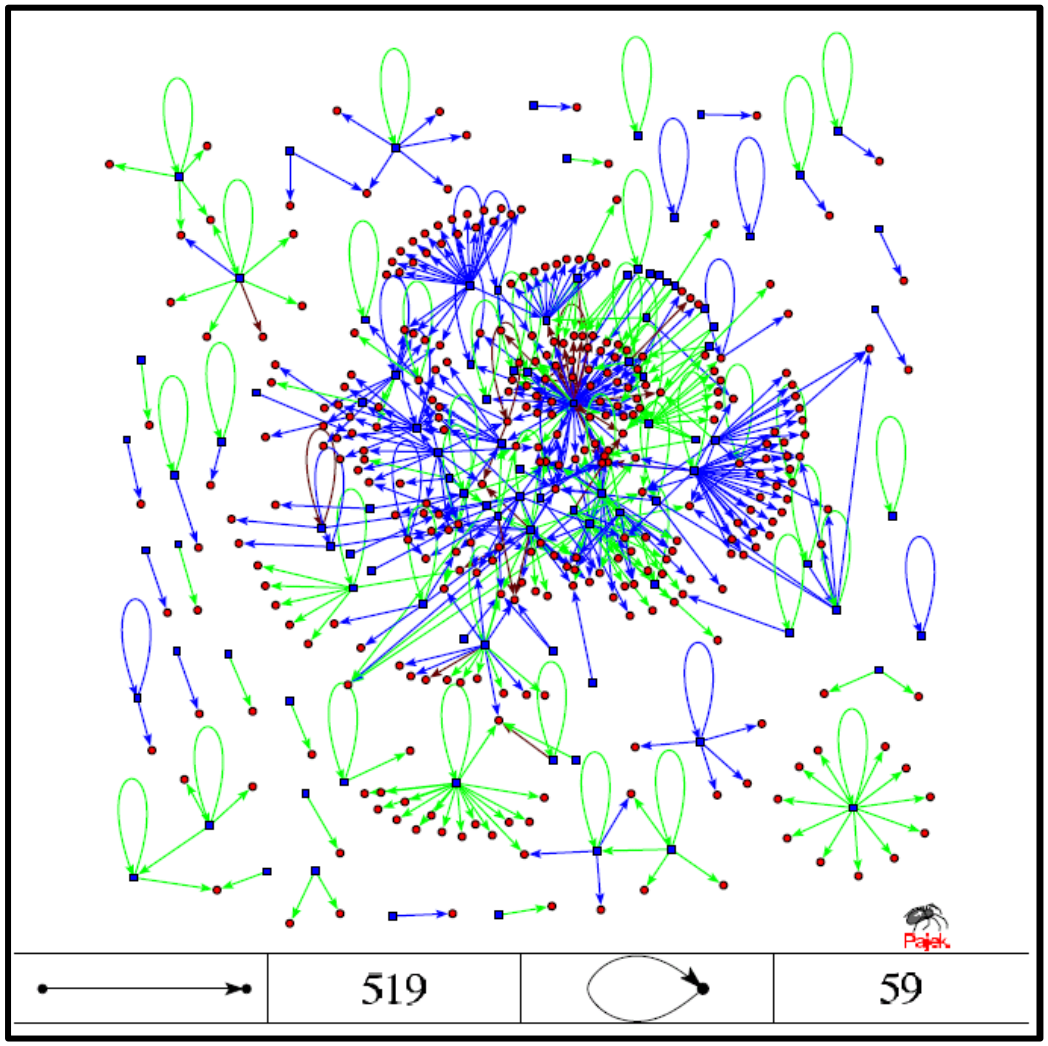
Network motifs in transcriptional networks

Example: *E. coli* transcriptional regulatory network



Blue diamonds represent transcription factors (TF)
red circles denote the regulated operons.
The links are color-coded according to their function:

- blue-activator
- green-repressor
- brown-activator or repressor effect

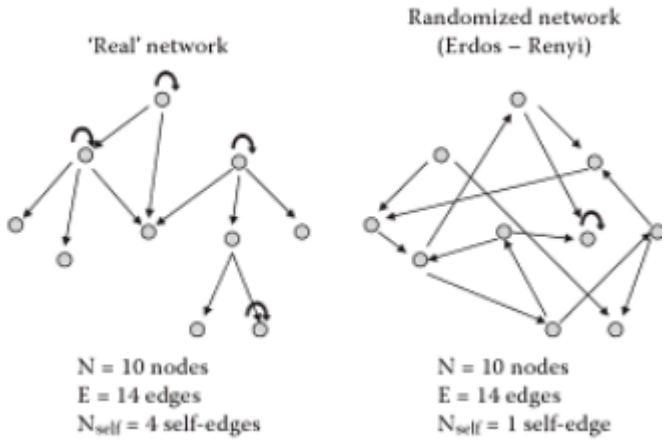
The number of the two types of elementary links*, i.e.,* the autoregulatory loops and the directed links, is listed at the bottom of the panel

Extracted from Dobrin *et al.,* 2004, BMC Bioinformatics 5:10

Detection of network motifs

Comparison with a randomized network : network with the same characteristics as the real one, *i.e.*, same number of nodes and edges but where the connections between nodes are made at random.

Network motif = a pattern that occurs in the real network significantly more often than in the randomized network



Basic idea: patterns that occur in the real network more often than in the randomized one must have been preserved over evolution against mutations that randomly change edges (example, one mutation in the promoter sequence can abolish the binding of a transcriptional factor and the loss of the edge in the network)

Autoregulatory loop: a network motifs

Two types of loops

- Negative autoregulation : when a transcription factor X represses its own transcription by binding to its own promoter to inhibit mRNA synthesis.

  $\Rightarrow$ The higher the concentration of X, the lower its production rate

- Positive autoregulation : when a transcription factor X activates its own transcription by binding to its own promoter.

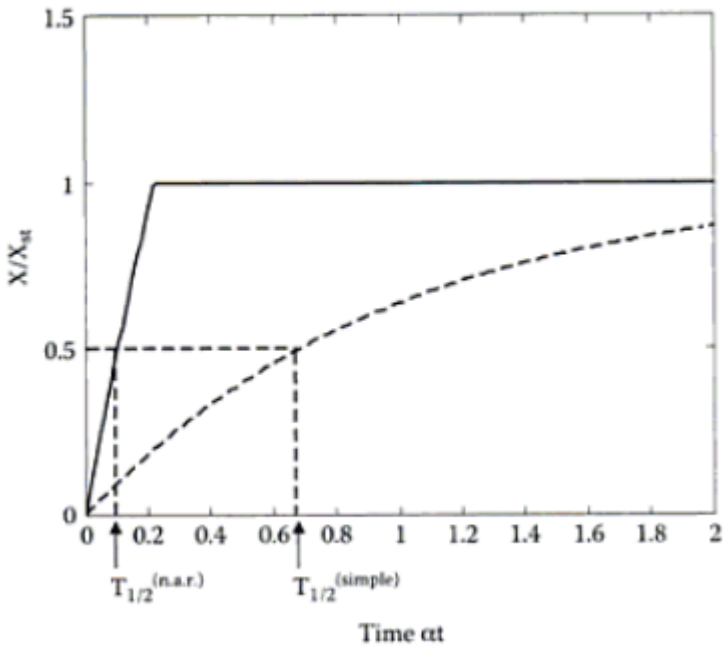
  $\Rightarrow$ The dynamics is initially slow, but as X accumulates, the production rate increases

Dynamics of X is described by its synthesis rate and its degradation/dilution rate

General form of the equation:

$$\frac{dX}{dt} = \text{synthesis}(X) - \text{degradation}(X)$$

Negative Autoregulation



Comparison of the dynamics of negatively autoregulated gene product (full line) and simply regulated gene product (dashed line) which reaches the same steady state and possesses the same degradation rate:

$$\beta = 5,\ \beta_{simple} = 1,\ \alpha = 1$$

The response time corresponds to the time the protein level reaches 50% of the steady state (the time required to reach halfway between the initial and final levels)

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Negative autoregulation speeds up the response time and increases robustness of the steady state expression level with respect to fluctuations in the production rate β

Positive Autoregulation

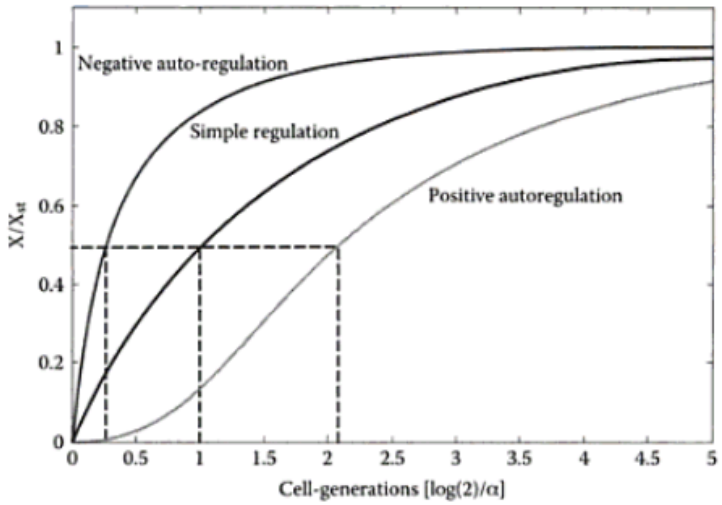

FIGURE 3.5 Dynamics of a negatively autoregulated gene, a simply regulated gene and a positively autoregulated gene. The negatively and positively autoregulated genes have a Hill input function with Hill coefficient n = 1. Shown is protein concentration normalized by its steady-state value, X/X_{st}, following an increase in production rate. Time is in cell generations, or for actively degraded proteins, $\log(2)/\alpha$, where α is the protein degradation/dilution rate. The response time is found by the intersect of the dynamics with a horizontal line at $X/X_{st} = 0.5$.

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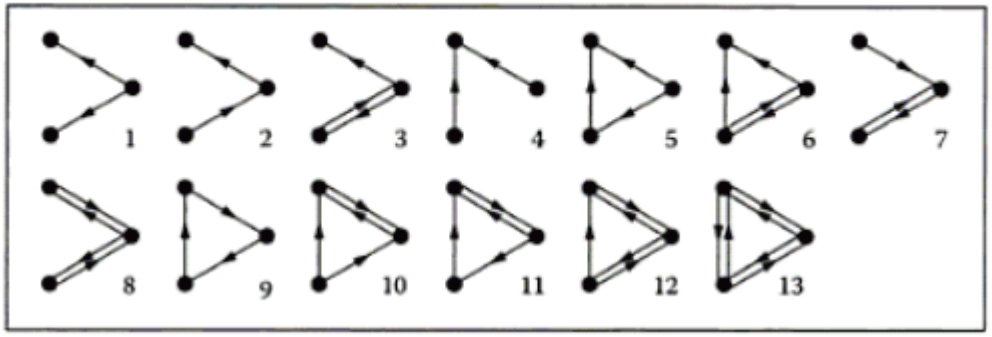
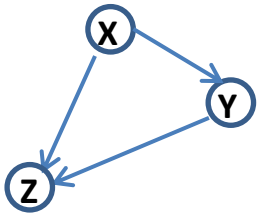
Positive autoregulation slows the response time compared to simple regulation.
The dynamics starts slowly and the production rates increase as the level of X built up.
If the value of β_{max} is high with respect to the degradation rate α, the system can become bi-stable.
Once the gene is activated, it is locked in a state of high expression and it maintains itself ON, even after the original signal of activation has disappeared.
(used for example in developmental transcription network to assure irreversible decision (cell type))

Feed-forward loop

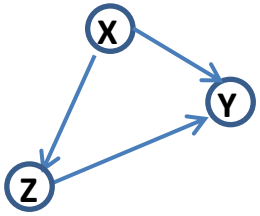
Focus on patterns with 3 nodes : there are 13 possible patterns



The subgraph 5 is the feed-forward loop (FFL)



The subgraph 9 is the feedback loop



Feed-forward loop

In transcriptional networks of many organisms the FFL is the only significant network motif of the 13 possible three-nodes motifs (comparison made in a corresponding randomized network).

In the *E. coli* transcriptional network used in Alon (2007, *An introduction to systems Biology))*, 42 FFL are identified and zero three-node feedback loop.

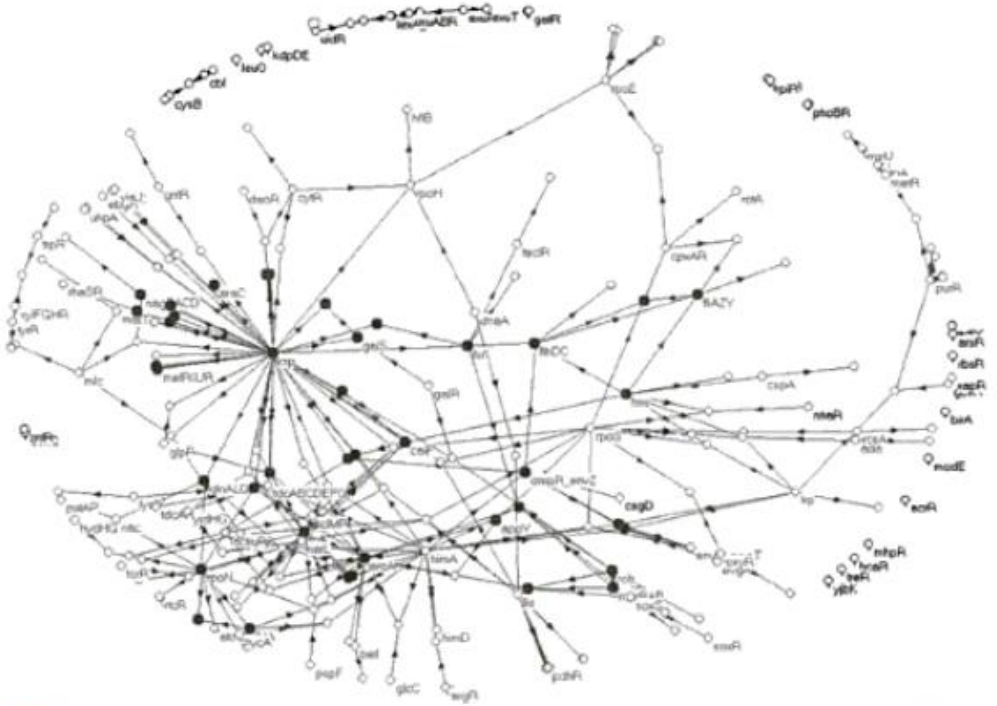


FIGURE 4.2 Feed-forward loops in the *E. coli* transcription network. Black nodes participate in FFLs.

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Feed-forward loop

Question : why such loop have been selected ?

A FFL is composed of a transcription factor X that regulates the gene of a second transcriptional Y factor, and both factors X and Y regulate the gene Z. Each regulation can be either an activation or a repression. Thus, there are 8 possible types of FFLs that can be classified in two groups :

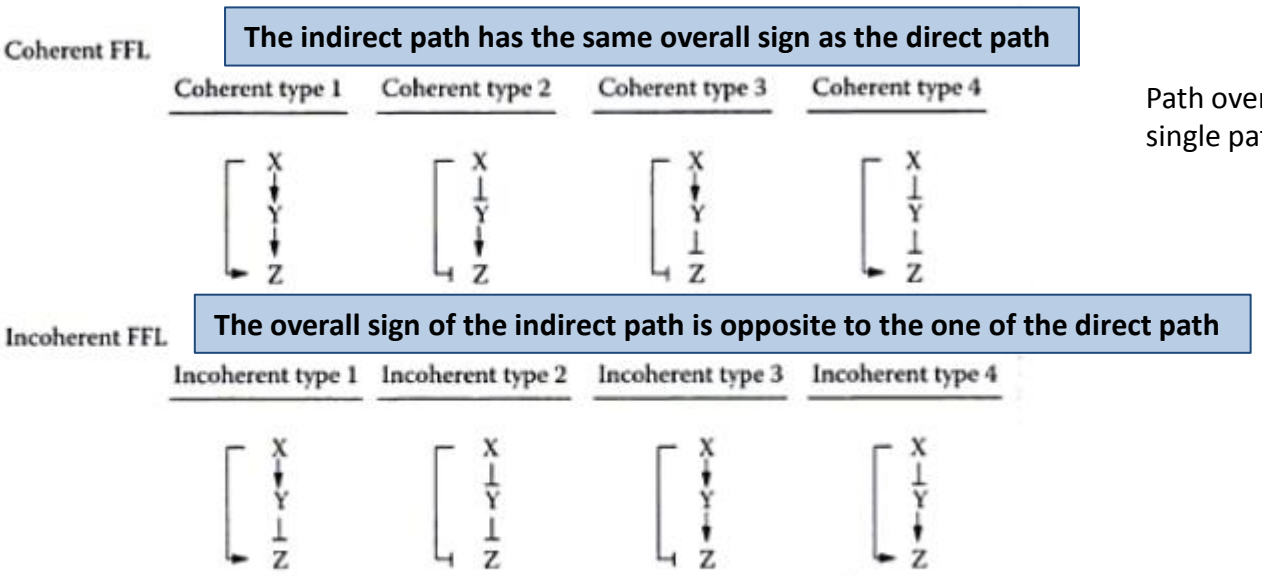

FIGURE 4.3 The eight sign combinations (types) of feed-forward loops. Arrows denote activation and ⊣ symbols denote repression.

Path overall sign = multiplication of the single path sign :

plus plus -> plus
plus minus -> minus
minus minus -> plus

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Feed-forward loop

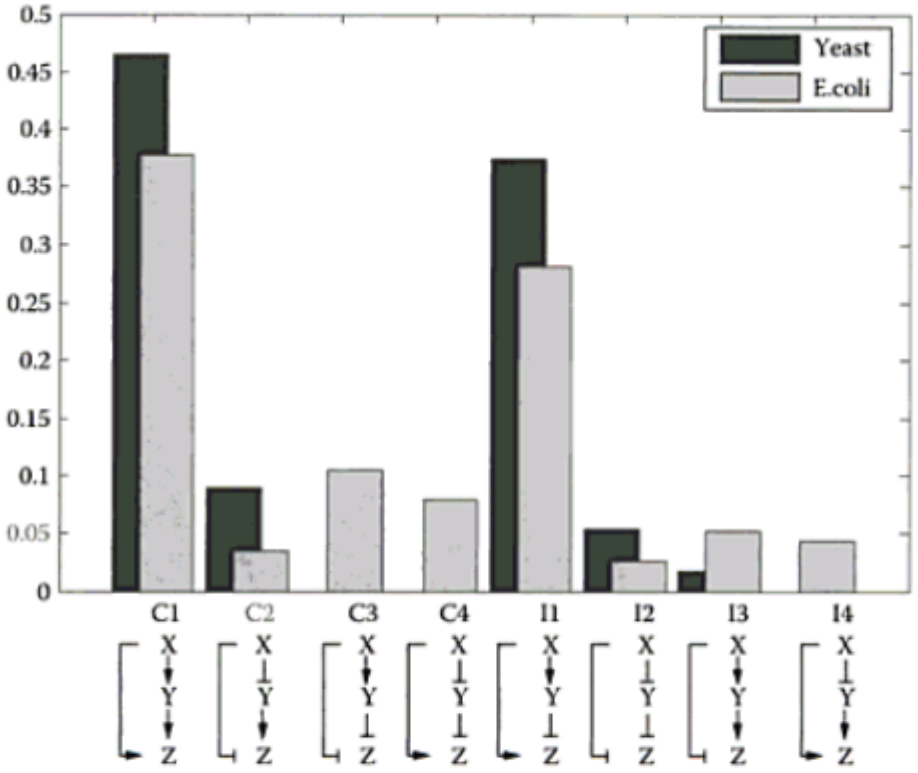

FIGURE 4.4 Relative abundance of the eight FFL types in the transcription networks of yeast and *E. coli*. FFL types are marked C and I for coherent and incoherent. The *E. coli* network is based on the Ecocyc and RegulonDB databases and has about twice as many edges as in the network of Figure 2.3. (From Mangan et al., 2006.)

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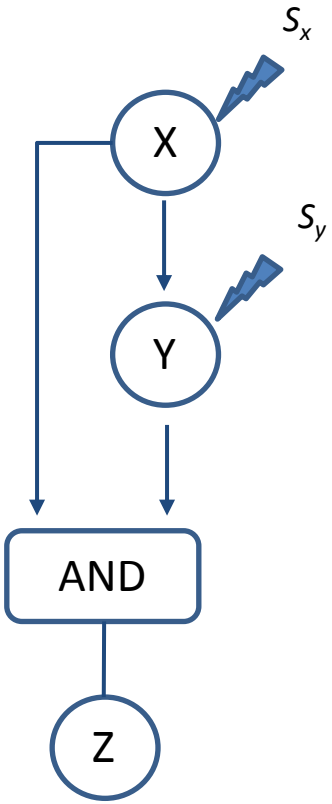
The different types of FFLs show different abundance, the C1-FFL where the three regulations are positive is the most frequent type, followed by the I1-FFL

Dynamics of the C1-FFL with AND gate



The activation of Z requires the binding of both active forms of X and Y (X* and Y*)

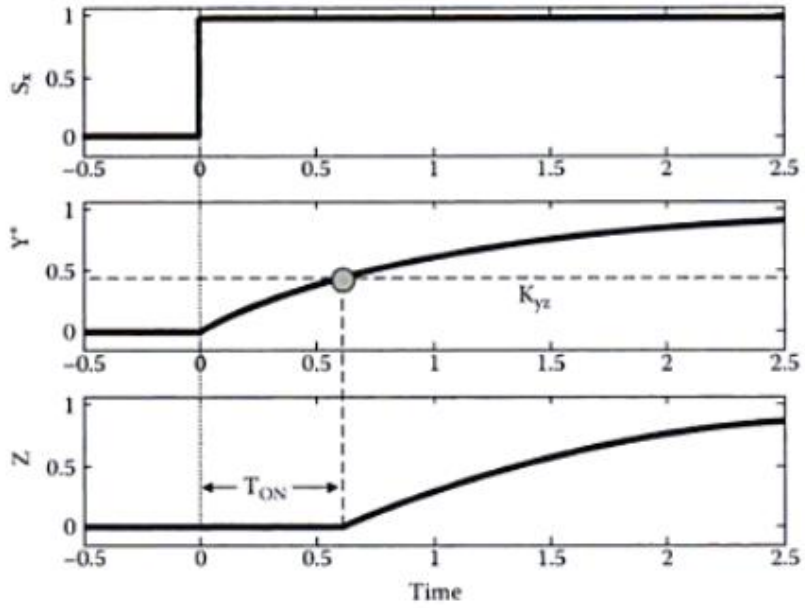


FIGURE 4.7 Dynamics of the coherent type-1 FFL with AND logic following an ON step of S_x at time t = 0 in the presence of S_y. The activation threshold of Z by Y is K_{yz} (horizontal dashed line). The production and degradation rates are $\alpha_y = \alpha_z = 1$, $\beta_y = \beta_z = 1$. The delay in Z production is T_{ON}.

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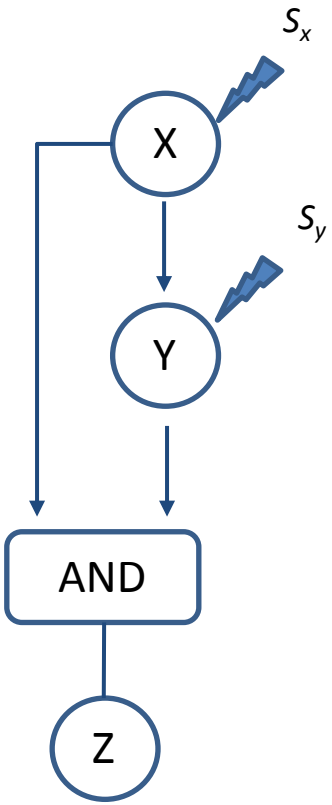
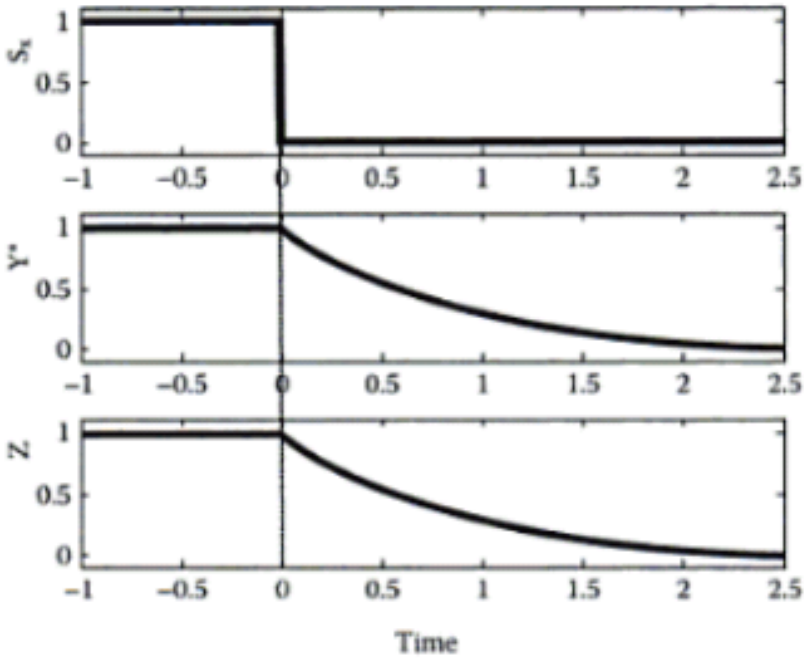
A strong signal S_x triggers the activation of X (step-like simulation) that rapidly transit to its active form X*. X* binds to the promoter of gene Y that initiates the synthesis of protein Y . The protein Y needs to exceed a specific level before it could activate Z (activation threshold K_{yz}) and the signal S_y must be present to turn Y in its active form Y*. The result is **a delay** in the production of Z

Dynamics of the C1-FFL with AND gate



When the signal S_x stops, there is **no delay** and the production of Z stops at once.



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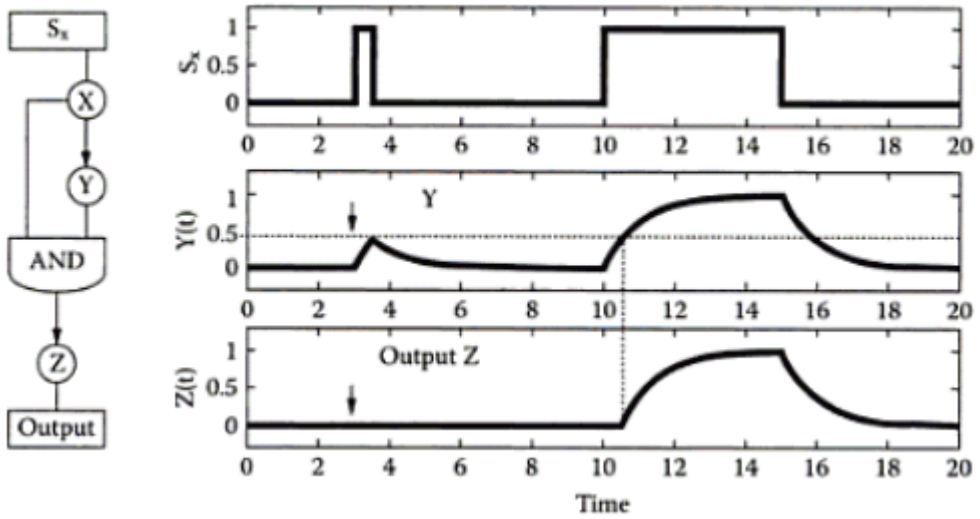
When the activation signal of X, S_x , is removed, X becomes inactive and unbinds from the promoters of Y and Z genes. As activation of Z requires both active form X* and Y*, activation of Z is stopped.

Dynamics of the C1-FFL with AND gate

This type of loop is a persistence detector for ON pulse and is called a Sign Sensitive Delay Element.

- If an ON pulse of S_x is shorter than the delay time required to Y to reach the concentration K_{yz}, the protein Z is not produced.
- Only ON pulse that exceeds the delay time will result in Z production
- But, the loop reacts immediately to the OFF pulse



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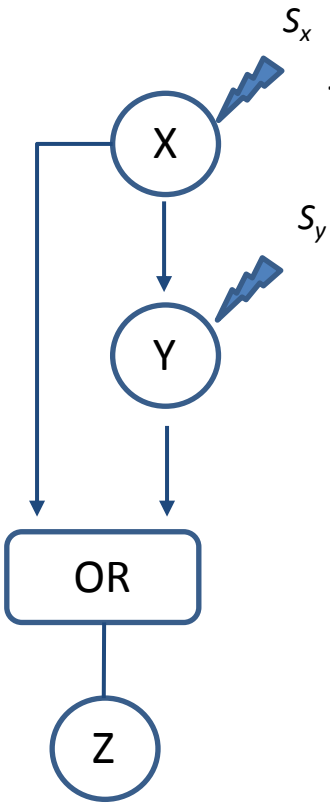
Evolution may have selected C1-FFL when the cell requires such a protection function. Indeed, the environment of the cell often suffers fluctuations. Sometimes, stimuli can be present as a brief pulses that should not elicit a response.

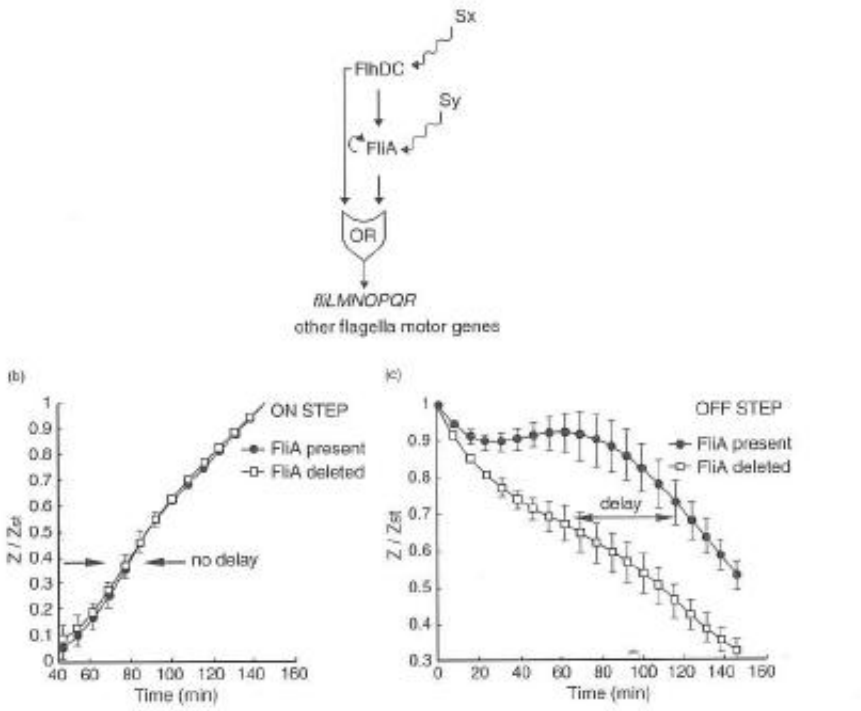
Dynamics of the C1-FFL with OR gate



The activation of Z requires the binding of only one of the active forms of X and Y (X* or Y*)

- After an ON pulse of S_x, Z is immediately activated as only one of the two active forms is required. So there is no delay for the activation.
- But, after an OFF pulse, it will be a delay before the deactivation of Z (Y* can activated Z without X*. The concentration of Y* should fall under the threshold K_{yz}, value under which Y* is not able to activate Z. The time for the decay process to reach a concentration of Y* smaller than K_{yz} corresponds to the delay time.
- a C1-FFL with a OR gate can maintain the expression of Z even if the input signal is momentarily lost. So it is also a sign-sensitive delay element.
- such a dynamic has been experimentally demonstrated in the flagella system of *E. coli*.

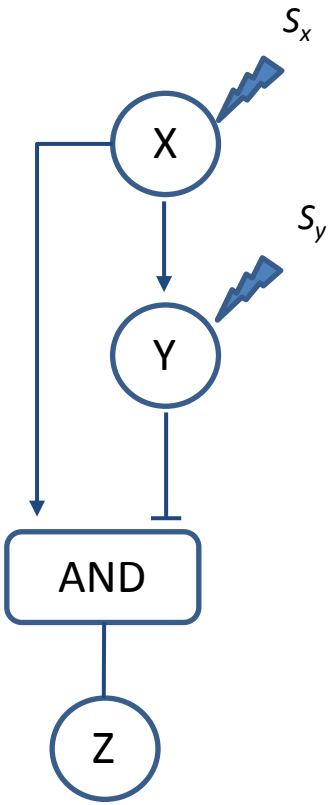
Dynamics of the C1-FFL with OR gate



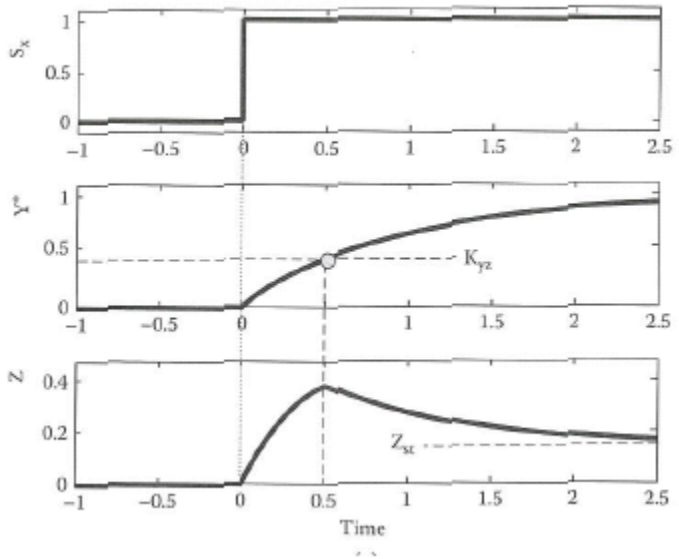
From Kalir *et al.* (2005) *Molecular System Biology*, doi: 10.1038:mbs4100010

Example of the C1-FFL OR gate in the flagella system of *E. coli*. The input signals S_x are environmental signals like glucose limitation, osmotic pressure ,temperature. The second signal S_y is a check point that is triggered when the first motors are completed. In b), after an ON step on S_x, the activity of the promoter *fliL* is measured by means of a green-fluorescent protein used as a reporter. In c) the promoter dynamics is reported after an OFF step of S_x in the presence of S_y. Black circles: wild type bacterium, white circles: mutant in which the gene *fliA* was depleted

Dynamics of the I1-FFL with AND gate



This loop is made of two antagonist regulation paths: X activates Z but also Y that in turn repressed Z.



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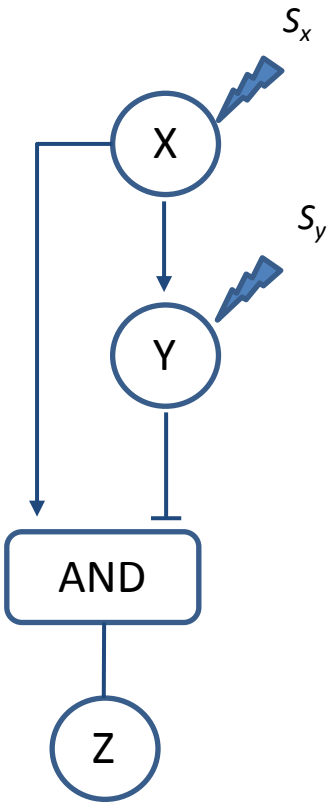
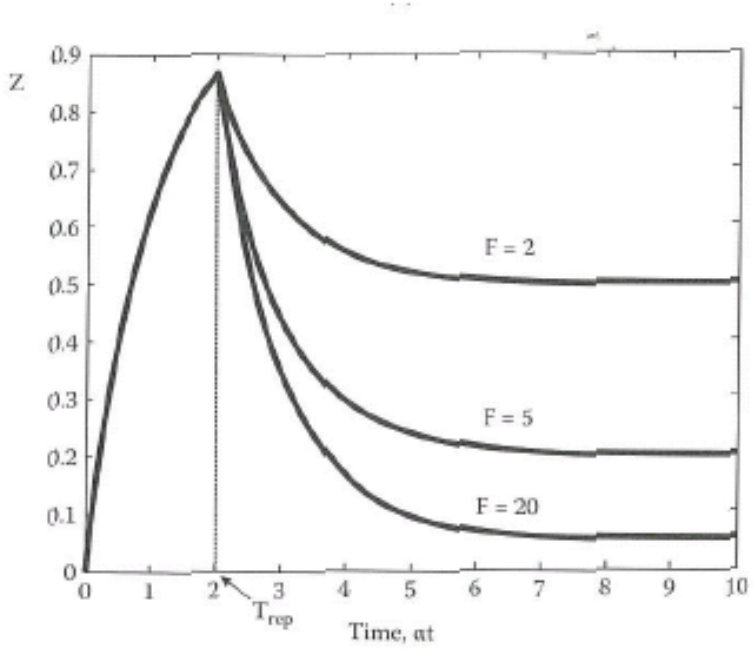
Upon an ON step of S_x, regulator X becomes activated (X*), binds the promoter of gene Z whose transcription starts leading to the synthesis of protein Z. The active form X* binds also the promoter of gene Y and activates the production of protein Y. When the concentration of protein Y* reaches the threshold K_{yz} (repression coefficient), Y* starts to repress the expression of gene Z and the level of protein Z decreased. Then, the I1-FFL AND gate can generate a pulse of Z production.

Dynamics of the I1-FFL with AND gate





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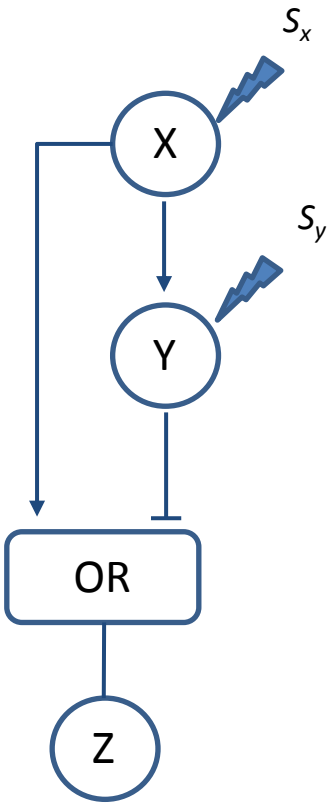
F is the repression factor

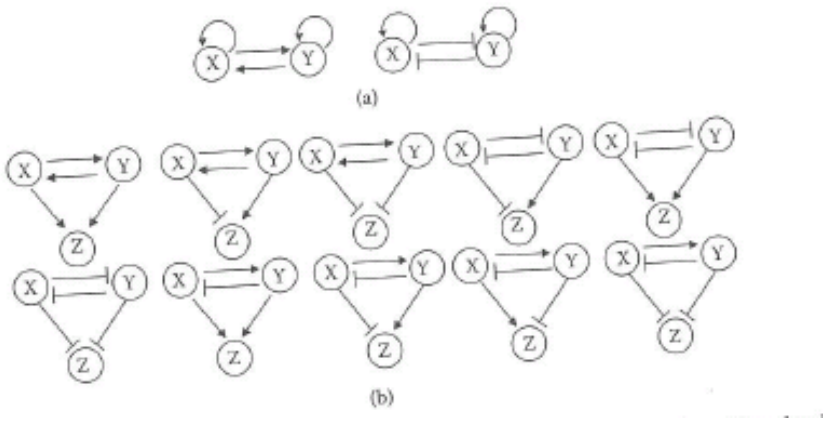
- When the repressor Y has a strong inhibitory effect on Z production (F>>1), the dynamic of Z has a pulse-like shape.
- In addition, it can accelerate the response time of the system when compared to a simple-regulation circuit (not shown).
- After an OFF step of S_x, there is an immediate shutoff of Z production
- Thus, I1-FFL with AND gate is a sign-sensitive response regulator

Dynamics of the I1-FFL with ORgate



The loop will have the same function as the one with the AND gate, but it has already be seen for the CC1-FFL OR gate, it can maintain the expression of Z even if the input signal is momentarily lost.

Feed-back loop



(a) Two-nodes feedback loops with autoregulation. A common network motif in developmental transcriptional networks
(b) The 10 different types of regulating feedback motifs

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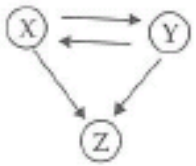
A feedback loop can be either positive or negative according to the number of negative interactions. If this number is even, then the feedback loop is positive. Otherwise, the feedback loop is negative (odd number of negative interactions).

Feed-back loop

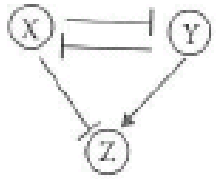
Double-positive feedback loop:



The gene Z is regulated as shown by the two transcriptional factors that activates each other. This loop will have two stable steady states:
A signal that causes the production of proteins X and Y can irreversibly lock the system into a state where X and Y are ON and activate each other. This case of bi-stable switch is called a lock-on mechanism.

	X	Y	Z
Steady state 1	ON	ON	ON
Steady state 2	OFF	OFF	OFF

Double-negative feedback loop:



The gene Z is regulated as shown by the two transcriptional factors that repressed each other. This loop will have two stable steady states:
Here, the loop expresses X either Y.

	X	Y	Z
Steady state 1	ON	OFF	OFF
Steady state 2	OFF	ON	ON

Feed-back loop

Maintenance of homeostasis

Almost all homeostatic control mechanisms are negative feedback mechanisms. These mechanisms change the variable back to its original state or “ideal value”.

Example: Control of blood sugar (glucose) by insulin:

When blood sugar rises, receptors in the body sense a change . In turn, the control center (pancreas) secretes insulin into the blood effectively lowering blood sugar levels. Once blood sugar levels reach homeostasis, the pancreas stops releasing insulin.

Positive feedback mechanisms

A positive feedback mechanism is the exact opposite of a negative feedback mechanism. With negative feedback, the output reduces the original effect of the stimulus. In a positive feedback system, the output enhances the original stimulus.

Example of a positive feedback system is child birth. During labor, a hormone called oxytocin is released that intensifies and speeds up contractions. The increase in contractions causes more oxytocin to be released and the cycle goes on until the baby is born. The birth ends the release of oxytocin and ends the positive feedback mechanism.