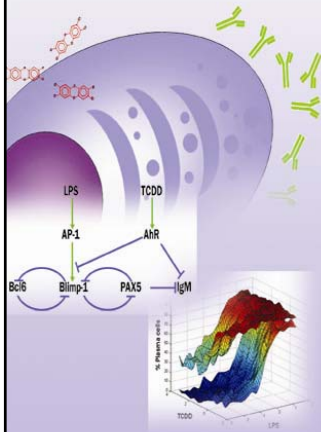


# Cellular Homeostasis, Adaptation and Steady-State Dose Response

September 25, 2008

Qiang Zhang

Division of Computational Biology  
The Hamner Institutes for Health Sciences



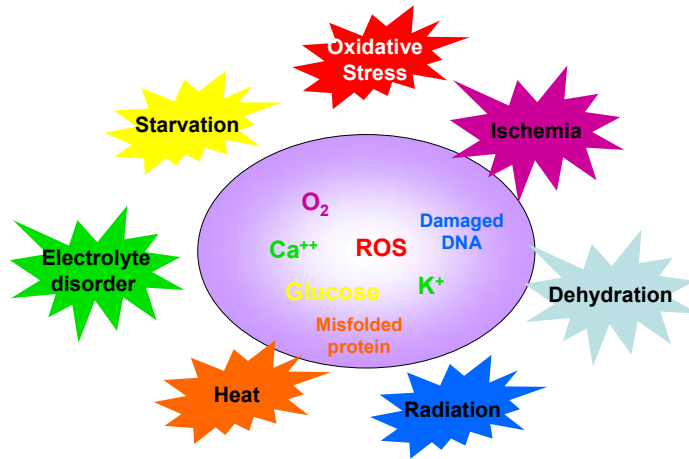
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## Concepts

- **Homeostasis** is the capability of a biological system to maintain a relatively stable internal milieu in a fluctuating external environment.
- Homeostatic control operates at all levels of biological organizations.
  1. **Whole-body level:** blood glucose concentration, blood pressure, body weight
  2. **Tissue/organ level:** tissue O<sub>2</sub> pressure, organ size and mass
  3. **Cellular level:** reactive oxygen species (ROS), ions, mis-folded proteins, DNA damages, metabolites, osmolarity
- **Adaptive cellular stress response** is the process by which a homeostatic mechanism is activated to restore the internal state of the cell after initial perturbation.

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## Cellular Stresses

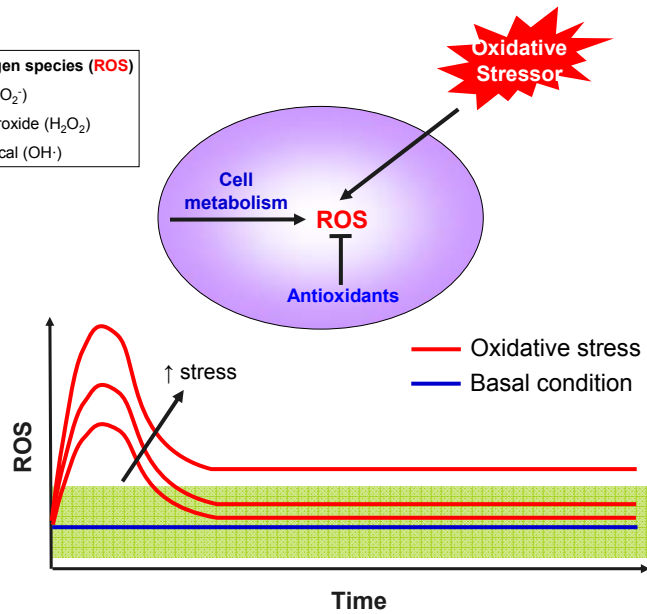


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## Adaptive Cellular Stress Response

### Reactive oxygen species (ROS)

- Super oxide ( $O_2^-$ )
- Hydrogen peroxide ( $H_2O_2$ )
- Hydroxyl radical ( $OH\cdot$ )

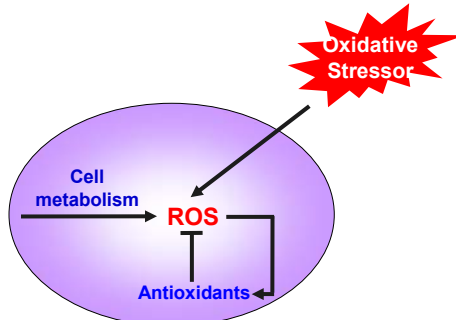


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# Adaptive Cellular Stress Response

## Reactive oxygen species (ROS)

- Super oxide ( $O_2^-$ )
- Hydrogen peroxide ( $H_2O_2$ )
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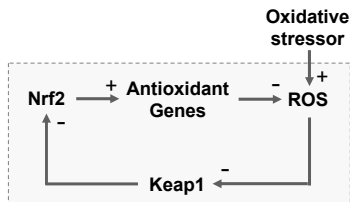


- What is the circuit design that underlies these adaptive responses to perturbations?
- Is there anything in common between the homeostatic control mechanisms handling different types of cellular stress?

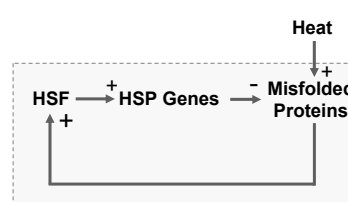
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# Negative Feedback-mediated Anti-Stress Gene Regulatory Networks

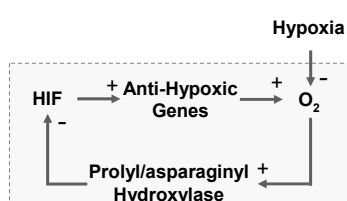
## A Oxidative Stress Response



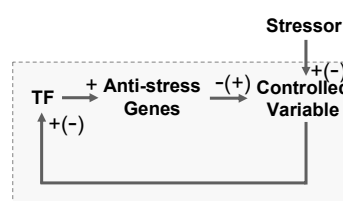
## B Heat Shock Response



## C Hypoxic Response



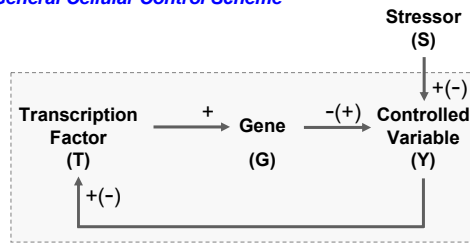
## D General Cellular Control Scheme



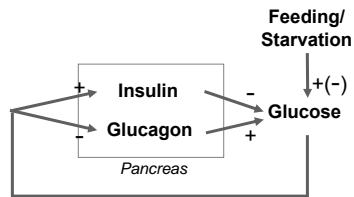
TF: Transcription factor

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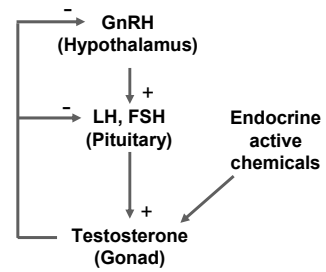
### General Cellular Control Scheme



### Blood Glucose Homeostasis

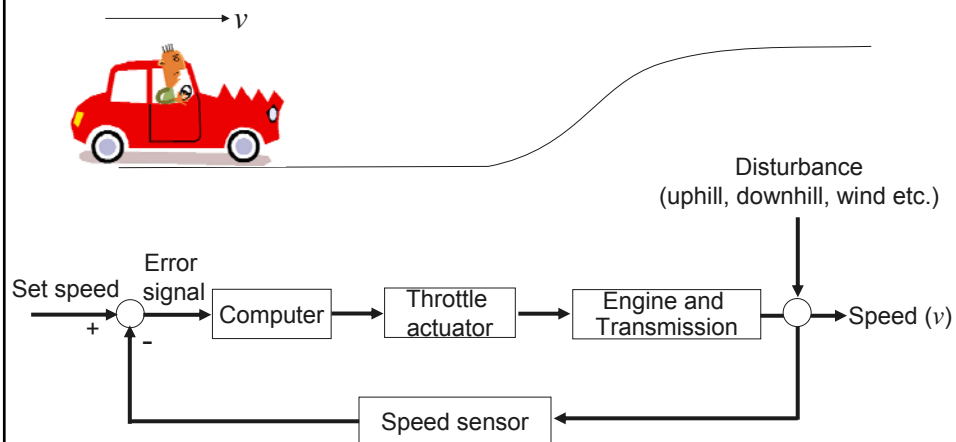


### Sex Hormone Homeostasis

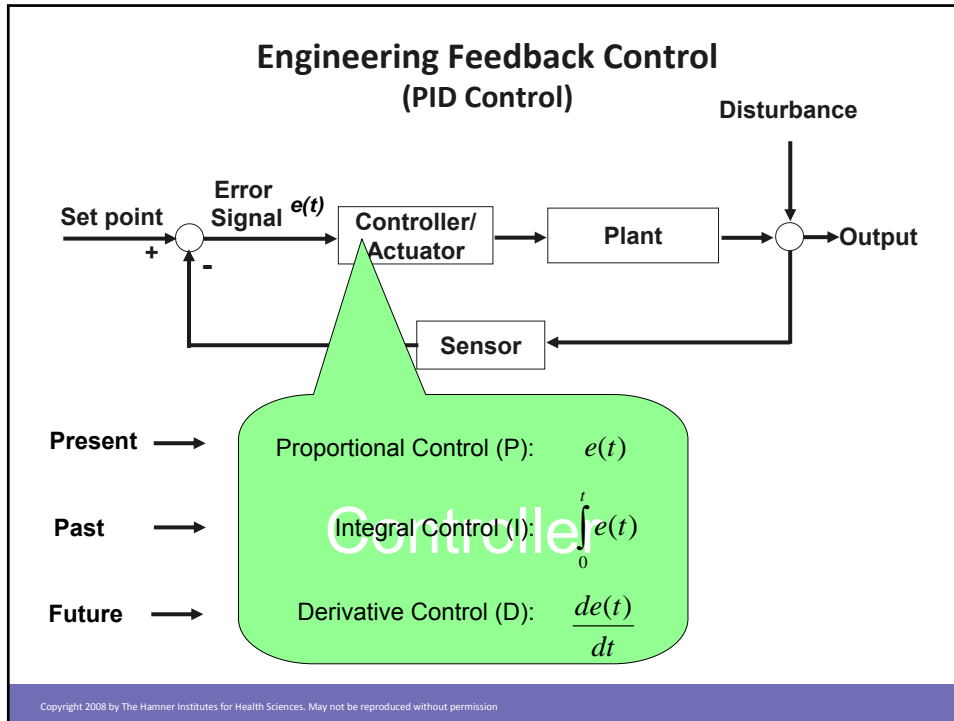


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### Automobile Cruise Control System



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### Objectives

*General Cellular Control Scheme*

1. What is nature's design principle to maintain robust homeostasis in response to perturbations by stressors?
2. How do the steady-state dose response curves look in such a feedback system and why?
3. What circuit parameters quantitatively affect the shape of dose response curves?

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## Response Coefficient (Logarithmic Gain) and Shape of Dose Response Curve

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**X**

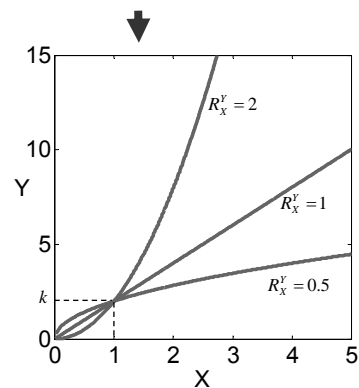
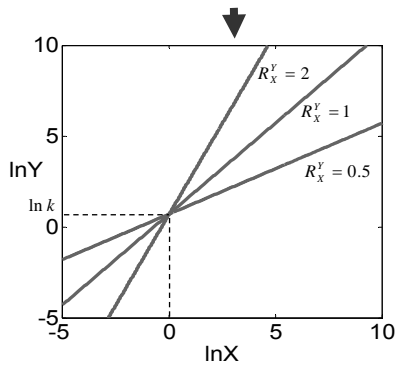
Response coefficient (logarithmic gain)

$$R_x^Y = \lim_{\Delta X \rightarrow 0} \frac{\Delta Y/Y}{\Delta X/X} = \frac{dY/Y}{dX/X} = \frac{d \ln Y}{d \ln X}$$

**Y**

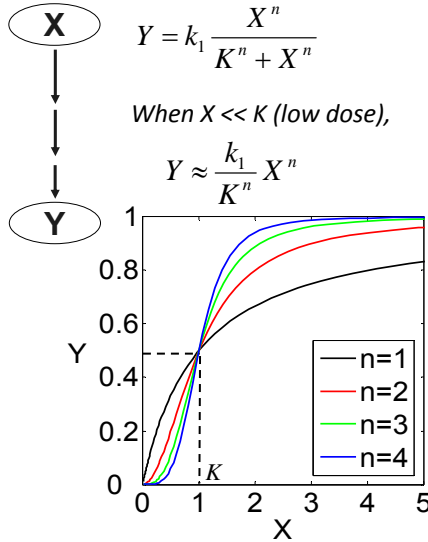
Assume  $R_x^Y$  remains constant

$$\ln Y = R_x^Y \ln X + \ln k \quad \longrightarrow \quad Y = kX^{R_x^Y}$$



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## From Response Coefficient to Hill Coefficient



Response coefficient

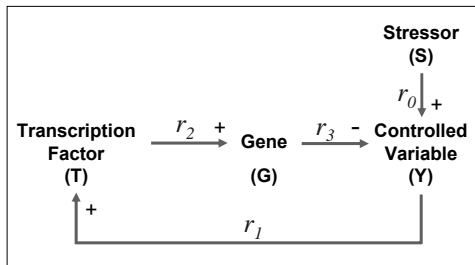
$$R_X^Y = \frac{dY/Y}{dX/X} = \frac{d \ln Y}{d \ln X}$$

$$Y = kX^{R_X^Y}$$

- Indeed, the low-dose part of a Hill function ( $n > 1$ ) or ultrasensitive curve is sublinear.
- At low doses, response coefficient is equal to Hill coefficient.
- Strictly speaking, ultrasensitivity occurs when response coefficient is significantly greater than 1 within some dose range.

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## Systems-Level Gains with Negative Feedback Regulation



According to Signal Transfer Analysis  
(Kholodenko et al, 1997)

$$R_S^Y = \frac{d \ln Y}{d \ln S} = \frac{r_0}{1 + |r_1 r_2 r_3|}$$

$$R_S^T = \frac{d \ln T}{d \ln S} = \frac{r_0 r_1}{1 + |r_1 r_2 r_3|}$$

$$R_S^G = \frac{d \ln G}{d \ln S} = \frac{r_0 r_1 r_2}{1 + |r_1 r_2 r_3|}$$

Local gain:

$$r_0 = d \ln Y / d \ln S$$

$$r_1 = d \ln T / d \ln Y$$

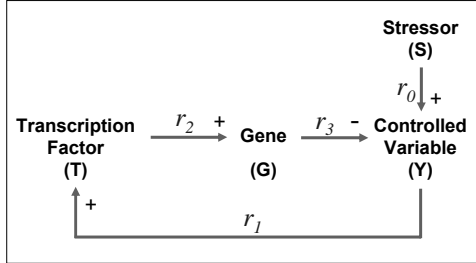
$$r_2 = d \ln G / d \ln T$$

$$r_3 = d \ln Y / d \ln G$$

Loop gain:  $R_{loop} = |r_1 r_2 r_3|$

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## Systems-Level Gains with Negative Feedback Regulation



$$\frac{dY}{dt} = k_0 \cdot S - k_1 \cdot G \cdot Y$$

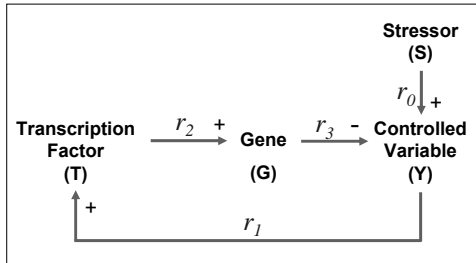
(At steady state)

$$Y = \frac{k_0}{k_1 \cdot G} \cdot S \rightarrow r_0 = 1$$

$$R_S^Y = \frac{d \ln Y}{d \ln S} = \frac{1}{1 + |r_1 r_2 r_3|} \quad R_S^T = \frac{d \ln T}{d \ln S} = \frac{r_1}{1 + |r_1 r_2 r_3|} \quad R_S^G = \frac{d \ln G}{d \ln S} = \frac{r_1 r_2}{1 + |r_1 r_2 r_3|}$$

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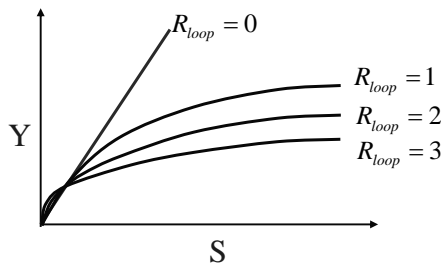
## Y vs. S Dose Response with Constant Local Gains



$$R_S^Y = \frac{d \ln Y}{d \ln S} = \frac{1}{1 + |r_1 r_2 r_3|} \leq 1$$

$$R_{loop} = |r_1 r_2 r_3|$$

$R_{loop}$	$R_S^Y$
0	1
1	0.5
2	0.333
3	0.25

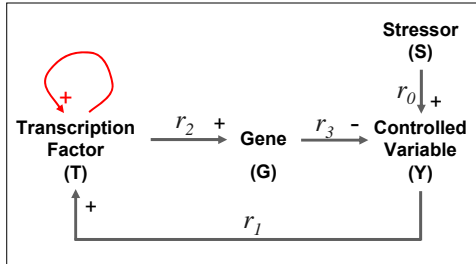


\* Assume all local gains remain constant with respect to S

- The higher the  $R_{loop}$ , the more superlinear the Y vs. S curve.
- For high homeostatic performance, high  $R_{loop}$  is preferred !

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## Enhancing Loop Gain for Strong Homeostasis



$$R_s^Y = \frac{d \ln Y}{d \ln S} = \frac{1}{1 + |r_1 r_2 r_3|}$$

$$R_{loop} = |r_1 r_2 r_3|$$

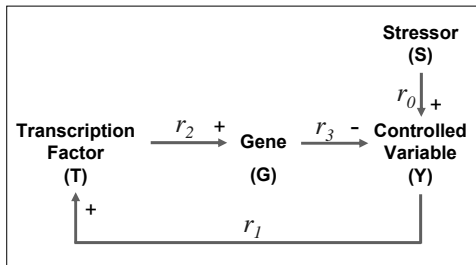
Increasing individual local gains will enhance loop gain, which requires ultrasensitive motifs.

1)  $\uparrow r_1$

- Homo-dimerization or homo-trimerization of transcription factor T (Heat shock factor 1 (HSF1) is a homo-trimer)
- Positive auto-regulation of T (Nrf2 for oxidative/electrophilic stress response is auto-regulated)
- Zero-order phosphorylation of T (Many stress-activated transcription factors are regulated by MAPK phosphorylation)

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## Enhancing Loop Gain for Strong Homeostasis



$$R_s^Y = \frac{d \ln Y}{d \ln S} = \frac{1}{1 + |r_1 r_2 r_3|}$$

$$R_{loop} = |r_1 r_2 r_3|$$

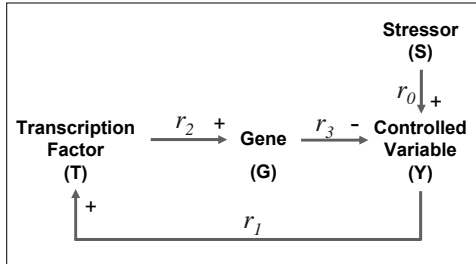
Increasing individual local gains will enhance loop gain, which requires ultrasensitive motifs.

2)  $\uparrow r_2$

- Cooperative binding of T to gene promoter (HSF binding to heat response element)
- Multimerization of anti-stress gene product (enzymes) (Antioxidant enzymes GR, SOD, GST, GS homodimer, GPx and CAT homotetramer)

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## Enhancing Loop Gain for Strong Homeostasis



$$R_S^Y = \frac{d \ln Y}{d \ln S} = \frac{1}{1 + |r_1 r_2 r_3|}$$

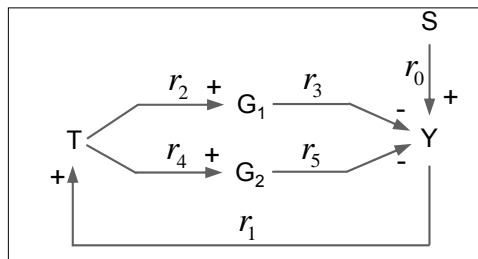
$$R_{loop} = |r_1 r_2 r_3|$$

Increasing individual local gains will enhance loop gain, which requires ultrasensitive motifs.

- 3)  $\uparrow r_3$
- Multiple genes involved in controlling Y

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## Enhancing Loop Gain for Strong Homeostasis



$$R_S^Y = \frac{1}{1 + |r_1 (r_2 r_3 + r_4 r_5)|}$$

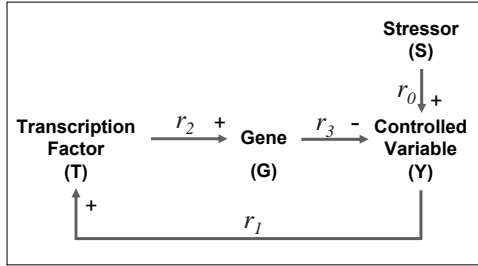
$$R_{loop} = |r_1 (r_2 r_3 + r_4 r_5)|$$

Increasing individual local gains will enhance loop gain, which requires ultrasensitive motifs.

- 3)  $\uparrow r_3$
- Multiple genes involved in controlling Y (multi-step signaling)
    - $G_1$  and  $G_2$  are subunits of a functional holo-enzyme (GCLC and GCLM are both Nrf2 targets, and they heterodimerize to form the holo-enzyme GCL).
    - $G_1$  and  $G_2$  are different enzymes for different but associated reactions that collectively control Y (GCL provides GSH, which are used as a cofactor by GPx to remove  $H_2O_2$ ).

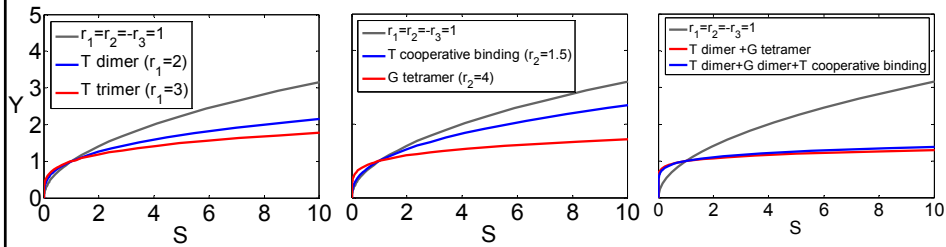
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## Y vs. S Dose Response with Constant Local Gains



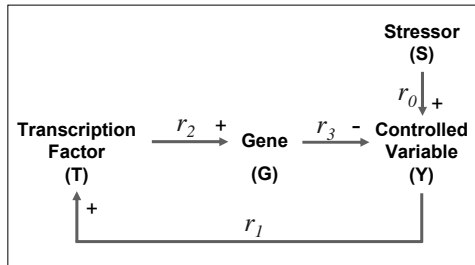
$$R_S^Y = \frac{d \ln Y}{d \ln S} = \frac{1}{1 + |r_1 r_2 r_3|}$$

$$R_{loop} = |r_1 r_2 r_3|$$



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## Gene Expression (G) vs. S Dose Response



$$R_S^G = \frac{d \ln G}{d \ln S} = \frac{r_1 r_2}{1 + |r_1 r_2|} \leq 1$$

When  $|r_1 r_2| \gg 1$

$$R_S^G \approx 1$$

$$\frac{dY}{dt} = k_0 \cdot S - k_1 \cdot G \cdot Y$$

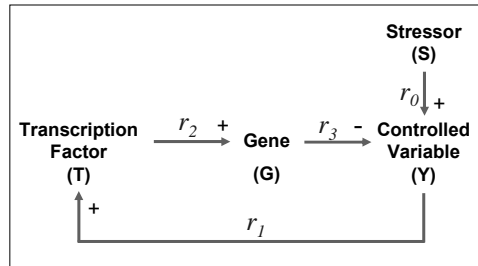
(At steady state)

$$Y = \frac{k_0 S}{k_1 G} \Rightarrow r_3 = -1$$

$R_{loop}$	$R_S^G$
0	0
1	0.5
2	0.667
3	0.75
4	0.8
$\infty$	1

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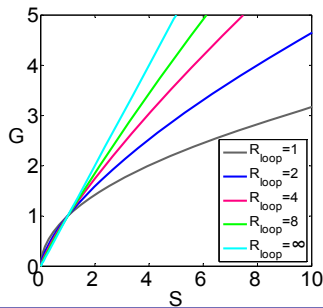
## Gene Expression (G) vs. S Dose Response



$$R_S^G = \frac{d \ln G}{d \ln S} = \frac{r_1 r_2}{1 + |r_1 r_2 r_3|} \leq 1$$

When  $|r_1 r_2| \gg 1$

$$R_S^G \approx 1$$



High loop gain, which is required for high homeostatic performance, tends to linearize the dose response for gene expression

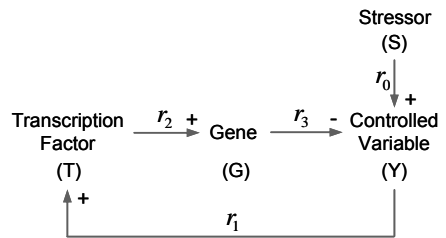
$R_{loop}$	$R_S^G$
0	0
1	0.5
2	0.667
3	0.75
4	0.8
$\infty$	1

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## Dose Response When Considering Maximum Gene Induction and Enzyme Saturation

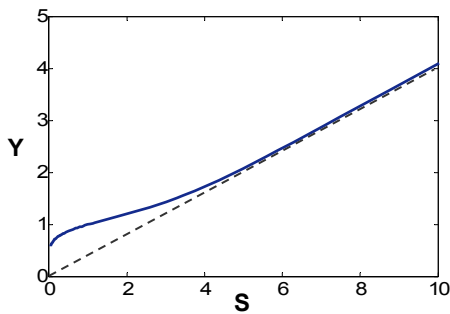
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## Moving toward Maximum Gene Induction



$$R_S^Y = \frac{1}{1 + |r_1 r_2 r_3|}$$

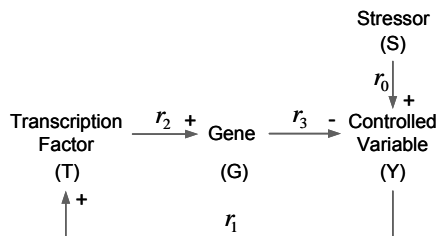
- when activation of T → maximum,  $r_1 \rightarrow 0$
- when induction of G → maximum,  $r_2 \rightarrow 0$
- As  $r_1$  and  $r_2 \rightarrow 0$ ,  $R_S^Y \rightarrow 1$ , i.e., linear response



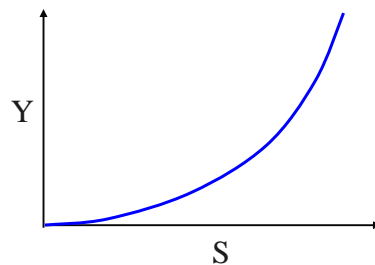
Y vs. S is first superlinear, then sublinear, then linear.

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## Moving toward Enzyme Saturation



- When the amount of Y is very high, it starts to saturate the enzyme G, which functions to eliminate Y. Then a small % change in S would cause a large % change in Y.
- When G operating close to saturation, Y vs. S is sublinear.



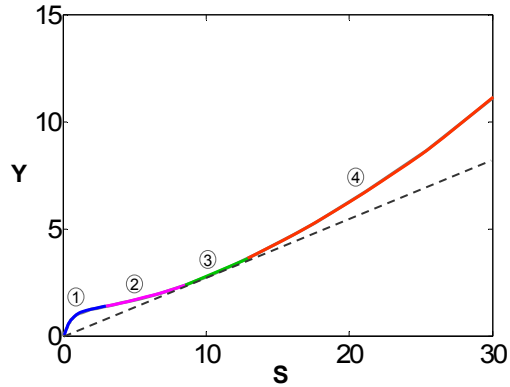
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## A More Likely Dose Response Curve

When considering maximum gene induction and enzyme saturation, the full-range Y vs. S dose response curve is more likely to transition through the following phases as S increases:

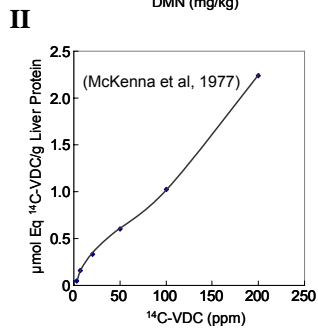
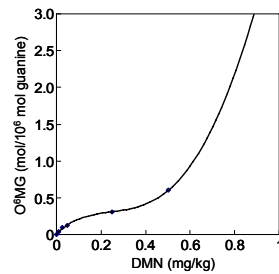
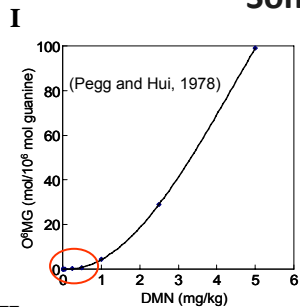
1. Superlinear controlled
2. Sublinear less controlled
3. Linear uncontrolled
4. Sublinear catastrophic

Low dose region is intrinsically nonlinear with active homeostatic control.

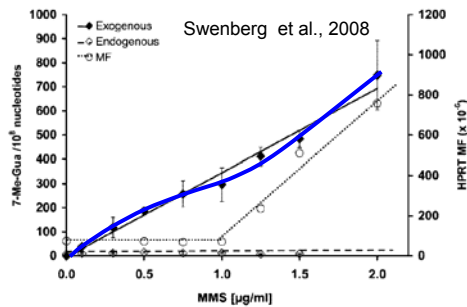


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## Some Biological Examples

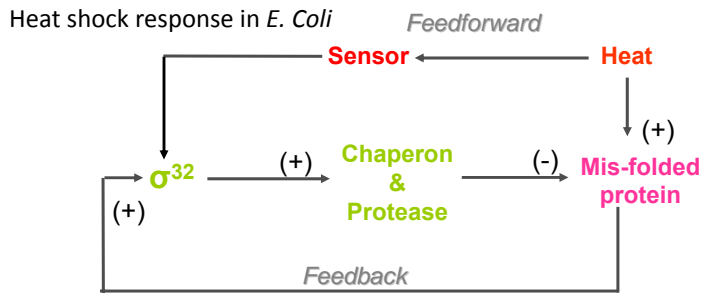


**III**

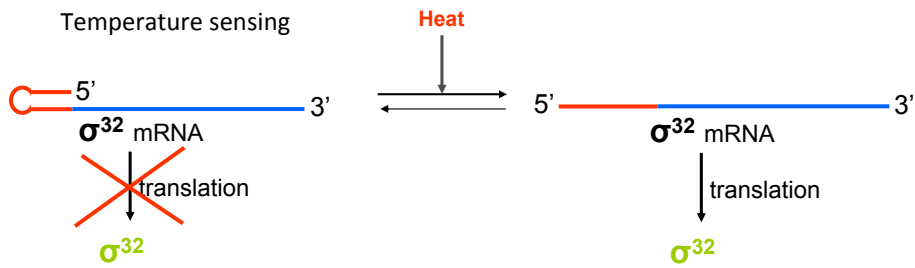


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## Feedforward Homeostatic Control

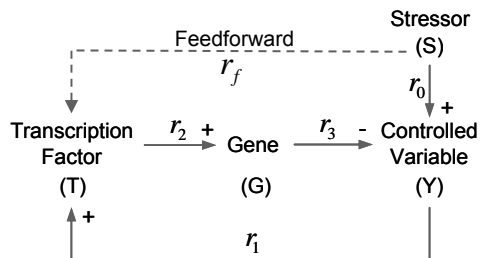


Temperature sensing



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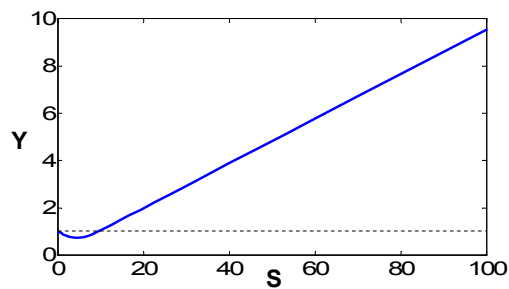
## Non-monotonic Dose Response with Feedforward Control



$$R_S^Y = \frac{r_0 - |r_f r_2 r_3|}{1 + |r_1 r_2 r_3|}$$

$$\text{When } r_0 < |r_f r_2 r_3|$$

$$R_S^Y < 0$$



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## Summary

1. Cellular homeostatic control is primarily mediated by gene regulatory networks organized in negative feedback loops.
2. Strong homeostatic performance requires high loop gain, i.e., ultrasensitive motifs to activate anti-stress genes.
3. Dose response curves for the controlled variable in a (-) feedback-mediated control system can transition sequentially through several distinct phases as stressor level increases: superlinear controlled, sublinear less controlled, linear uncontrolled, and sublinear catastrophic.
4. Feedforward control may produce nonmonotonic responses.
5. The low-dose region is intrinsically non-linear with active homeostatic control.

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## For details, see.....

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PLoS COMPUTATIONAL BIOLOGY

### Dose Response Relationship in Anti-Stress Gene Regulatory Networks

Qiang Zhang<sup>\*</sup>, Melvin E. Andersen

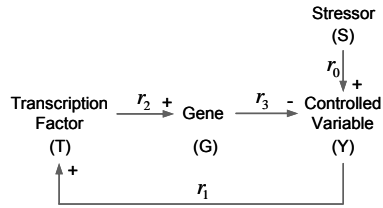
Division of Computational Biology, CIIT Centers for Health Research, Research Triangle Park, North Carolina, United States of America

To maintain a stable intracellular environment, cells utilize complex and specialized defense systems against a variety of external perturbations, such as electrophilic stress, heat shock, and hypoxia, etc. Irrespective of the type of stress, many adaptive mechanisms contributing to cellular homeostasis appear to operate through gene regulatory networks that are organized into negative feedback loops. In general, the degree of deviation of the controlled variables, such as electrophiles, misfolded proteins, and  $O_2$ , is first detected by specialized sensor molecules, then the signal is transduced to specific transcription factors. Transcription factors can regulate the expression of a suite of anti-stress genes, many of which encode enzymes functioning to counteract the perturbed variables. The objective of this study was to explore, using control theory and computational approaches, the theoretical basis that underlies the steady-state dose response relationship between cellular stressors and intracellular biochemical species (controlled variables, transcription factors, and gene products) in these gene regulatory networks. Our work indicated that the shape of dose response curves (linear, superlinear, or sublinear) depends on changes in the specific values of local response coefficients (gains) distributed in the feedback loop. Multimerization of anti-stress enzymes and transcription factors into homodimers, homotrimers, or even higher-order multimers, play a significant role in maintaining robust homeostasis. Moreover, our simulation noted that dose response curves for the controlled variables can transition sequentially through four distinct phases as stressor level increases: initial superlinear with lesser control, superlinear more highly controlled, linear uncontrolled, and sublinear catastrophic. Each phase relies on specific gain-changing events that come into play as stressor level increases. The low-dose region is intrinsically nonlinear, and depending on the level of local gains, presence of gain-changing events, and degree of feedforward gene activation, this region can appear as superlinear, sublinear, or even J-shaped. The general dose response transition proposed here was further examined in a complex anti-electrophilic stress pathway, which involves multiple genes, enzymes, and metabolic reactions. This work would help biologists and especially toxicologists to better assess and predict the cellular impact brought about by biological stressors.

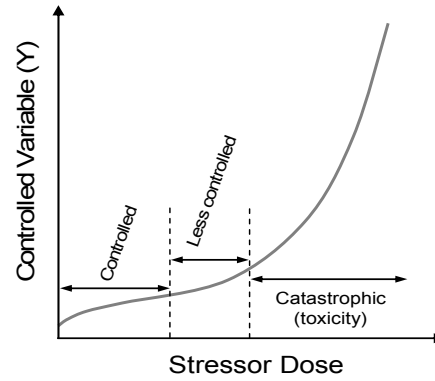
Citation: Zhang Q, Andersen ME (2007) Dose response relationship in anti-stress gene regulatory networks. PLoS Comput Biol 3(3): e24. doi:10.1371/journal.pcbi.0030024

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## Take Home Message



$$R_S^Y = \frac{r_0}{1 + |r_1 r_2 r_3|}$$



**Low-dose response is nonlinear**

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