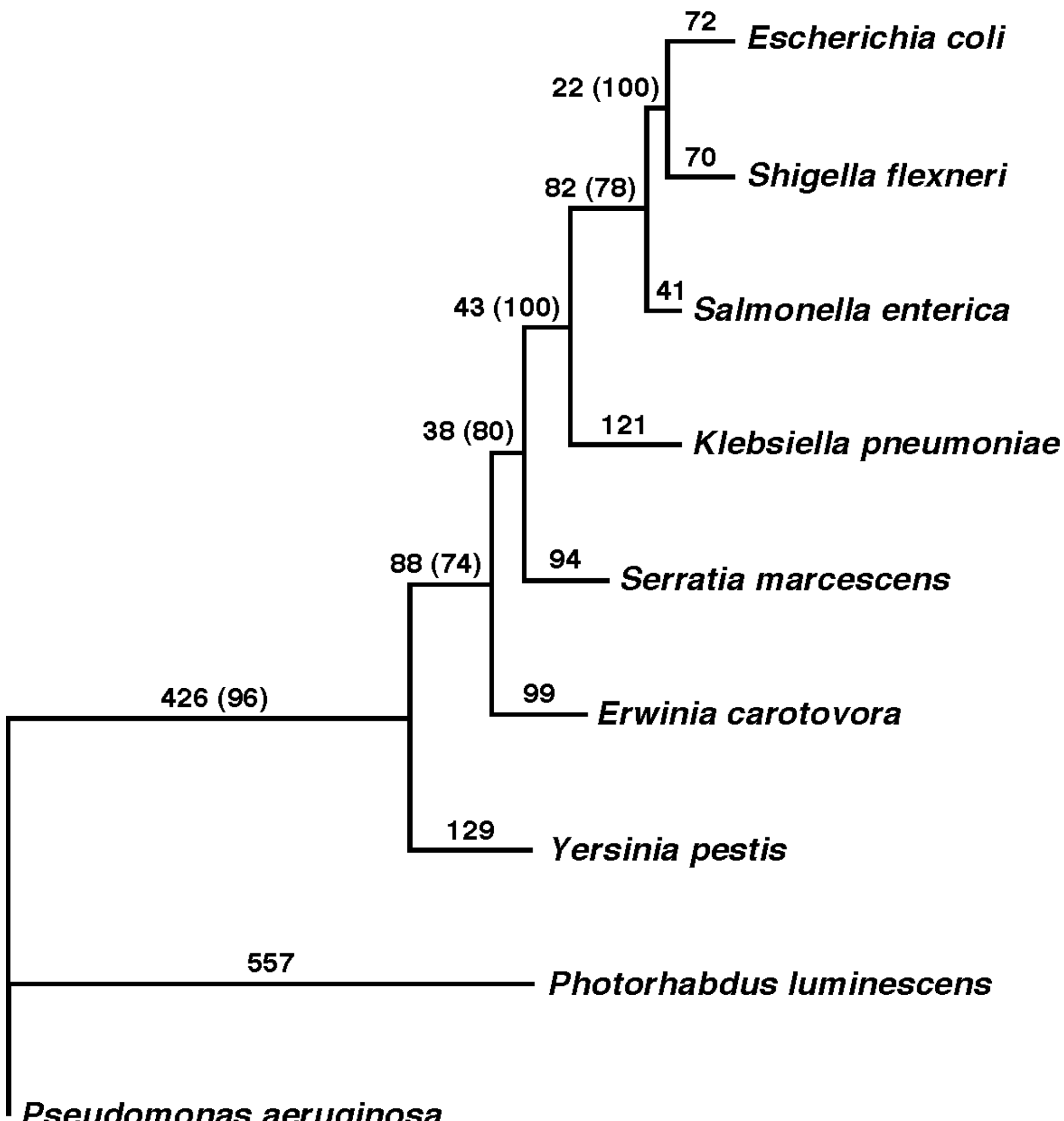
Different signals activate distinct response networks



What are the functional implications of different modes of signal integration?



Enteric bacteria as model organisms



Human symbiont or pathogen (urinary tract infections)

Human pathogen (diarrhea)

Human pathogen (gastroenteritis)

Free-living, human commensal, or pathogen (pneumonia, UTI)

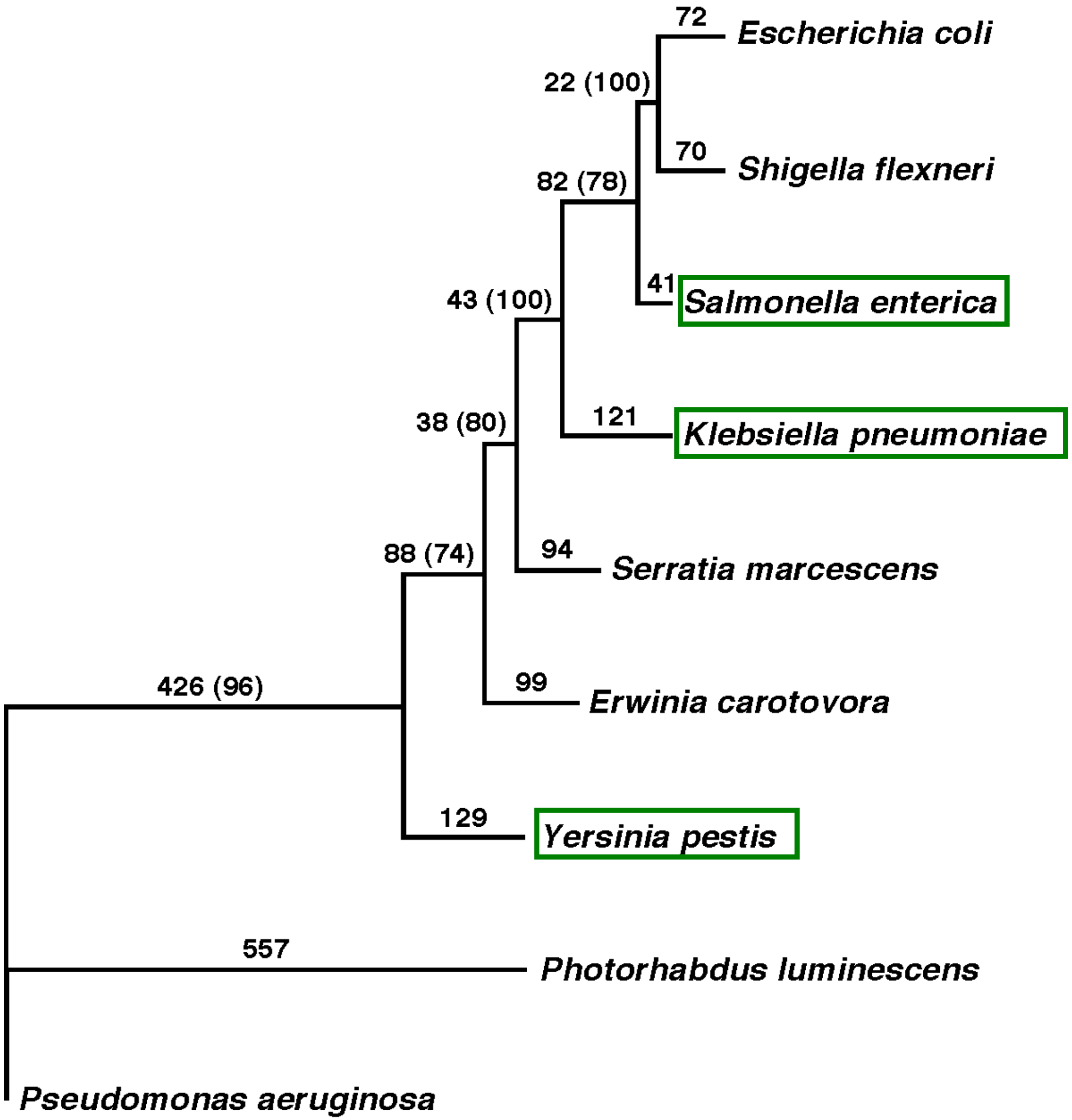
Human pathogen (UTI)

Plant pathogen

Human pathogen (plague)

Nematode symbiont; insect pathogen

Enteric bacteria as model organisms



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Nematode symbiont; insect pathogen

Two-component systems regulating polymyxin B resistance

Signal

↓

Sensor

↓

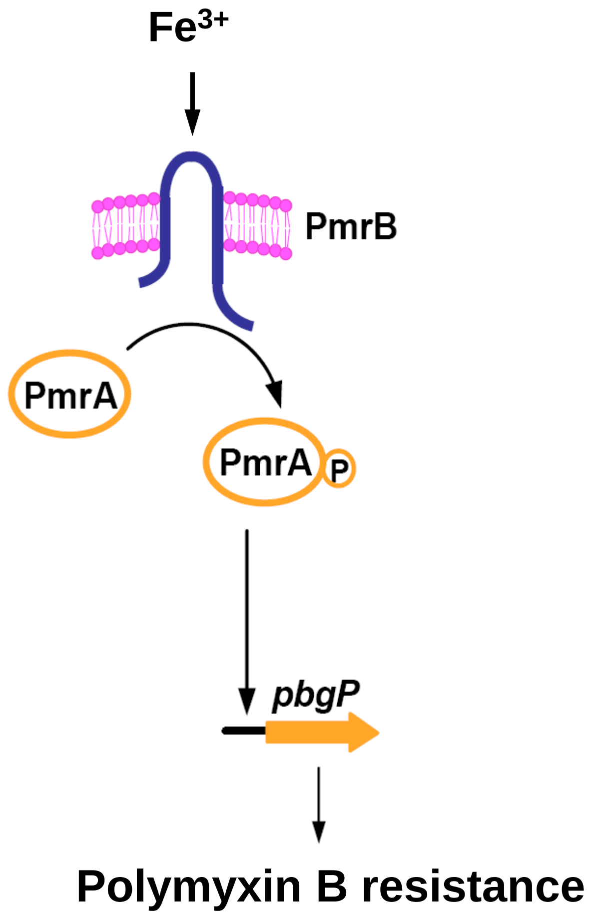
Regulator

↓

Effectors

↓

Response



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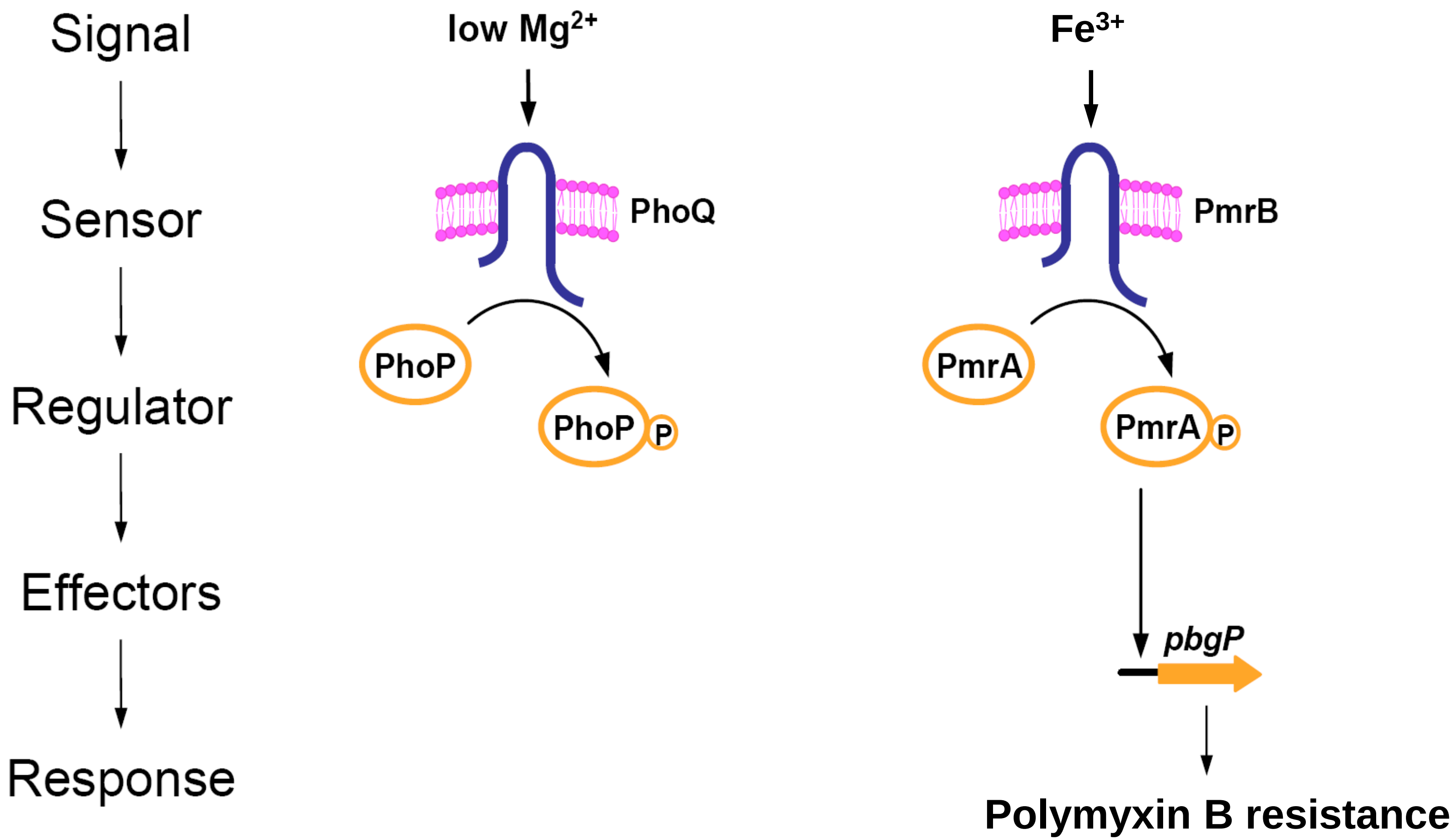

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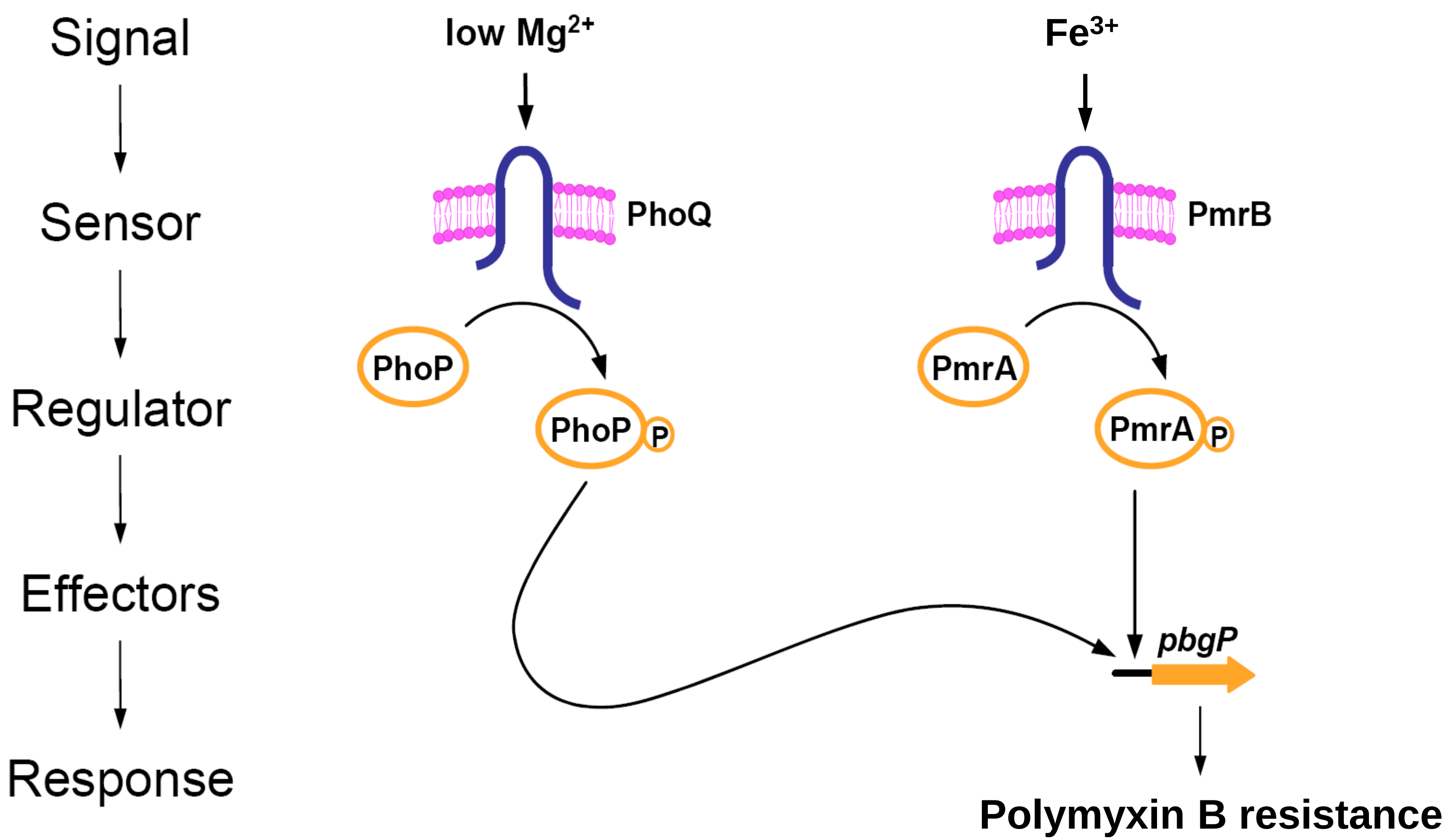
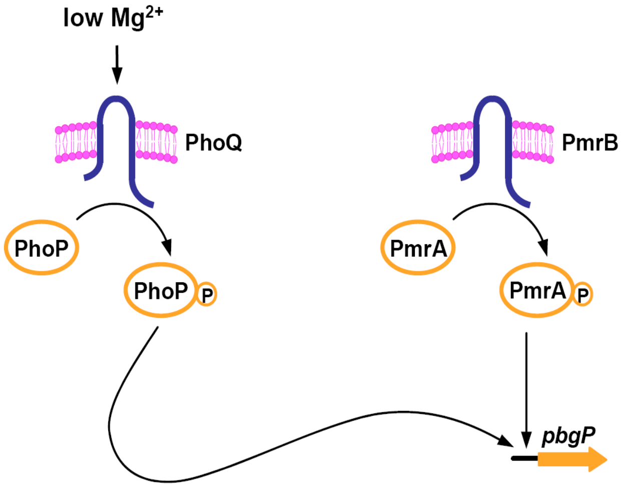


Direct regulation circuit

Yersinia pestis

Different species of enterics use distinct architectures to activate *pbgP* by low Mg^{2+}



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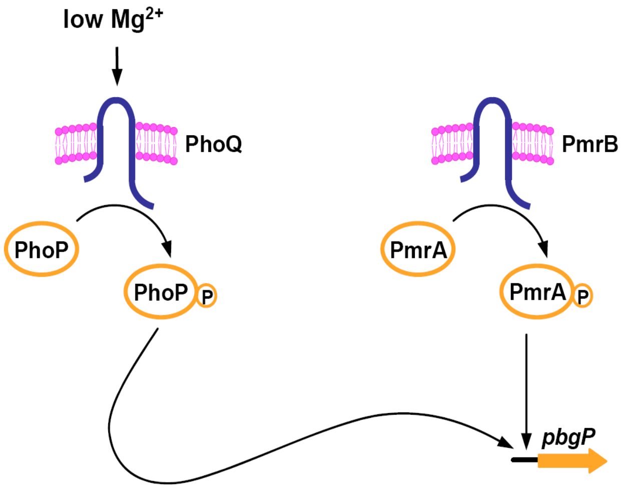

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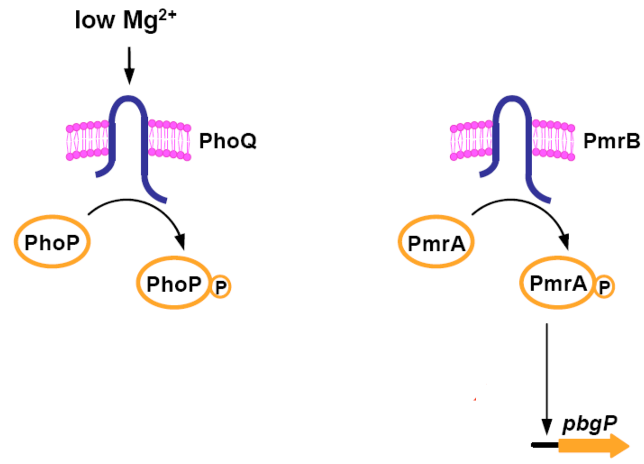

Salmonella enterica

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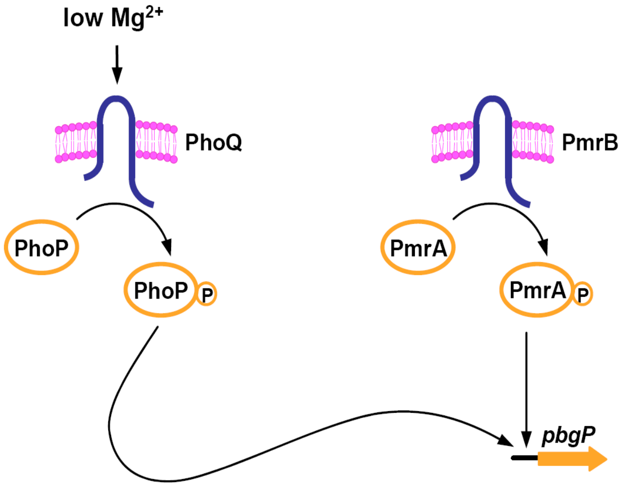

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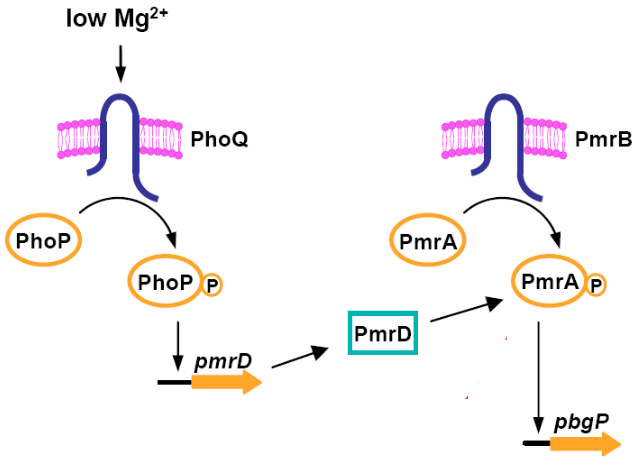

Connector-mediated pathway
Salmonella enterica

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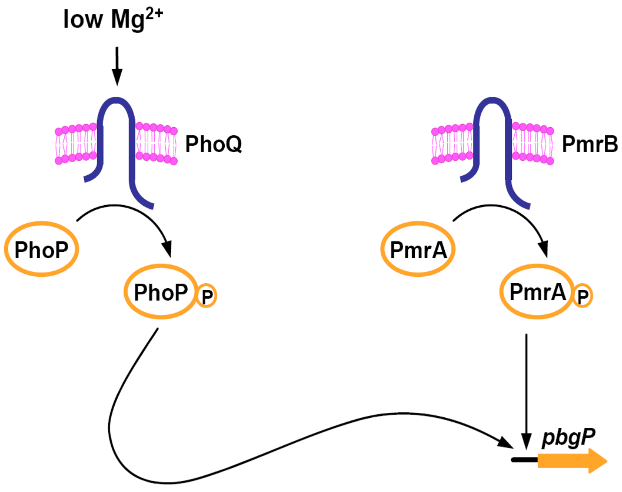

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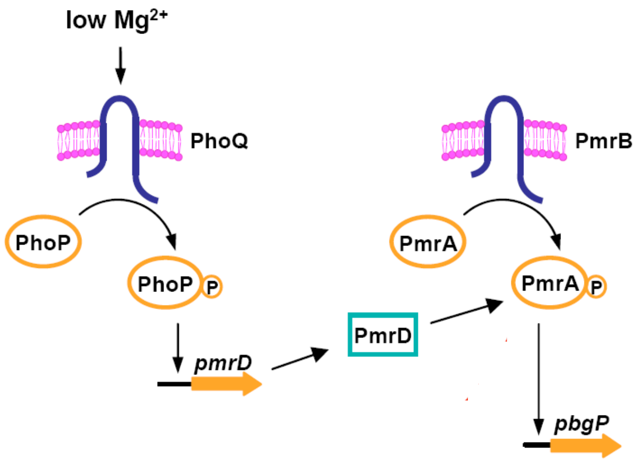
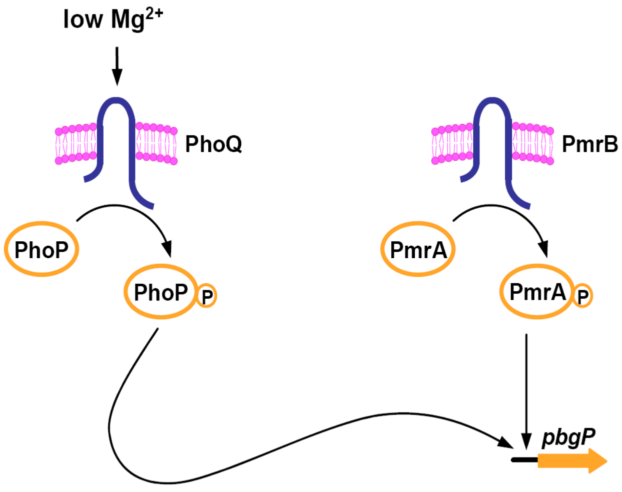


Connector-mediated pathway
Salmonella enterica

- How does the circuit architecture affect the **induction ratios**?

Different species of enterics use distinct architectures to activate *pbgP* by low Mg^{2+}



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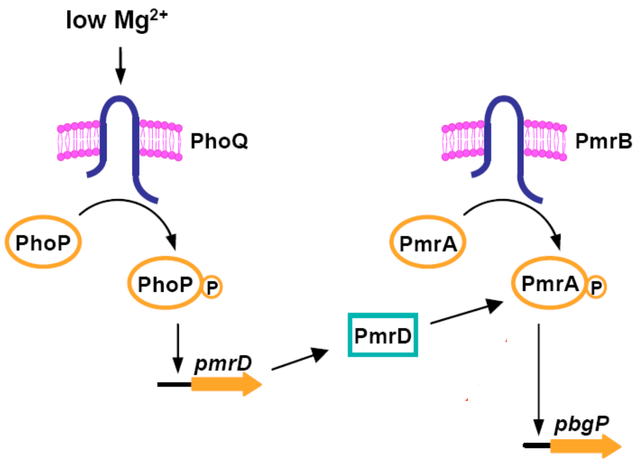
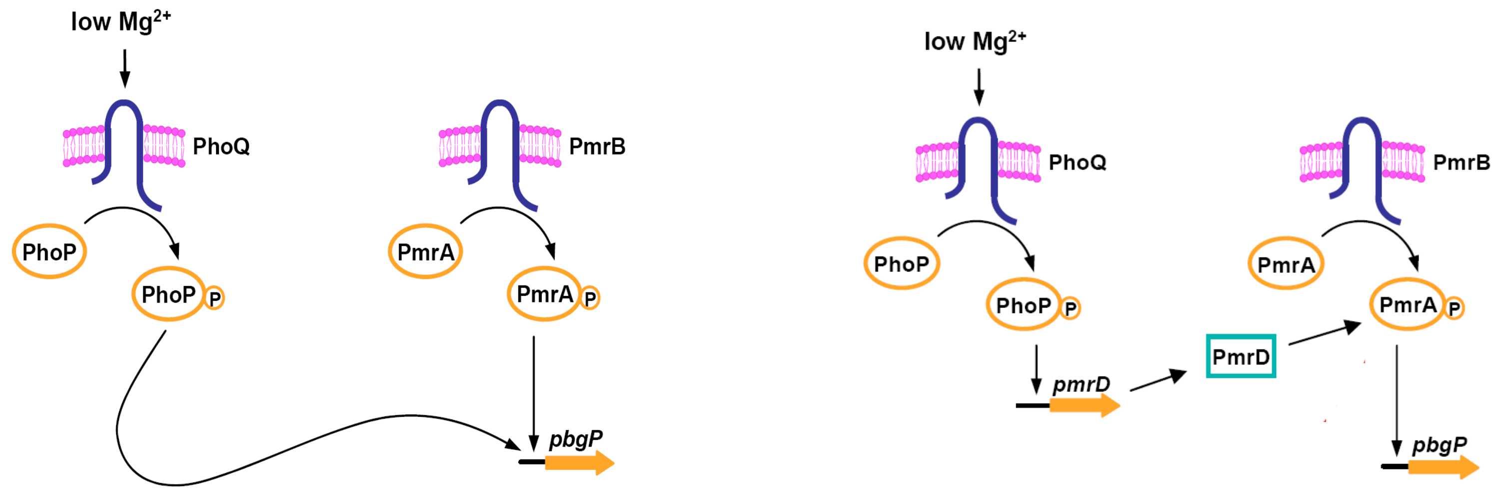


Connector-mediated pathway
Salmonella enterica

- How does the circuit architecture affect the **induction ratios**?
- What is the connection between the circuit architecture and **response timing**?

Different species of enterics use distinct architectures to activate *pbgP* by low Mg^{2+}



Direct regulation circuit
Yersinia pestis

Connector-mediated pathway
Salmonella enterica

- How does the circuit architecture affect the **induction ratios**?
- What is the connection between the circuit architecture and **response timing**?
- How does the circuit architecture influence the **response levels**?

Foundation of mathematical modeling: chemical kinetics

Chemical reaction

$$A \rightleftharpoons B$$

Foundation of mathematical modeling: chemical kinetics

Chemical reaction $A \rightleftharpoons B$

Mass action law

A is depleted with rate $k_1[A]$

B is depleted with rate $k_2[B]$

Foundation of mathematical modeling: chemical kinetics

Chemical reaction $A \rightleftharpoons B$

Mass action law

A is depleted with rate $k_1[A]$

B is depleted with rate $k_2[B]$

Mathematical formulation

$$\begin{cases} d[A]/dt = k_2[B] - k_1[A]; \quad [A](0) = A_0 \\ d[B]/dt = k_1[A] - k_2[B]; \quad [B](0) = B_0 \end{cases}$$

Foundation of mathematical modeling: chemical kinetics

Chemical reaction $A \rightleftharpoons B$

Mass action law

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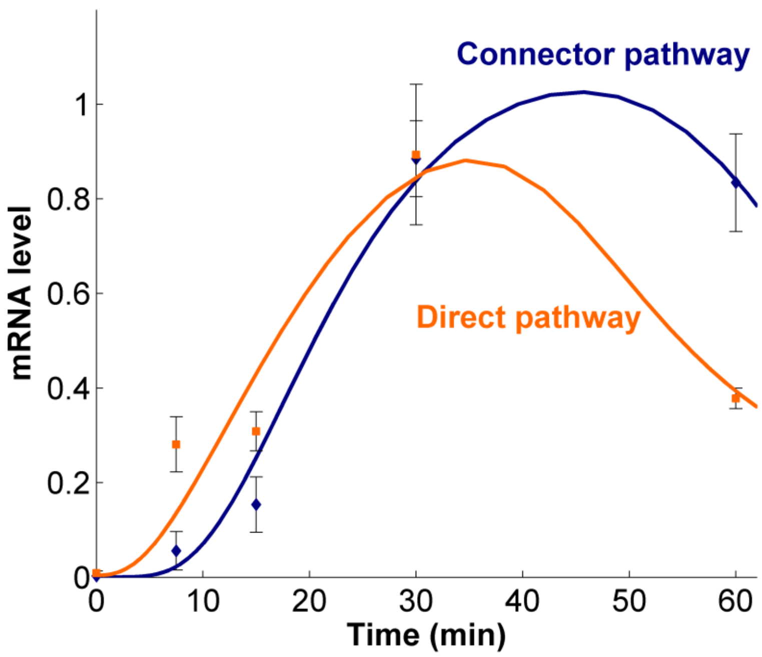
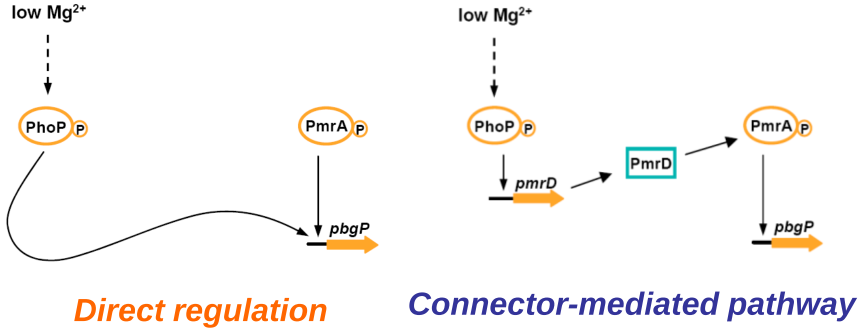
Solution

[A], [B] as functions of time

Pathway activation data can be used to fit and test the pathway mathematical models





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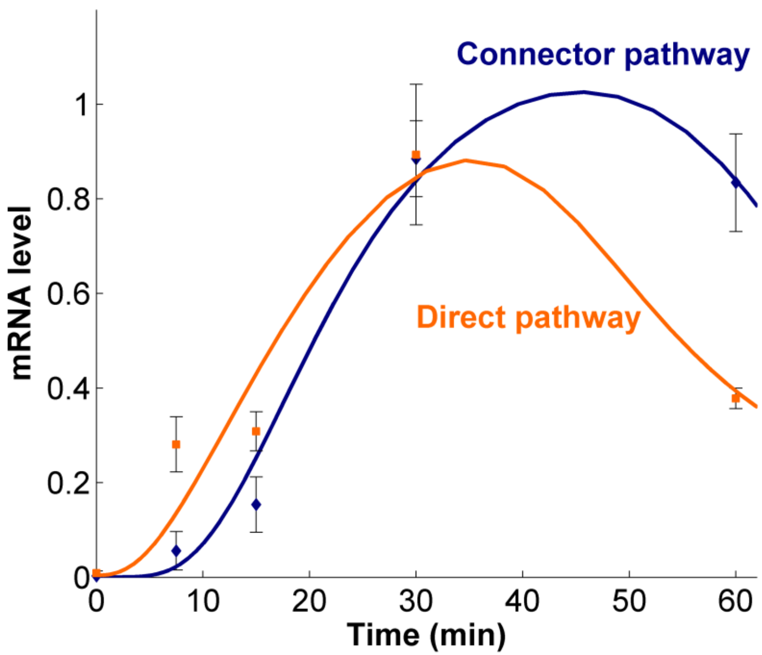

$$\frac{dX_1}{dt} = k_1^{indir}\frac{K_1^{indir}(X_3^2+X_4^2)}{1+K_1^{indir}(X_3^2+X_4^2)} - k_{-1}^{indir}X_1\ ; \qquad [1]$$

$$pbgP \text{ promoter} \xrightarrow{k_1^{indir}\frac{K_1^{indir}(X_3^2+X_4^2)}{1+K_1^{indir}(X_3^2+X_4^2)}} pbgP \text{ mRNA } (X_1) \xrightarrow{k_{-1}^{indir}} 0 \text{ (degradation + dilution)}$$

$$\frac{dX_2}{dt} = k_2\frac{K_2[\text{PhoP-P}]^2}{1+K_2[\text{PhoP-P}]^2} + k_{23}X_4 - k_{-2}X_2 - k_4X_2X_3\ ; \qquad [2]$$

$$pmrD \text{ promoter} \xrightarrow{k_2\frac{K_2[\text{PhoP-P}]^2}{1+K_2[\text{PhoP-P}]^2}} \text{PmrD } (X_2) \xrightarrow{k_{-2}} 0 \text{ (degradation + dilution)}$$

$$\text{PmrD } (X_2) + \text{PmrA-P } (X_3) \xleftrightarrow{k_4/k_{23}} \text{PmrD/PmrA-P } (X_4)$$

$$\frac{dX_3}{dt} = k_3 + k_{23}X_4 - k_{-3}X_3 - k_4X_2X_3\ ; \qquad [3]$$

$$\text{PmrA (const)} \xrightarrow{k_3} \text{PmrA-P } (X_3) \xrightarrow{k_{-3}} 0 \text{ (dephosphorylation + degradation + dilution)}$$

$$\text{PmrD } (X_2) + \text{PmrA-P } (X_3) \xleftrightarrow{k_4/k_{23}} \text{PmrD/PmrA-P } (X_4)$$

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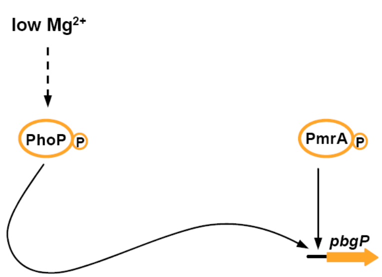
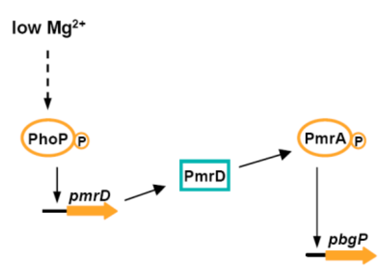
$$\text{PmrD/PmrA-P } (X_4) \xrightarrow{k_{-4}} 0 \text{ (degradation + dilution)}$$

$$\frac{dX_1}{dt} = k_1^{dir}\frac{K_1^{dir}[\text{PhoP-P}]^2}{1+K_1^{dir}[\text{PhoP-P}]^2} - k_{-1}^{dir}X_1\ . \qquad [6]$$

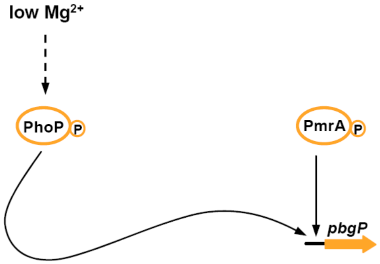


Direct regulation



Connector-mediated pathway

Fitted mathematical models predict steady-state output levels as functions of PhoP-P concentration



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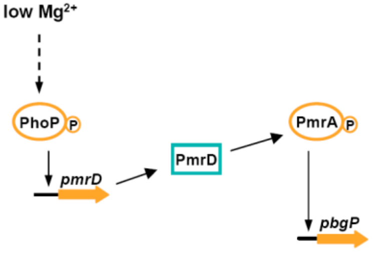

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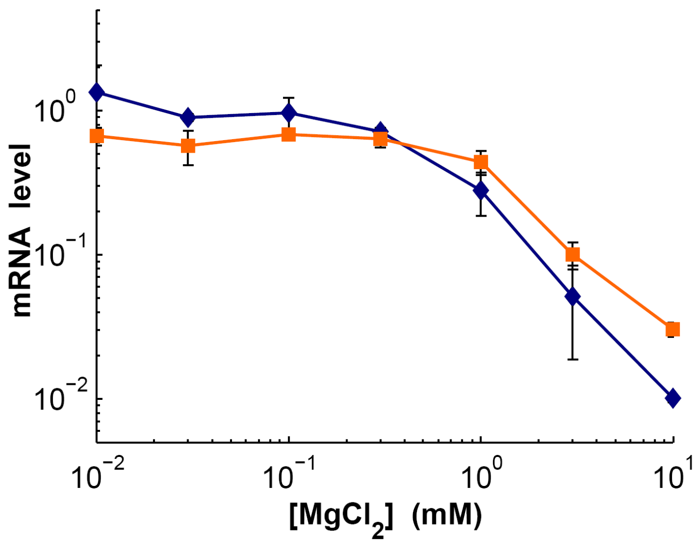

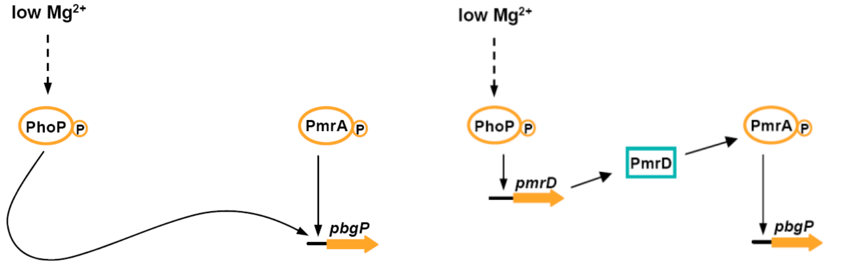

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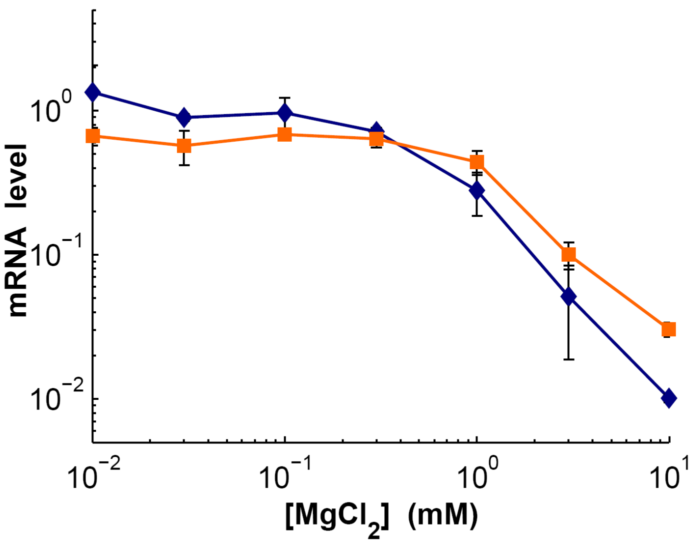

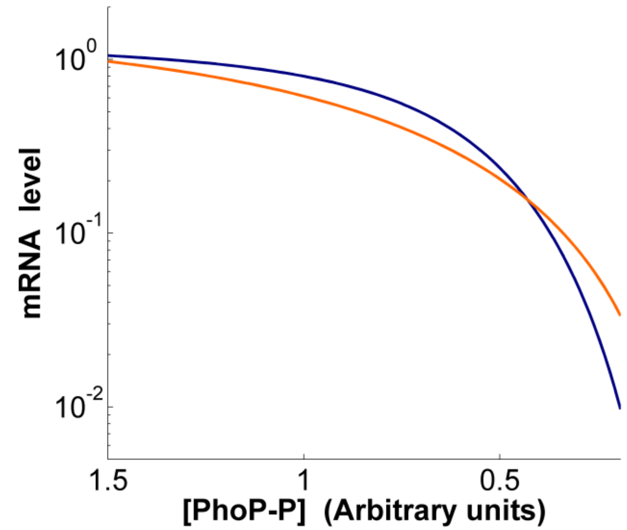

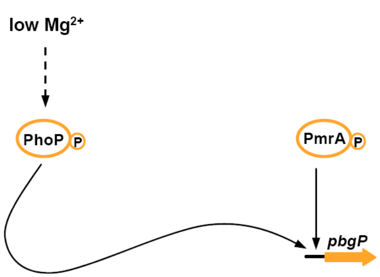

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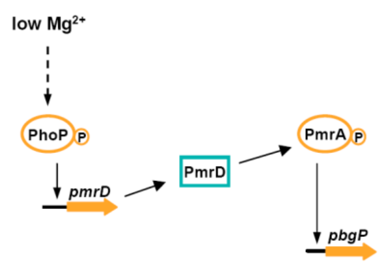
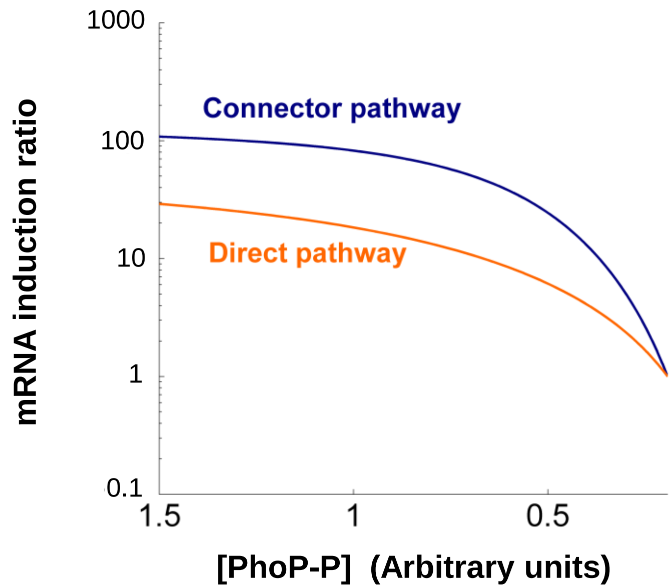
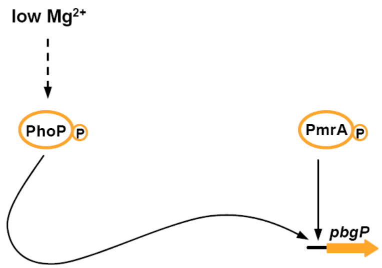


Connector-mediated pathway

Connector-mediated pathway promotes signal amplification





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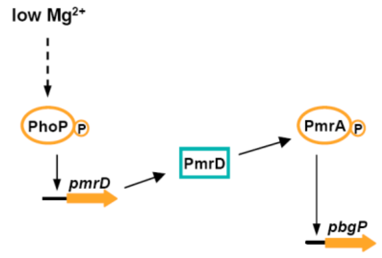
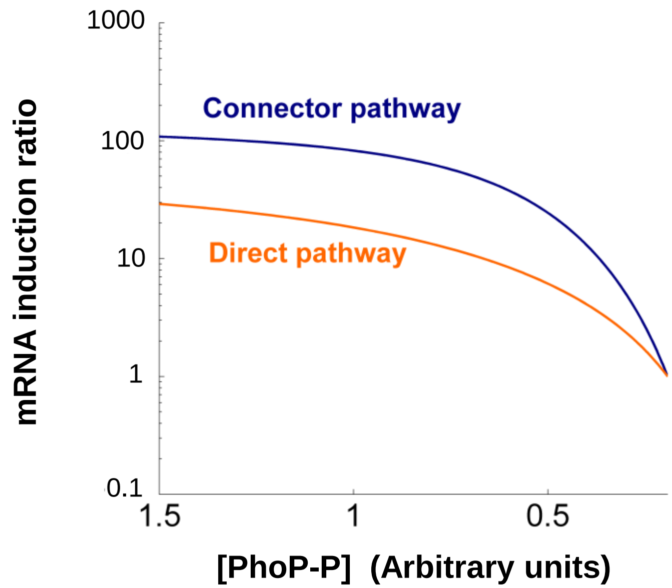


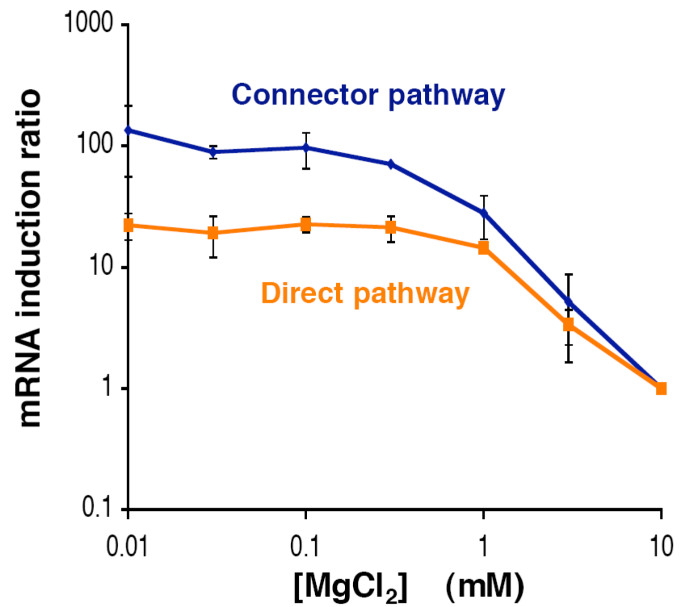
Connector-mediated pathway

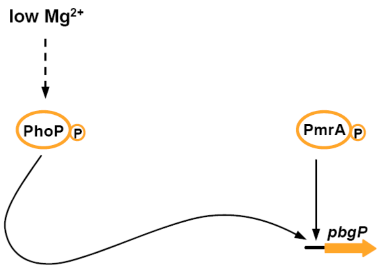
Induction ratio =

$$\frac{\text{mRNA level (inducing conditions)}}{\text{mRNA level (repressing conditions)}}$$

Connector-mediated pathway promotes signal amplification







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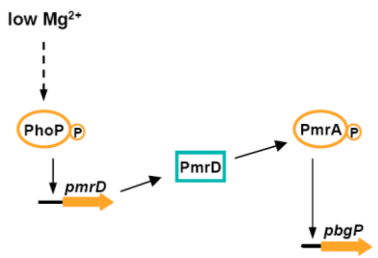

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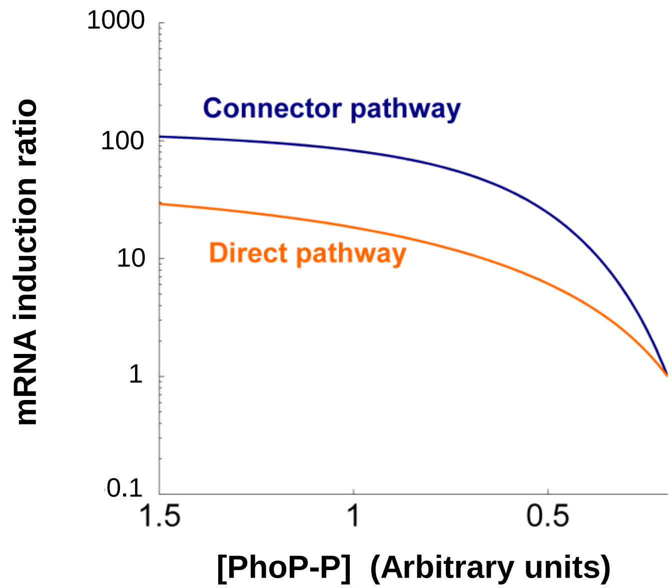
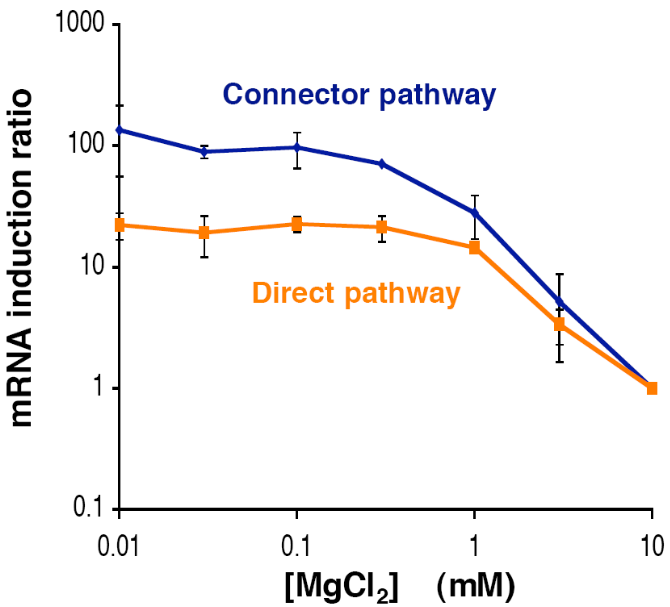
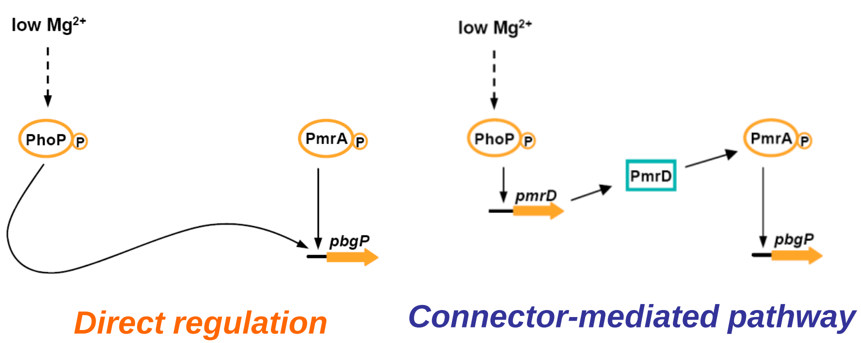
Connector-mediated pathway promotes signal amplification

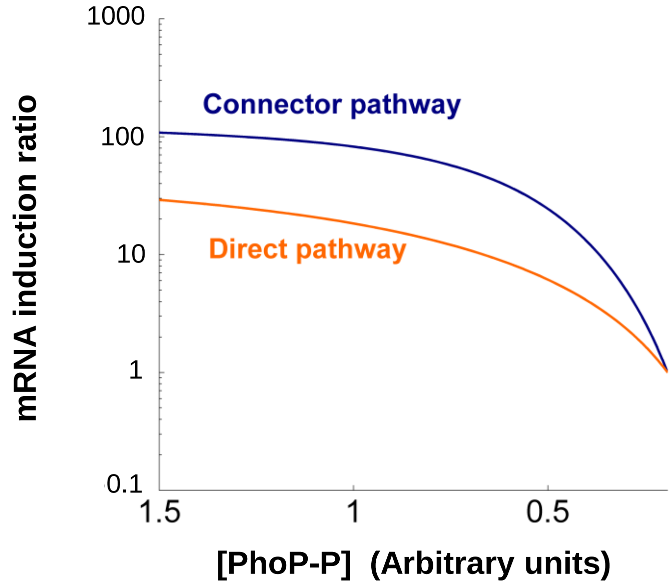


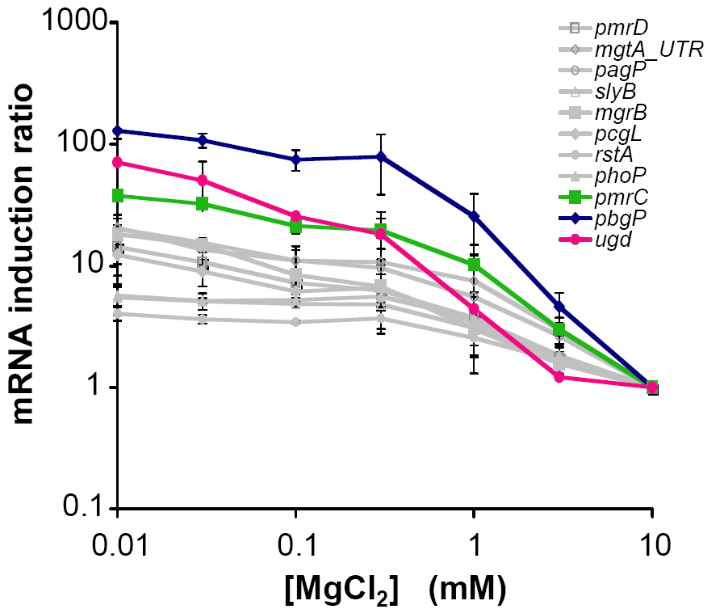


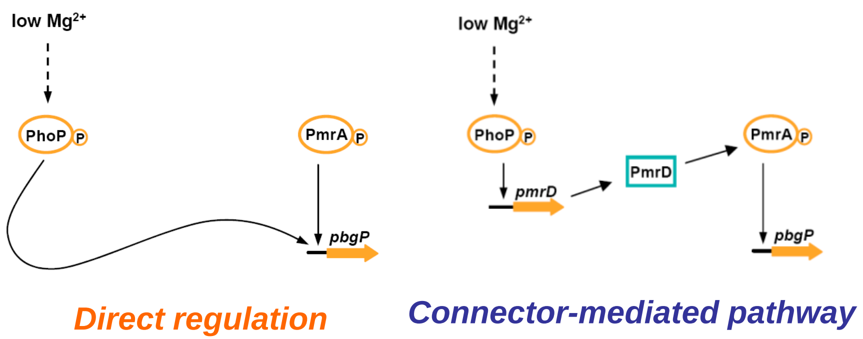


Mathematical result: signal amplification occurs in the ***connector-mediated pathway*** for sufficiently high signal levels

Connector-mediated pathway promotes signal amplification

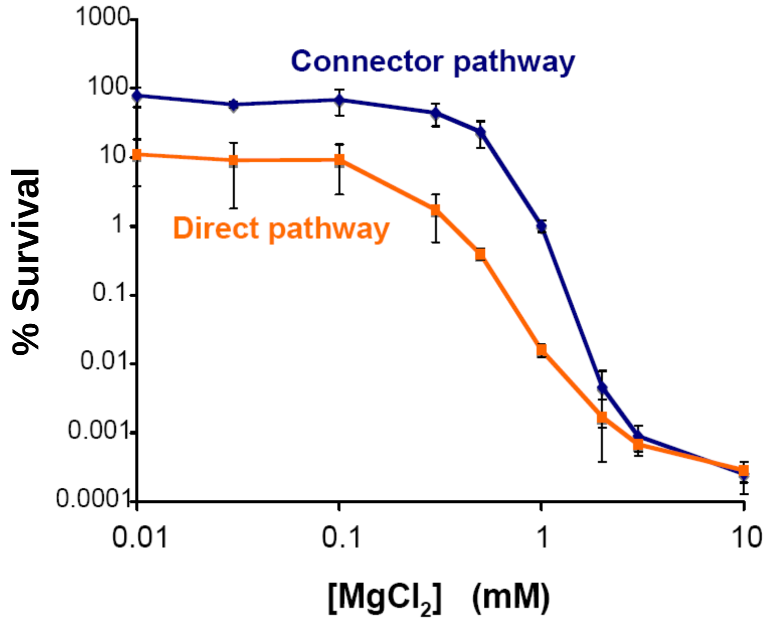


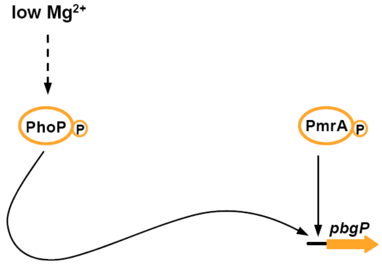




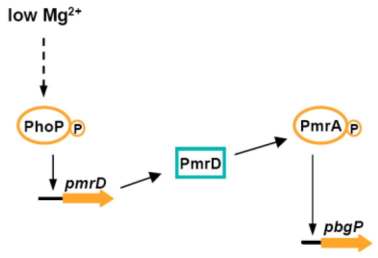
<u>Mathematical result</u>: signal amplification occurs in the ***connector-mediated pathway*** for sufficiently high signal levels

Connector-mediated pathway promotes heightened polymyxin B resistance



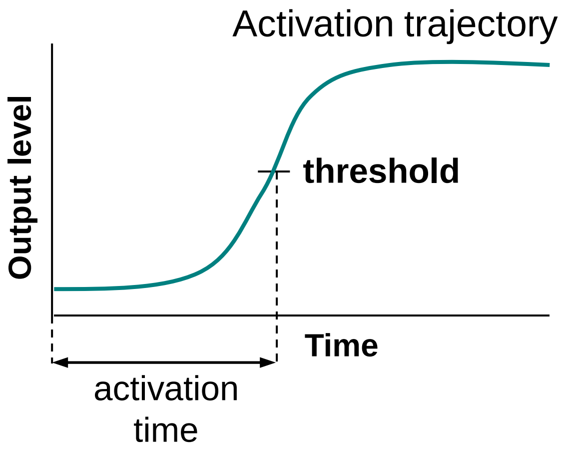


Direct regulation

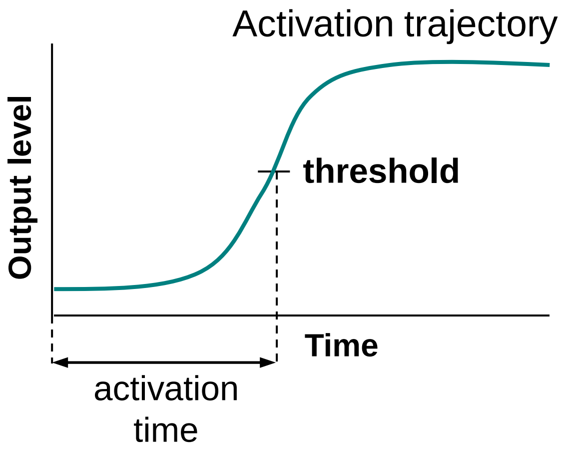


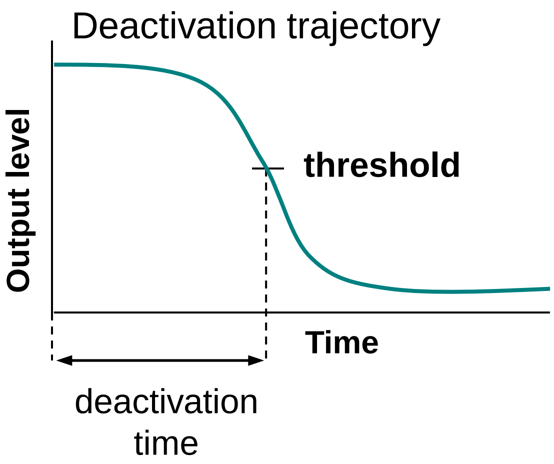
Connector-mediated pathway

Analysis of response timing

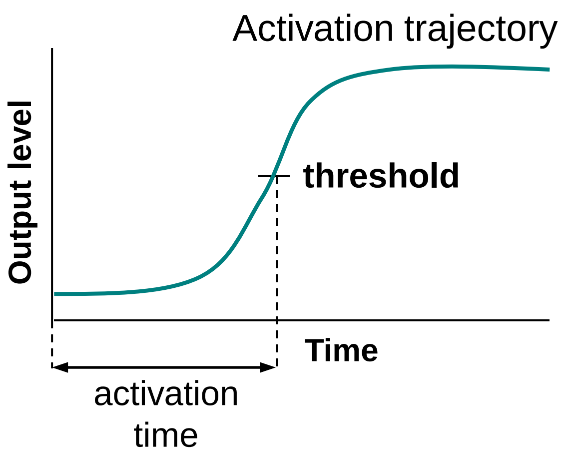


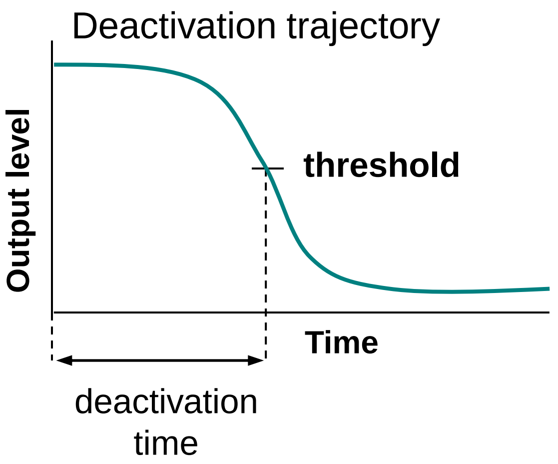
Analysis of response timing





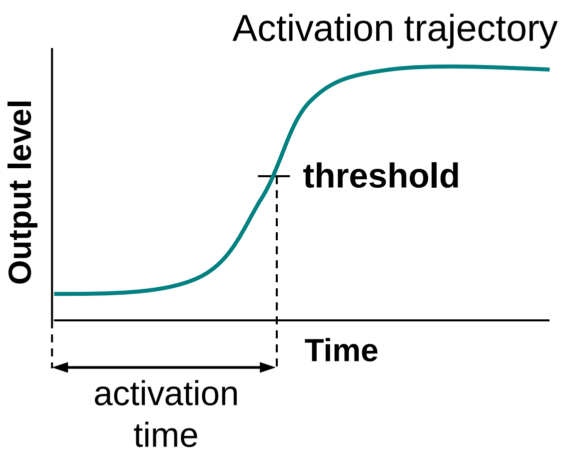
Analysis of response timing

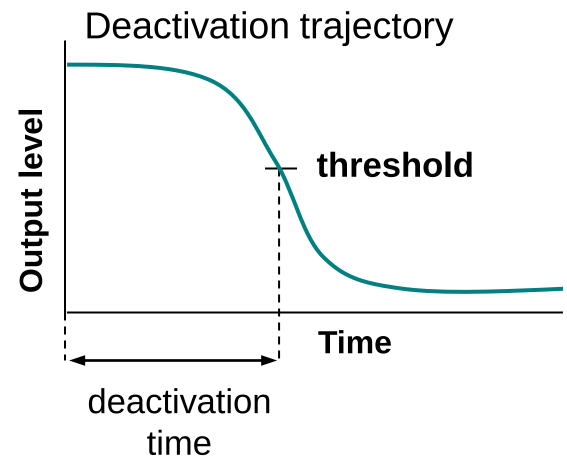


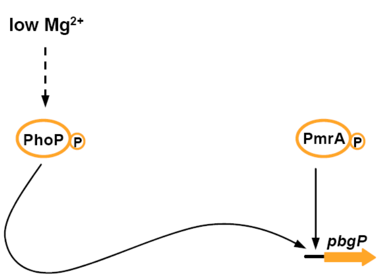


Activation (deactivation) delay: the difference in activation (deactivation) times between the **architecture of interest** and a **reference architecture**

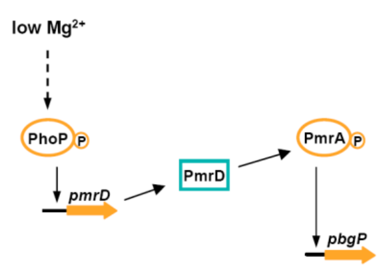
Analysis of response timing







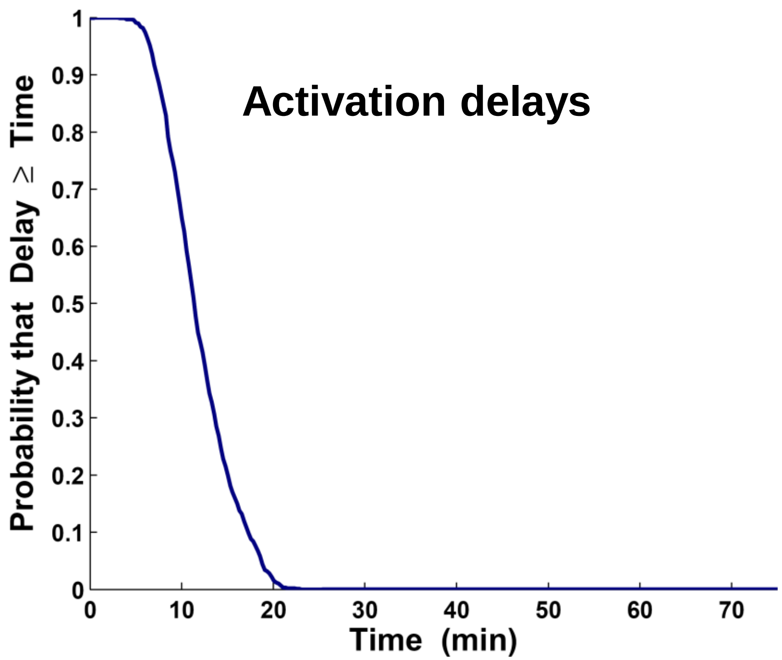
Direct regulation
(reference architecture)

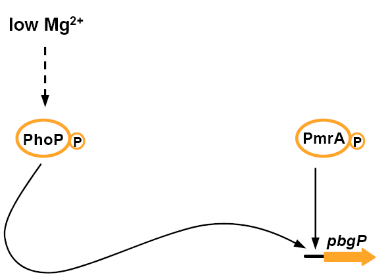


Connector-mediated pathway

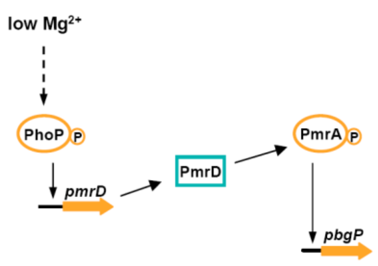
Activation (deactivation) delay: the difference in activation (deactivation) times between the **architecture of interest** and a **reference architecture**

Connector-mediated pathway exhibits expression delays



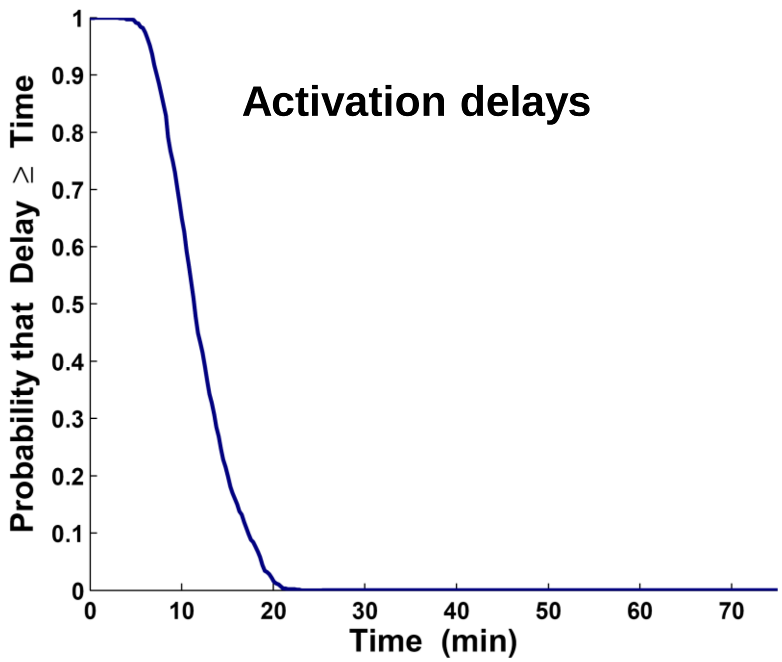


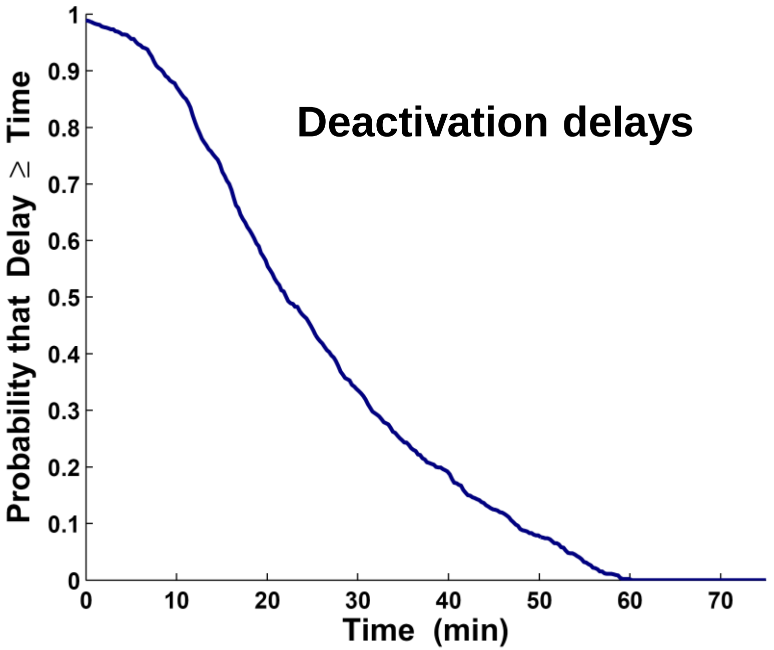
Direct regulation
(reference architecture)

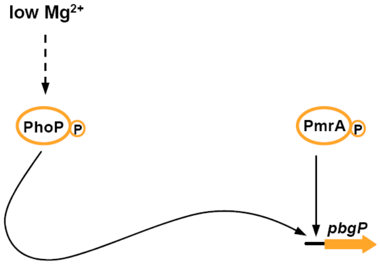


Connector-mediated pathway

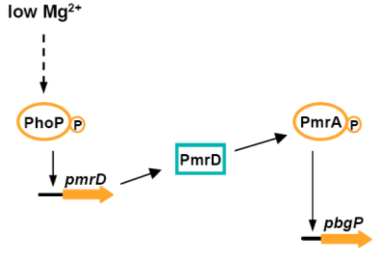
Connector-mediated pathway exhibits expression delays





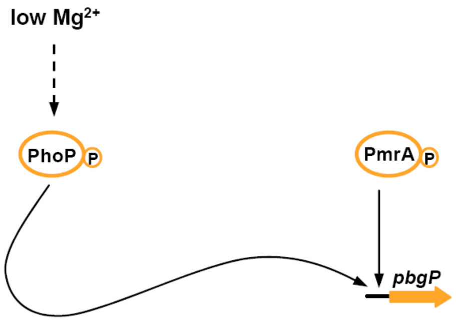


Direct regulation
(reference architecture)



Connector-mediated pathway

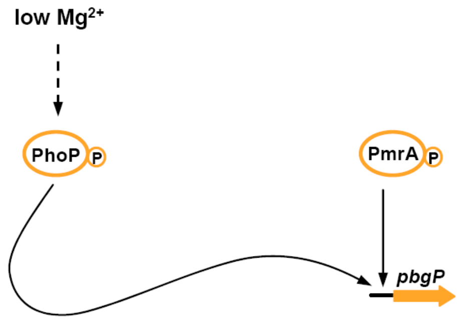
Feedforward connector loop is an intermediate stage between direct and connector-mediated regulation



Direct pathway

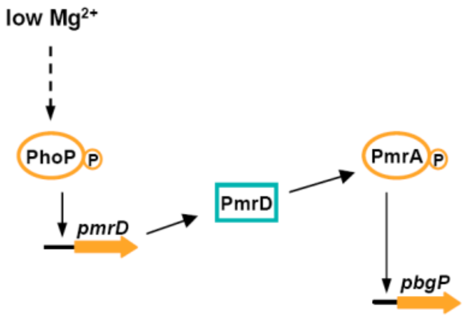
Yersinia pestis

Feedforward connector loop is an intermediate stage between direct and connector-mediated regulation



Direct pathway

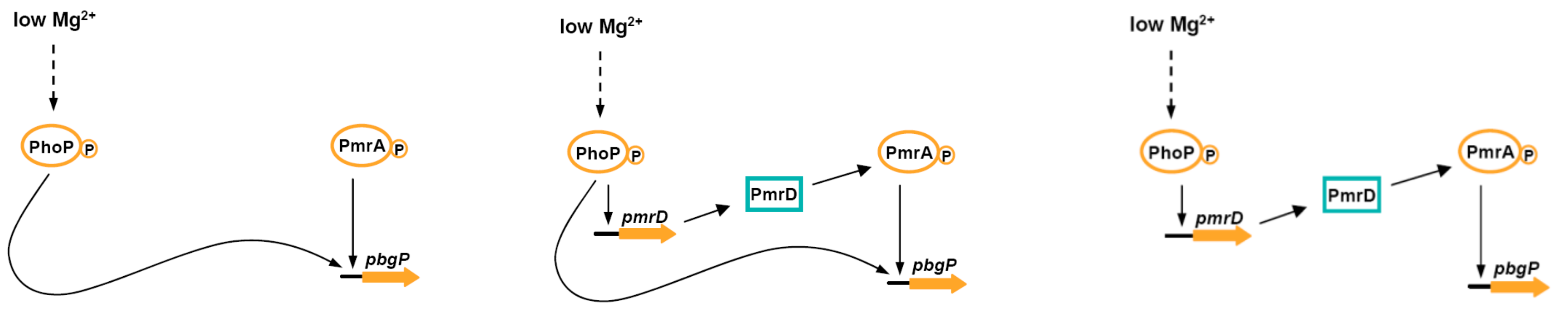
Yersinia pestis



Connector-mediated pathway

Salmonella enterica

Feedforward connector loop is an intermediate stage between direct and connector-mediated regulation

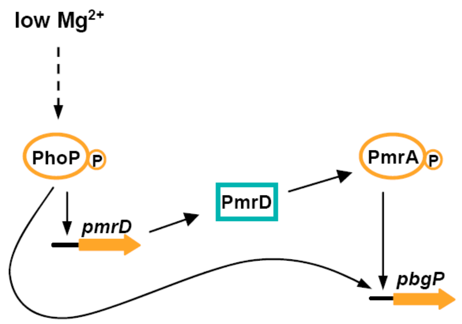


Direct pathway
Yersinia pestis

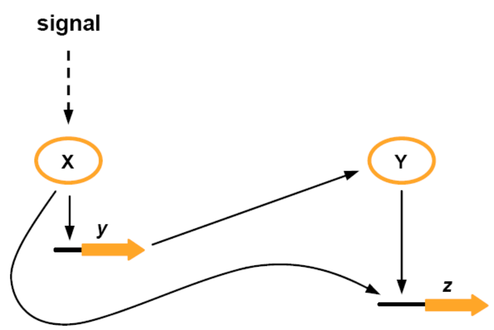
Feedforward connector loop (FCL)
Klebsiella pneumoniae

Connector-mediated pathway
Salmonella enterica

Feedforward connector loop resembles the ubiquitous feedforward loop

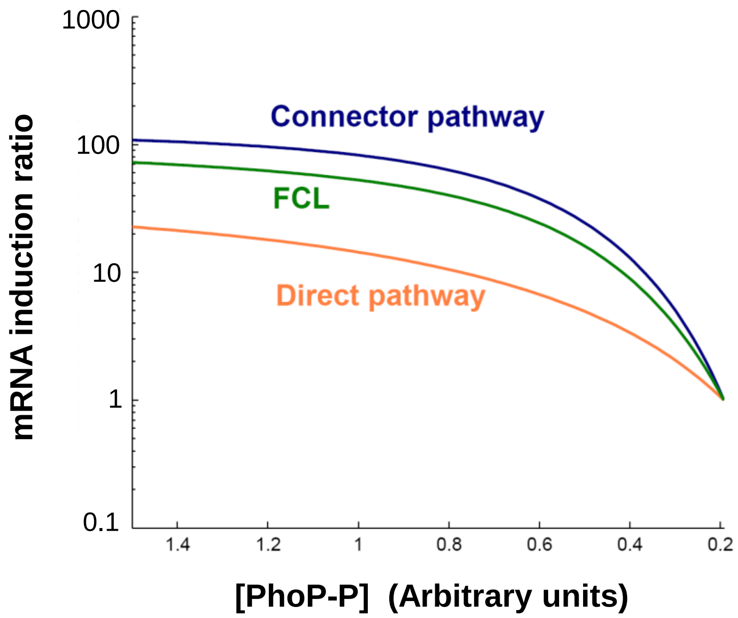


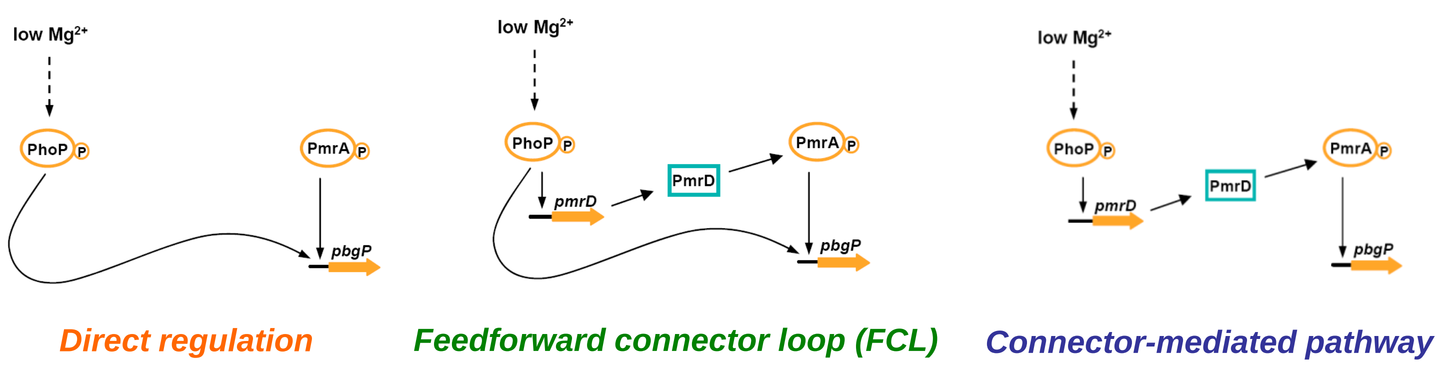
Feedforward connector loop (FCL)
Klebsiella pneumoniae



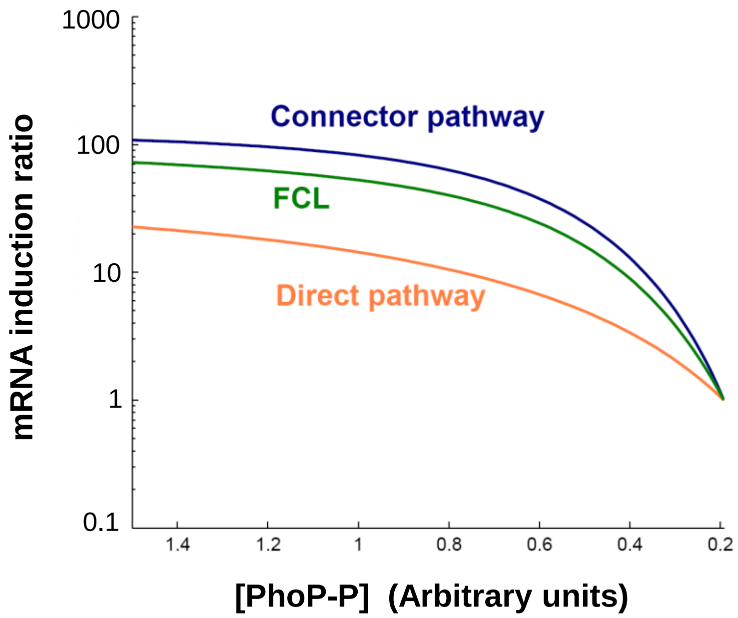
Feedforward loop (FFL)

Feedforward connector loop promotes signal amplification





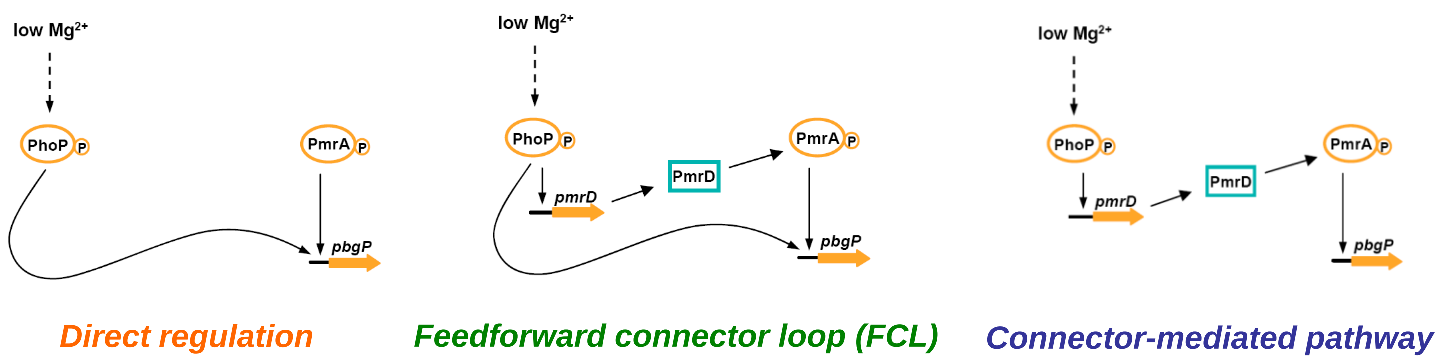
Feedforward connector loop promotes signal amplification



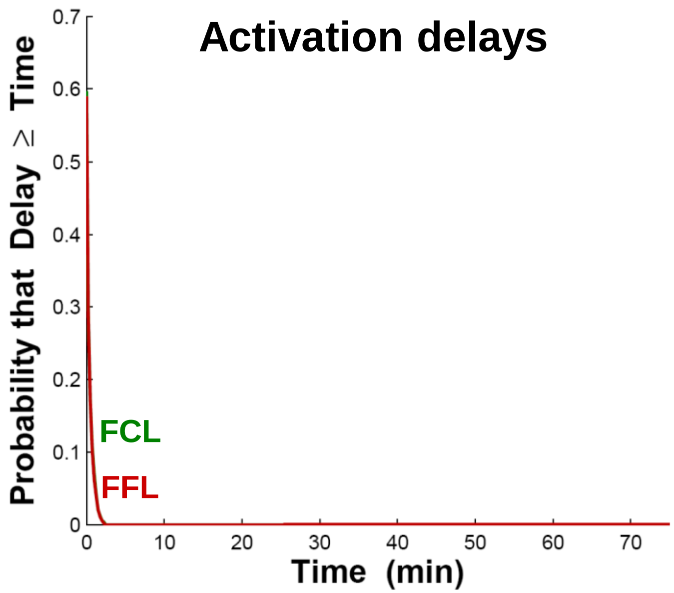
Mathematical results:

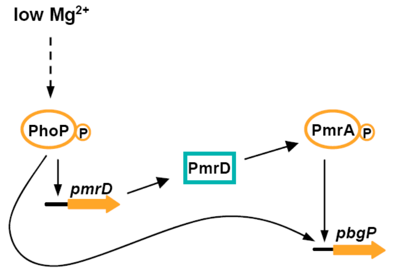
(a) Signal amplification occurs for the ***FCL*** for sufficiently high signal levels

(b) Signal amplification for the ***FCL*** is less pronounced than for the ***connector-mediated pathway***

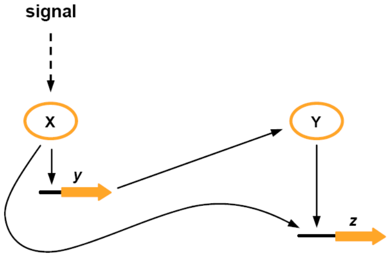


FCL and FFL demonstrate fast activation



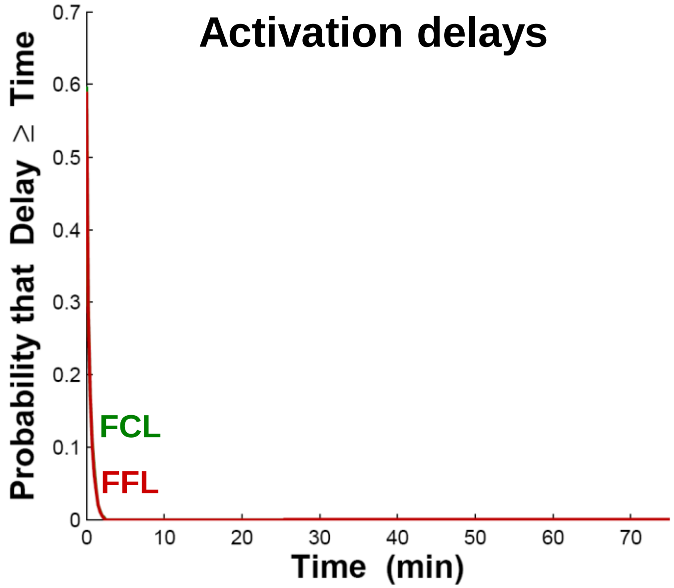


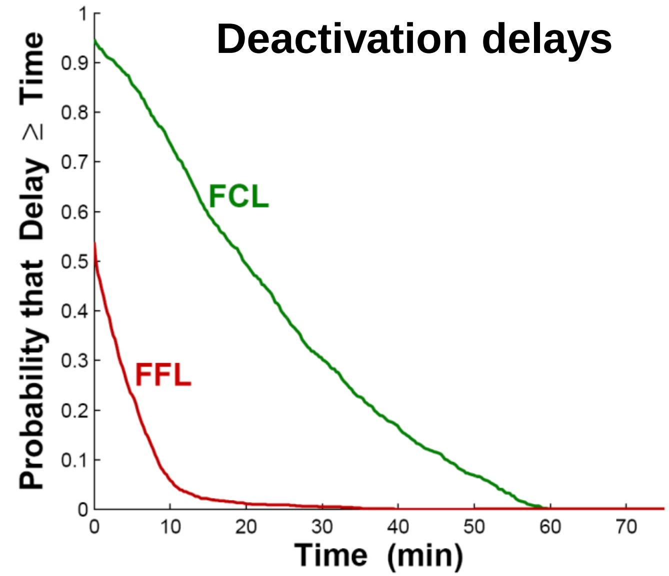
Feedforward connector loop (FCL)

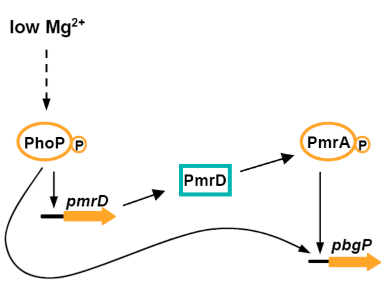


Feedforward loop (FFL)

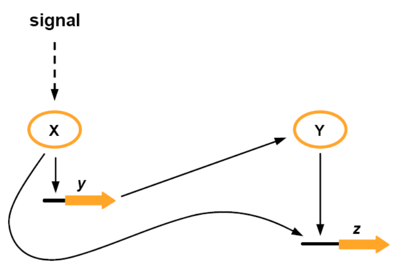
FCL exhibits larger deactivation delays than FFL





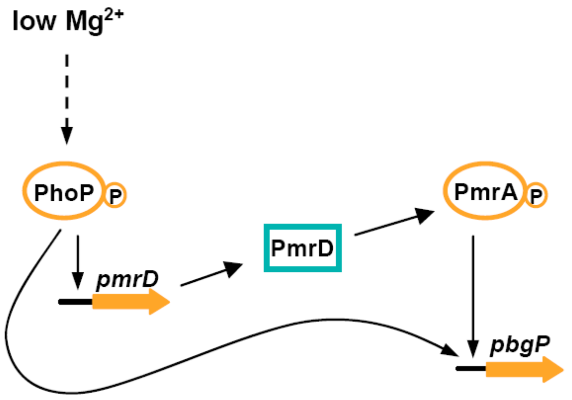


Feedforward connector loop (FCL)

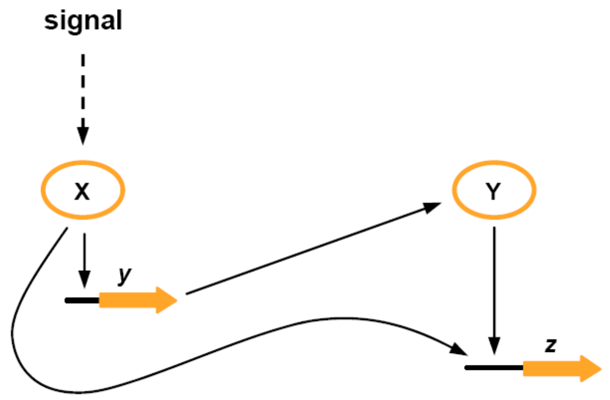


Feedforward loop (FFL)

What is the role of the direct branch of regulation in FCL and FFL?

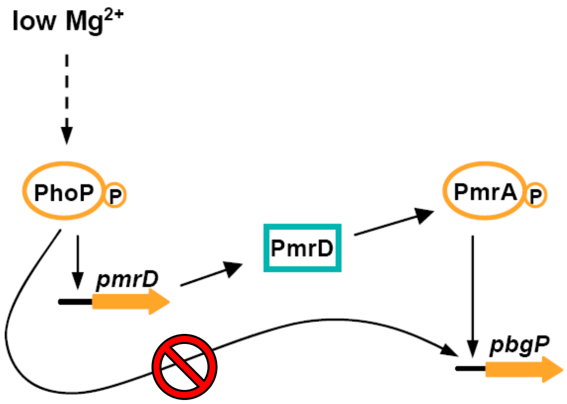


Feedforward connector loop (FCL)

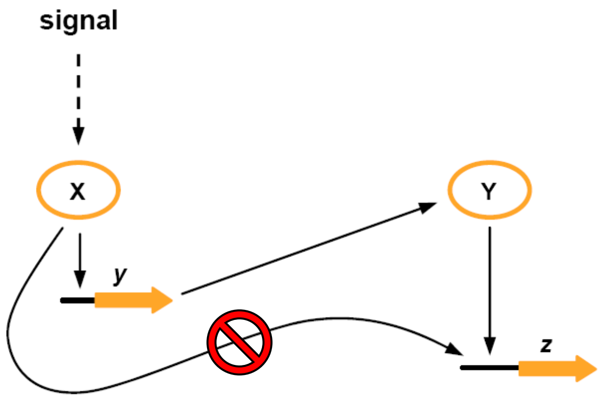


Feedforward loop (FFL)

What is the role of the direct branch of regulation in FCL and FFL?

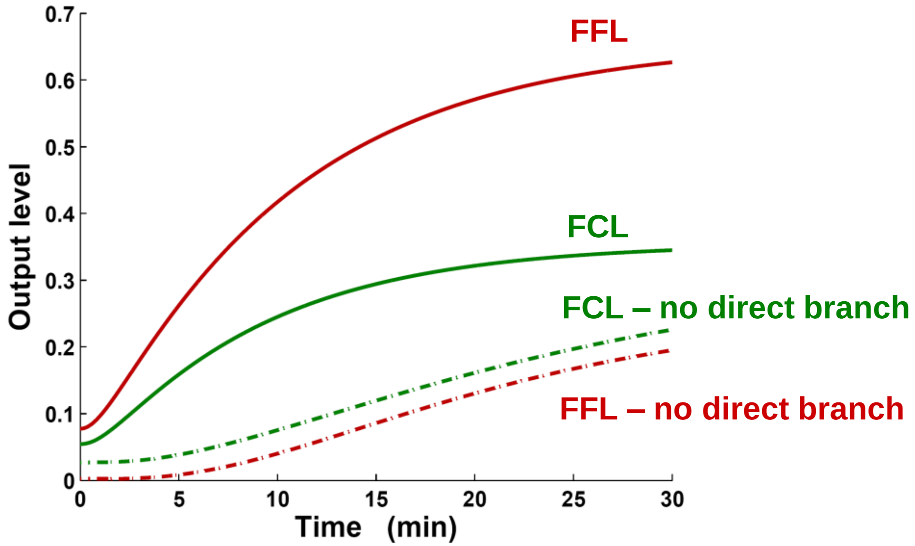


Feedforward connector loop (FCL)

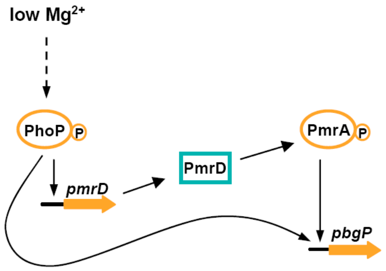


Feedforward loop (FFL)

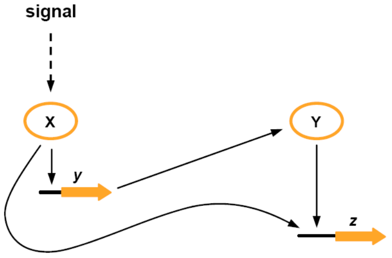
FCL and FFL display higher output levels than their cascade counterparts



Mathematical result:
having two regulation branches leads to higher output levels

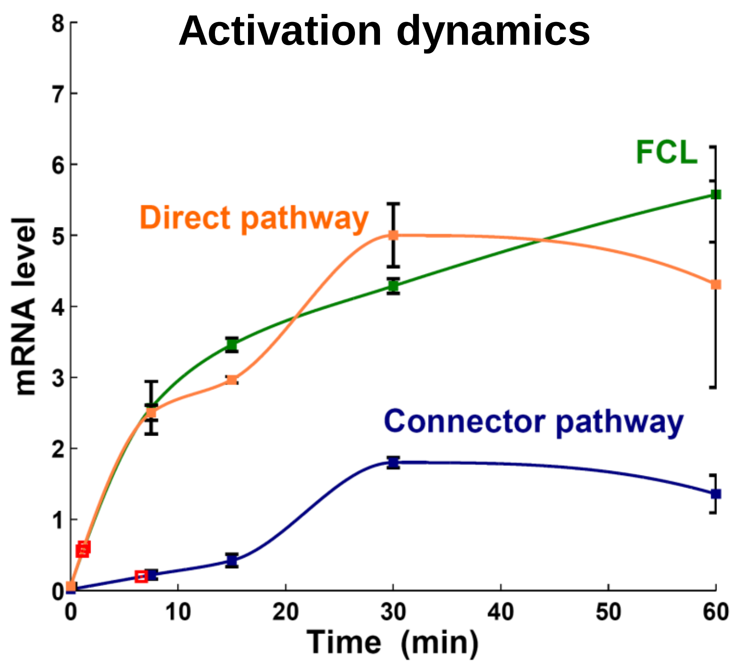


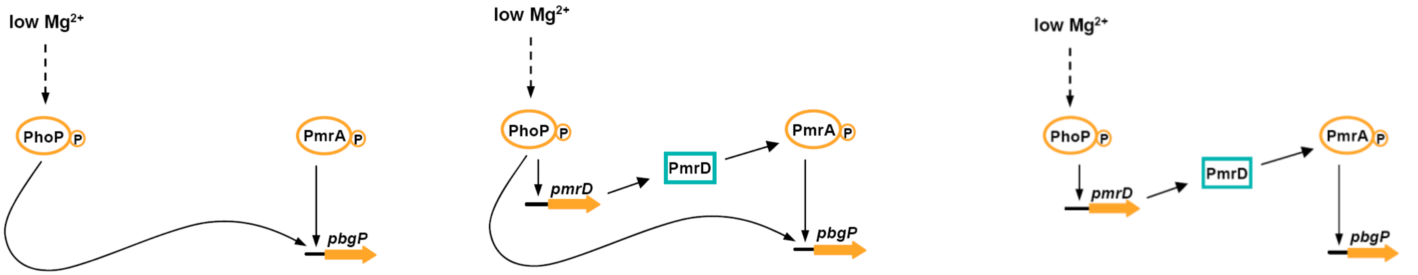
Feedforward connector loop (FCL)



Feedforward loop (FFL)

In vivo dynamics of regulatory architectures



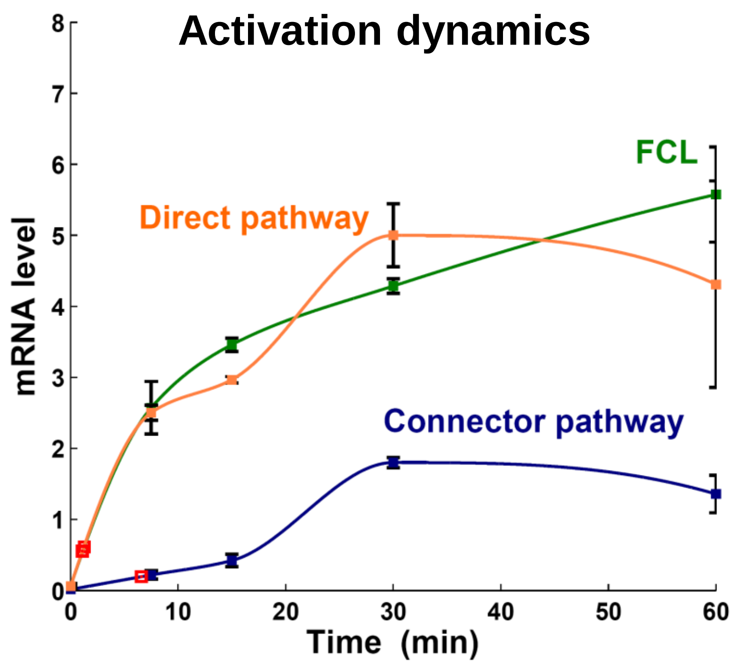


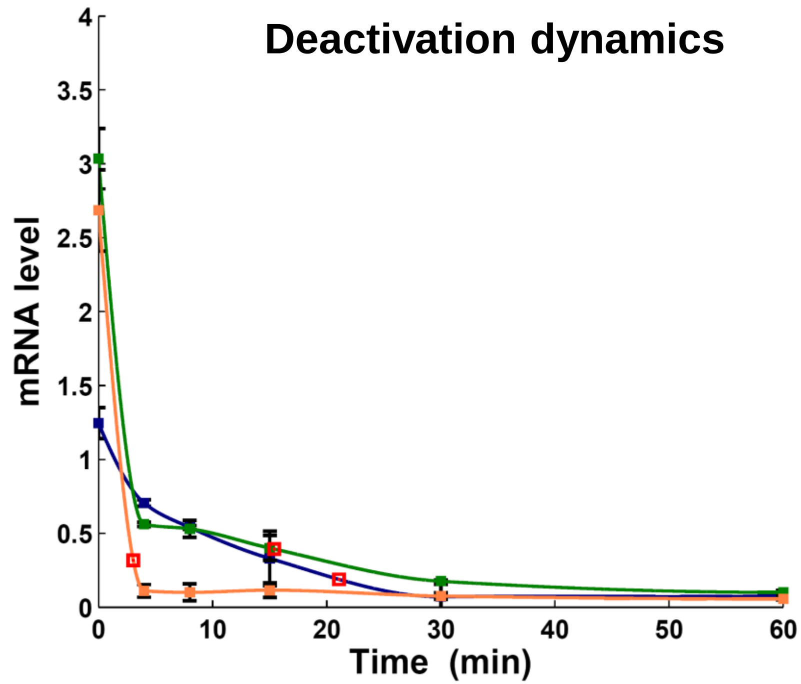
Direct regulation

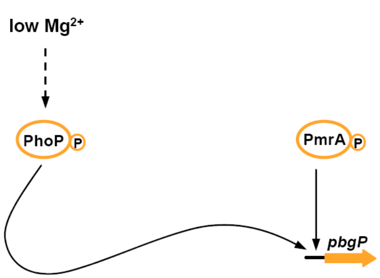
Feedforward connector loop (FCL)

Connector-mediated pathway

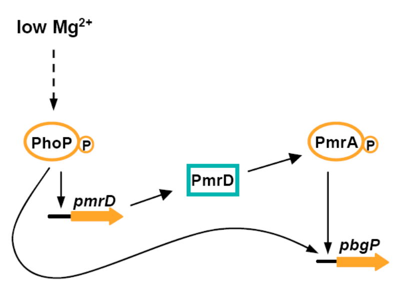
In vivo dynamics of regulatory architectures



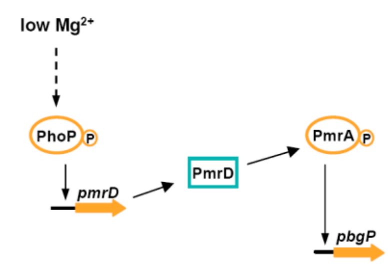




Direct regulation



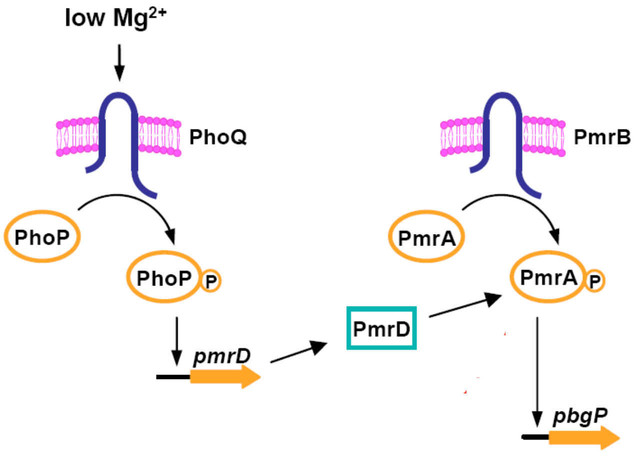
Feedforward connector loop (FCL)



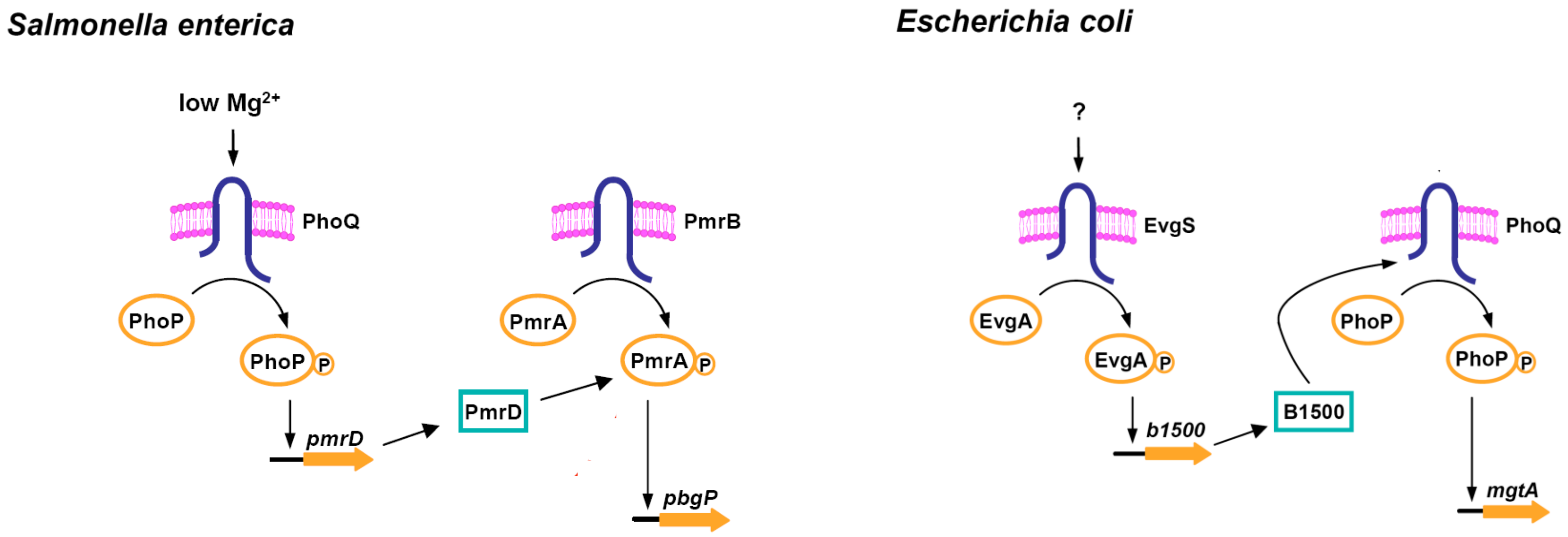
Connector-mediated pathway

Connector-mediated regulation is an emerging paradigm in bacterial gene expression control

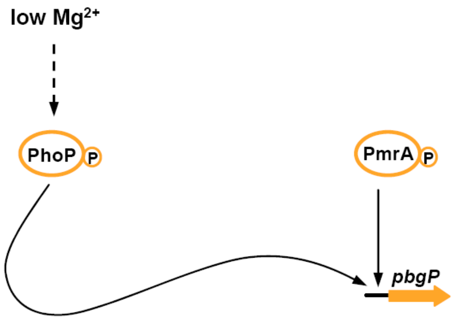
Salmonella enterica



Connector-mediated regulation is an emerging paradigm in bacterial gene expression control

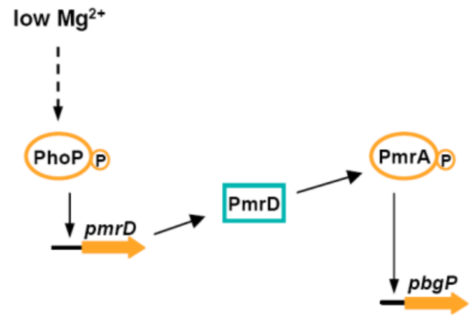


Conclusions



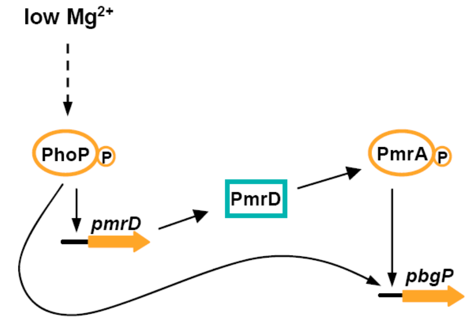
Direct pathway

Yersinia pestis



Connector-mediated pathway

Salmonella enterica

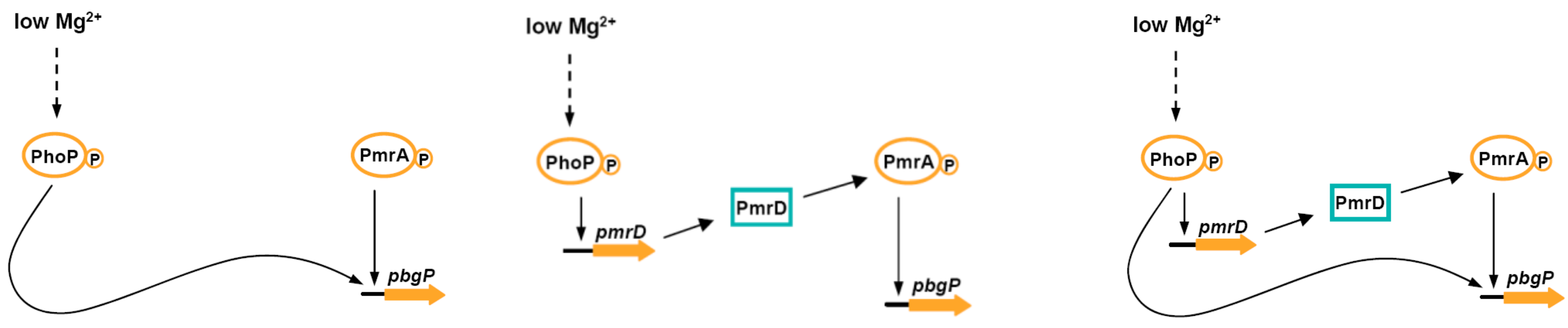


Feedforward connector loop (FCL)

Klebsiella pneumoniae

- **Properties of induction ratios, activation/deactivation timing, and response levels are determined by the structure of regulatory circuits**

Conclusions



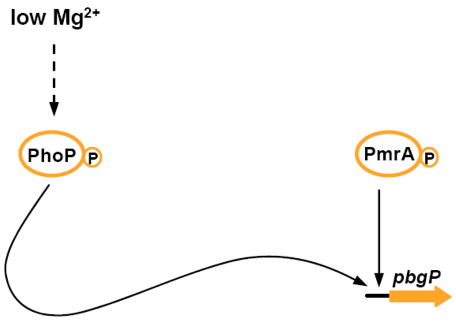
Direct pathway
Yersinia pestis

Connector-mediated pathway
Salmonella enterica

Feedforward connector loop (FCL)
Klebsiella pneumoniae

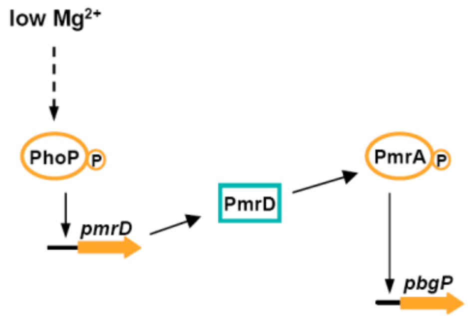
- **Properties of induction ratios, activation/deactivation timing, and response levels are determined by the structure of regulatory circuits**
- **Connector-mediated regulatory mechanisms confer special functional features on the regulatory circuits**

Conclusions



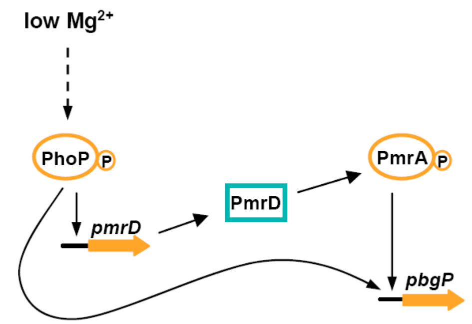
Direct pathway

Yersinia pestis



Connector-mediated pathway

Salmonella enterica



Feedforward connector loop (FCL)

Klebsiella pneumoniae

- **Properties of induction ratios, activation/deactivation timing, and response levels are determined by the structure of regulatory circuits**

- **Connector-mediated regulatory mechanisms confer special functional features on the regulatory circuits**

- **Different regulatory architectures may contribute to different lifestyles of bacterial species**

Acknowledgements

Akinori Kato

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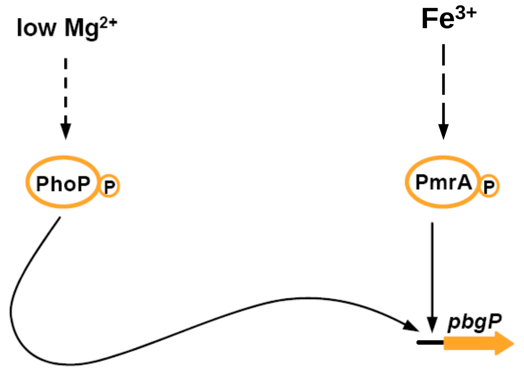
Groisman Lab

Henry Huang (Washington U)

Howard Hughes Medical Institute

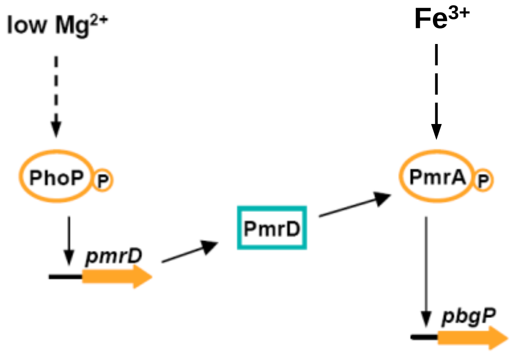
National Institutes of Health

Strong second input speeds up activation and slows down deactivation of the connector-mediated pathway

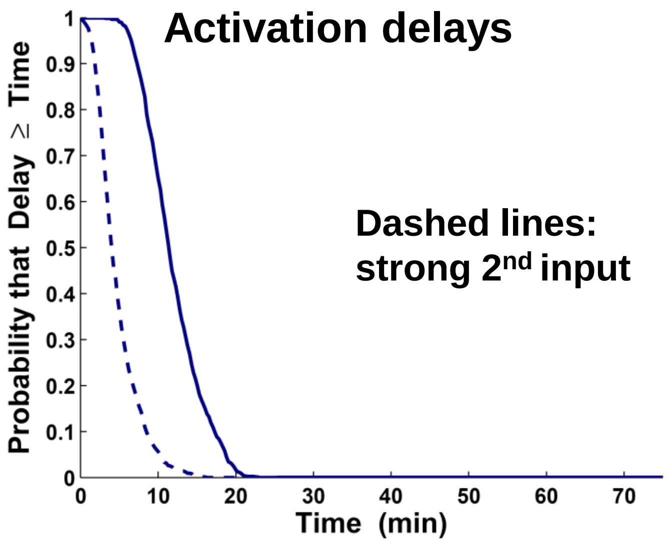


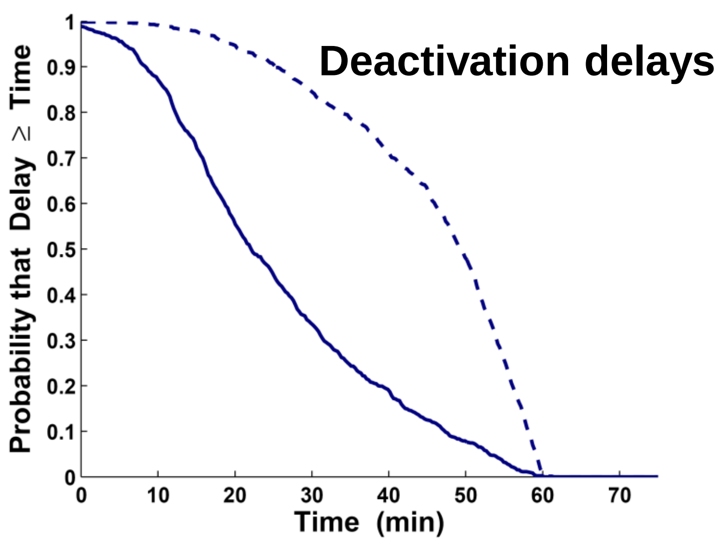
Direct regulation

(reference architecture)

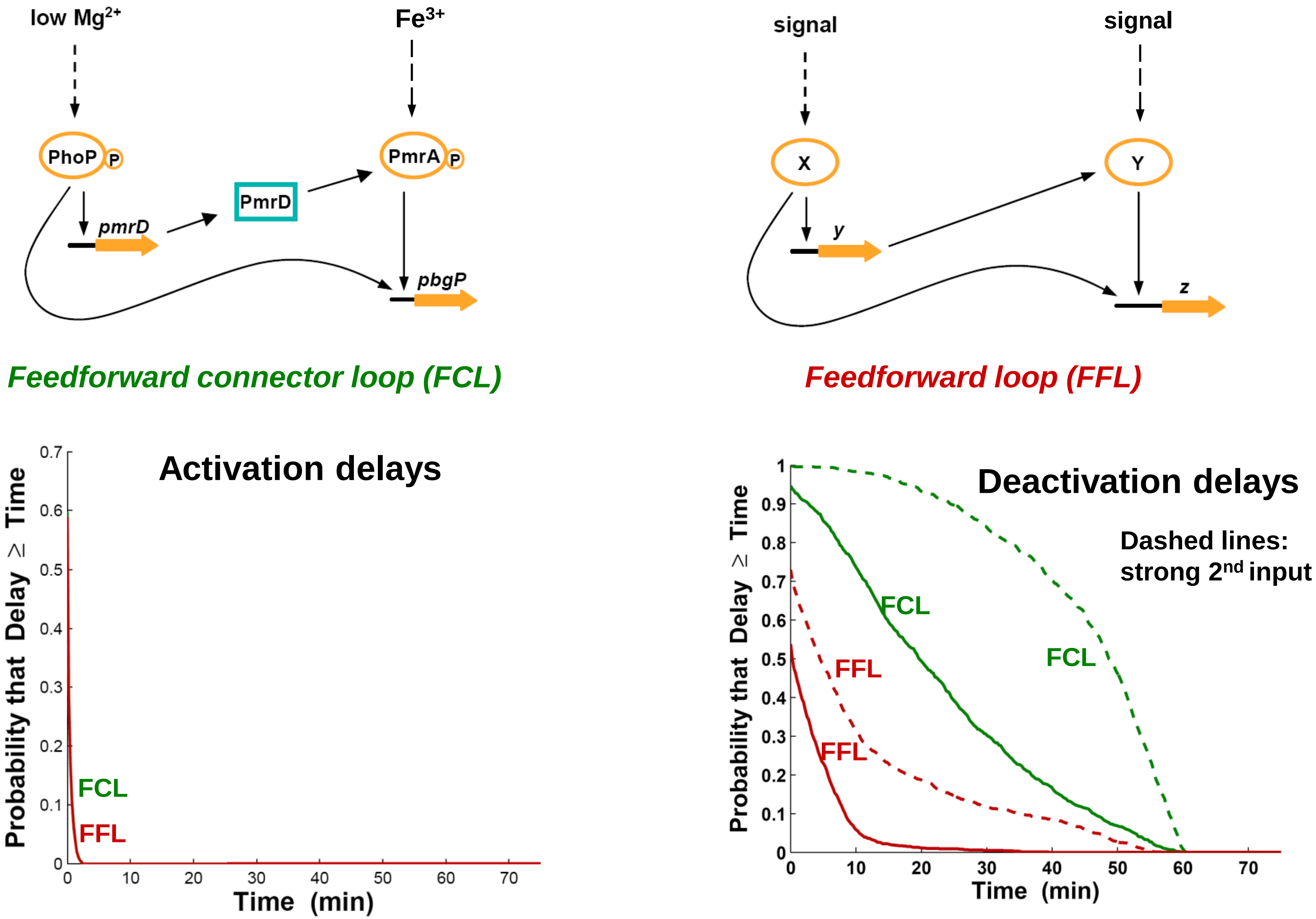


Connector-mediated pathway



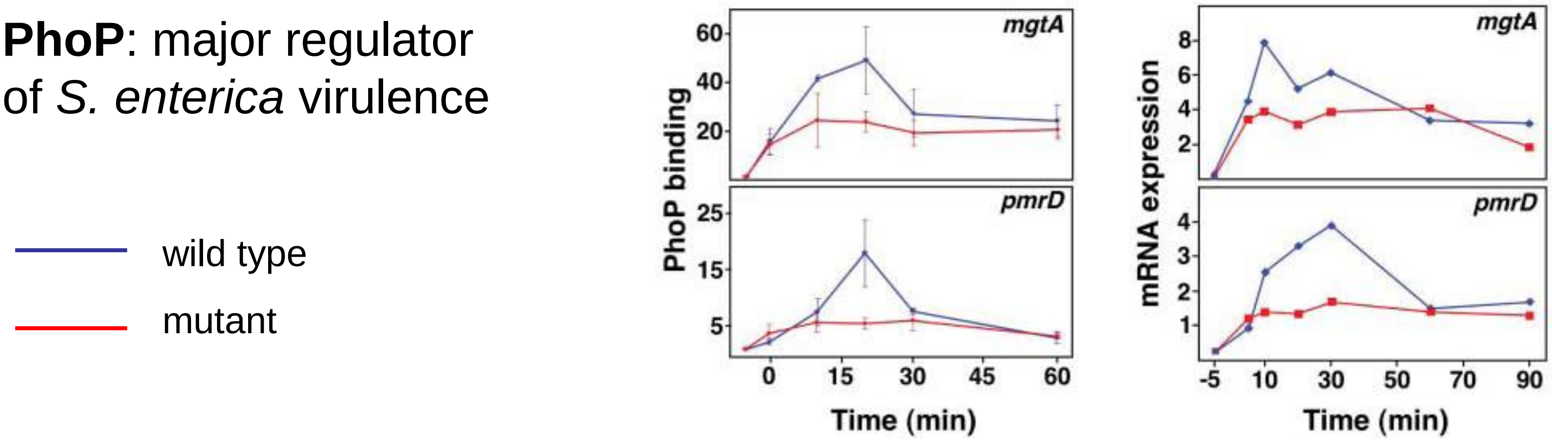


Strong activation of second input increases deactivation delays

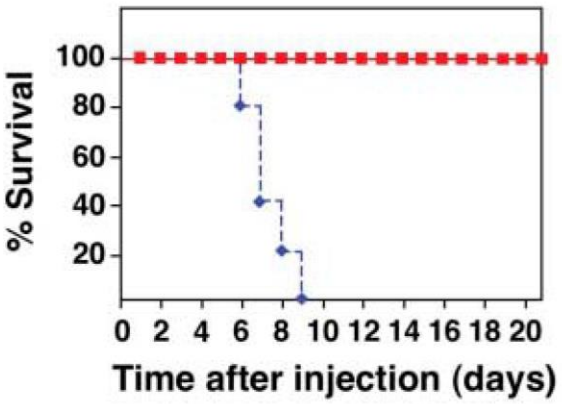


Shape of a dynamic response curve determines the ability of *Salmonella enterica* to cause disease

PhoP: major regulator of *S. enterica* virulence



S. enterica infection in a mouse model:



(Shin et al., *Science* 2006)