Biological significance of autoregulation through steady state analysis of genetic networks

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Abstract

Autoregulation of regulatory proteins is a recurring theme in genetic networks. Autoregulation is an important component of a genetic regulatory network besides protein–protein and protein–DNA interactions, stoichiometry, multiple binding sites and cooperativity. Although the biological significance of autoregulation has been studied before, its significance in presence of other mechanisms is not clearly enumerated. We have analyzed at steady state the significance of autoregulation in presence of other molecular mechanisms by considering hypothetical genetic networks. We demonstrate that autoregulation of a regulatory protein can impart amplification to the response. Further, autoregulation of an activator binding to the DNA as a dimer can introduce bistability, thus forcing the system to reside in two distinct steady states. In combination with autoregulation, cooperative binding can further increase the sensitivity and can yield a highly ultrasensitive response. We conclude that autoregulation with the help of other molecular mechanisms can impart distinct system level properties such as amplification, sensitivity and bistability. The results are further discussed in relation to various examples of genetic networks that exist in biological systems.

Keywords: Gene; Activator/repressor; Binding sites; Dimerization; Cooperativity; Bistability; Amplification and sensitivity

1. Introduction

Genetic regulatory networks are complex systems consisting of various interactions and molecular mechanisms that are essential for their operation. These mechanisms include protein–DNA and protein–protein interactions, multiple binding sites, cooperativity and autoregulation of regulatory proteins. Regulatory protein, either as an activator or repressor, binds to the upstream activation sequence (UAS) or upstream repression sequence (URS) respectively, to constitute protein–DNA binding (Lohr et al., 1995). Further, the regulatory protein can also be regulated through protein–protein interaction by other regulatory elements in the system (Melcher and Xu, 2001). Genes can have multiple binding sites for specific transcriptional activator or repressor, which can influence the expression profile (Lohr et al., 1995; Melcher and Xu, 2001). The binding characteristic for the second site can alter depending on the binding state of the first site constituting cooperativity (Lohr et al., 1995; Magnuson and Yarmolinsky, 1998). The above mechanisms are further compounded by autoregulation of the regulatory proteins.

The synthesis of an autoregulatory protein is controlled by the same switch that it regulates (Simpson et al., 2003). The feedback constituted by autoregulation can either be positive (activation) or negative (inhibi-
tion). They are ubiquitous and are observed in genetic systems like bacteriophage-lambda (Ackers et al., 1982) and GAL (Verma et al., 2003) regulatory networks. Various roles have been attributed to autoregulation, such as amplification of gene expression, buffering response of genes to environmental changes (Sonnelborn et al., 1965) and maintaining constant protein concentration independent of cell size and growth parameters (Simpson et al., 2003; Sompayrac and Maasbe, 1973; Huber and Adhya, 1987; Becskei and Serrano, 2000). Further, autoregulation also provides stability to the genetic switches (Becskei and Serrano, 2000; Savageau, 1974) and reduce the time necessary to reach the steady state concentration (Rosenfeld et al., 2002).

Autoregulation typically does not operate independently of other mechanisms in a regulatory network. For example, a regulatory protein, which is autoregulated, can either bind as a monomer or a dimer to the upstream activation sequence (UAS) of a gene. While expression from Tyr R gene in Escherichia coli (Bhattachy et al., 2003) and SOCS3 gene in human (Ausnhammer et al., 1999) is regulated by autoregulation of proteins binding as a monomer, rho gene in E. coli (Huber and Adhya, 1987) and Lac gene in Kluyveromyces lactis (Zachariae and Breunig, 1993) are regulated by autoregulation of proteins binding as a dimer. Further, autoregulation can exist for a regulatory protein, which binds to the multiple binding sites with cooperativity. TRA gene in F-plasmid (Fekete and Frost, 2002) and TOR gene in E. coli (Ansardi et al., 2000) are regulated by autoregulated proteins interacting with multiple binding sites. Autoregulation is, thus, known to play an important role in regulating various genetic networks (Simpson et al., 2003) and there are myriad examples of gene expression systems with an autoregulatory component in addition to other mechanisms. Autoregulation as a mechanism exists in genetic networks, where the transcriptional effectors bind either as a monomer or dimer. Further, these mechanisms may include multiple binding sites resulting in a complex network. At this stage, it may be pertinent to ask a question regarding the significance of autoregulation at the system level in presence of various other mechanisms. The past studies do not explicitly mention the role of autoregulation in relation to the presence of other mechanisms such as stoichiometry, multiple binding site, cooperativity, etc.

Mathematical models can help in understanding the natural complexity arising due to autoregulation. Various strategies that are used to model genetic networks, includes steady state and dynamic modeling. Steady state models require only equilibrium dissociation constants and total component concentration as parameters. Further, these parameters are relatively easy to determine through in vitro experiments. Whereas, dynamic models require kinetic rate constants for transcription, which are difficult to obtain. Although dynamic analysis yields temporal details, steady state models are more common and have been used to simulate genetic networks such as lactose operon (Isaacs et al., 2005), bacteriophage-lambda (Ackers et al., 1982), GAL regulatory network (Verma et al., 2003), etc.

In this article, we employ steady state analysis to quantify the effect of autoregulation by considering various examples of isolated genetic switches. Researchers have demonstrated that isolated gene networks coupled with proper quantification, can be used to elucidate the key properties of in vivo functional genetic networks (Chung and Stephanopoulos, 1996). The analysis includes systems wherein an autoregulated regulatory protein binds as (i) monomer, (ii) dimer, and (iii) dimer with cooperativity to the UAS or URS of a gene. We have analyzed the effect of both positive and negative autoregulation. Our analysis clearly indicates that positive autoregulation can yield bistable response, while negative autoregulation yields stable but a less sensitive response. Moreover, the bistability is only observed when the regulatory protein acts as a dimer.

2. Methodology

A steady state analysis for expression of a gene with and without autoregulation for the following systems was considered:

1. Monomer binding of transcriptional activator to the upstream activation sequence (UAS) of a gene having one binding site.
2. Dimer binding of transcriptional activator to the UAS of a gene having one binding site.
3. Dimer binding of transcriptional activator to the UAS of a gene having two binding sites with cooperativity.
4. Dimer binding of transcriptional repres sor to the upstream repression sequence (URS) of a gene having two binding sites with cooperativity.

Fig. 1 shows the schematic representation of the gene expression systems discussed above. Steady state analysis assumes equilibrium binding between all interactions and molar balances of all the component concentrations. In case of autoregulation, the concentration of the transcriptional activator at steady state is related to the fractional protein expression ($f_p$) of the gene it activates. Let the transcriptional factor “$A$” be present at a steady state having a concentration of $A_0$. The steady state con-
Fig. 1. (a) Schematic representation for gene expression having one binding site with and without autoregulation. The mRNA molecules are synthesized from transcription of gene (D) and are translated to protein (A). Protein (A) dimerizes with dissociation constant $K_1$. The monomer (A) and dimer (A$_2$) binds to upstream activation sequence (D) with dissociation constant $K_d$ for transcriptional expression of gene. Note that if the transcriptional activator (A$_2$) does not interact with the gene ‘D’ the system lacks autoregulation mechanism. (b) Schematic representation of positive/negative autoregulation for expression of gene having two binding sites. The mRNA molecules are synthesized from transcription of gene (D) and are translated to protein (A). Protein (A) dimerizes with dissociation constant $K_1$. The dimer (A$_2$) molecule binds to first binding site (D) with dissociation constant $K_d$ and with second binding site by $K_d$/$m$ for transcriptional expression of gene. The term ‘m’ quantifies cooperativity. Note that if the transcriptional activator (A) does not interact with the gene ‘D’ the system lacks autoregulation mechanism.

The molar balances for the total gene and activator concentration are as follows:

$$[D]_t = [D] + [DA]$$  \hspace{1cm} (4)
$$[A]_t = [A] + [DA]$$  \hspace{1cm} (5)

The probability of protein expression is defined as the ratio of concentration of the activator bound DNA to the total DNA concentration. Thus

$$f = \frac{[DA]}{[D]_t}$$  \hspace{1cm} (6)

Eqs. (2)–(6) can be used to relate total activator concentration to fractional protein expression as follows:

$$[A]_t = \frac{f}{1 - f}K_d + f [D]_t$$  \hspace{1cm} (7)

The effect of autoregulation ($A_0$ or $A_0$) can be incorporated by relating $[A]_t$ from Eq. (7) to $[A]_0$ using Eq. (1) as follows:

$$[A]_t = \frac{f}{1 - f}K_d + f [D]_t$$  \hspace{1cm} (8)

Eqs. (7) and (8) relate the input concentration ($A_0$ or $A_0$) required for a specific output fractional expression ($f$) for cases without and with autoregulation, respectively.

Case 2. Activator as a dimer

In this case, transcriptional activator (A) dimerizes before binding to the DNA (see Fig. 1a). The equations representing all the molecular interactions are as follows:

$$A + A \leftrightarrow A_2$$  \hspace{1cm} (9)
K is Eqs. (16) and (17), thus, represent the input–output relationship for the cases without and with autoregulation of transcriptional activator and its binding to UAS of the gene, respectively. The total molar balances for the DNA and activator protein are as follows:

\[ [D]_k = [D] + [DA_2] \]  
\[ [A]_0 = [A] + 2[A_2] + 2[DA_2] \]  

The probability of transcription is defined as

\[ f = \frac{[DA_2]}{[D]} \]  

Eqs. (9)–(15) can be used to relate the total activator concentration required to achieve a specific fractional protein expression \( f \) as follows:

\[ [A]_0 = \sqrt{\frac{f}{1-f}}(K_1K_4) + \frac{2f}{1-f}K_4 + 2f/[D] \]  

Further, in the presence of autoregulation Eq. (16) can be modified by substituting for \([A]_0\) using Eq. (1):

\[ [A]_0 = \sqrt{\frac{f}{1-f}}(K_1K_4d) + \frac{2f}{1-f}K_4d + 2f/[D] \]  

Eqs. (16) and (17), thus, represent the input–output relationship for the cases without and with autoregulation of transcriptional activator binding to DNA as a dimer.

**Case 3.** Activator binds as a dimer to the gene having two binding sites with cooperativity

Here, the transcriptional activator binds as a dimer to the gene \( (D) \) having two binding sites (see Fig. 1b). The dissociation constant for binding to the first site is \( K_4 \) and for the cooperative binding to the second site is assumed to be \( K_4/m \), where factor \( m \) quantifies cooperativity (enhanced affinity of binding for the second binding site). The following equations represent the molecular interactions:

\[ D + A_2 \leftrightarrow DA_2 \]  
\[ K_4 = \frac{[D][A_2]}{[DA_2]} \]  
\[ DA_2 + A_2 \leftrightarrow DA_2A_2 \]  
\[ K_4 = \frac{[DA_2][A_2]}{[DA_2A_2]} \]  

The molar balances for DNA and the activator protein are as

\[ [D]_k = [D] + [DA_2] + [DA_2A_2] \]  
\[ [A]_0 = [A] + 2[A_2] + 2[DA_2] + 4[DA_2A_2] \]  

The probability of transcription is defined as follows:

\[ f = \frac{[DA_2] + [DA_2A_2]}{[D]} \]  

The mass balance equations can be solved simultaneously to yield input–output relationship by relating \( A_0 \) and \( f \) for this case in absence of autoregulation. As before, the relationship obtained by including Eq. (1) will yield the input–output relationship for the case with autoregulation.

**Case 4.** Repressor binds to the gene having two binding sites as a dimer.

In this case, the repressor molecule binds to the upstream repression sequence (URS) of a gene for regulation of transcription (see Fig. 1b). All the equations for this case are similar to that listed in case-3 except Eq. (23) defining \( f \), the fraction for transcriptional expression. Since regulator \( (A) \) is a repressor in this case, the transcription occurs only when the operator gene is free. Thus

\[ f = \frac{[D]}{[D]_k} \]  

For each of the four cases, the equations were solved simultaneously by using function solve algorithm of MATLAB-12 (The Math Works Inc., USA) and equation solve algorithm of Mathematica 4.1. The total molar balances on DNA and activator/repressor were independently verified by adding the concentrations of various complexes.

3. **Model parameters**

The dissociation constants for protein–protein and DNA–protein interactions were taken in the physiological range as given in the figure legend. The value of \([D]_k\) was fixed for a model organism with cellular volume 70 \( \mu \)m\(^3\), equal to the cellular volume of a haploid yeast cell (Sherman, 1991) having single copy per genome.

4. **Results**

The steady state response curves for the four cases were obtained by solving the model equations. For each
Fig. 2. Fractional protein expression through a positively autoregulated gene expression system as predicted by the steady state analysis when the transcriptional activator binds to the operator site (binding site) as a monomer to the gene having one binding site. Curve (i) shows response for the unautoregulated system and (ii), (iii) and (iv) show expression responses for autoregulated system with $q=10^{-1}$, $100$- and $1000$-fold changes in the activator concentrations due to autoregulation. Numbers next to curves show values of Hill coefficients. It can be noted that autoregulation brings about amplification by shifting the response curve to the left. Parameter values are as follows; $K_d = 2.0 \times 10^{-10}$ M, $K_1 = 1.0 \times 10^{-7}$ M, $m=30$ and $D_t = 2.37 \times 10^{-11}$ M.

In case, the fractional transcriptional expression was plotted with respect to the concentration at the onset of transcription ($A_0$) to represent autoregulation. To compare the performance of the network without autoregulation, the fractional expression was plotted with respect to $A_t$ excluding Eq. (1).

Fig. 3 shows the fractional transcription for the case when transcriptional activator binds as a monomer (Case 1). In absence of autoregulation, the response shows a typical Michaelis–Menten type response with a Hill coefficient of one (curve i). Whereas, the response obtained in the presence of autoregulation is sensitive. Further, the Hill coefficient (as a measure of sensitivity) increases with higher degree of autoregulation (i.e., increasing value of $q$). The curves also shift to the left indicating that the expression switches on at a lower concentration of the activator required to switch on the expression. Thus, autoregulation causes not only an increase in the sensitivity but also amplifies the activator signal.

Fig. 3. (a) Fractional protein expression through a positively autoregulated gene expression system when the transcriptional activator binds as a dimer to the promoter of the gene having one binding site. Curve (i) shows response for the unautoregulated system and curves (ii)–(iv) show expression responses for autoregulated system with $q=10^{-1}$, 100- and 1000-fold changes in the activator concentration due to autoregulation. (b) A bistable response is observed due to positive autoregulation at the value of $q=100$. Non-linearity is caused due to ultrasensitive response and autoregulation yields bistable response. Parameter values are as follows; $K_d = 2.0 \times 10^{-10}$ M, $K_1 = 1.0 \times 10^{-7}$ M, $m=30$ and $D_t = 2.37 \times 10^{-11}$ M.
Fig. 4. Fractional protein expression through an autoregulated gene expression system at different total concentrations of activator, when the activator binds as a dimer to gene having two binding sites with cooperativity. Curve (i) shows response for unautoregulated system and curves (ii)–(iv) show the expression responses for autoregulated with $q = 10$, $100$, and 1000-fold changes in the activator concentrations due to autoregulation and with a cooperativity of $m = 30$. It can be noted that bistability can be observed for responses obtained for $q = 10$, 100 and 1000. Parameter values are as follows; $K_d = 2.0 \times 10^{-10}$ M, $K_1 = 1.0 \times 10^{-7}$ M, $m = 30$ and $D_t = 2.37 \times 10^{-11}$ M.

Fig. 3a shows the transcriptional response when activator dimerizes and binds to the operator site (i.e., Case 2). In this case even in the absence of autoregulation, the response is sensitive (with a Hill coefficient of 1.9). Autoregulation further, increases the sensitivity of the response to 5.2 at $q = 10$. It can be noted that on further increasing the value of $q$ (higher degree of autoregulation), the response becomes switch like with a bistable response (see Fig. 3b). A bistable response has a distinct concentration of activator at which it switches on and a different concentration at which it switches off. The figure also shows that a certain concentration of activator at the onset of expression is essential to switch on the transcription. Thus, a leaky transcription is necessary in the off state for the operation of such positive autoregulatory systems.

Fig. 4 shows the transcriptional response when the activator dimerizes and binds to the gene having two binding sites with cooperativity (i.e., Case 3). Even in this case, the response is highly sensitive in absence of autoregulation (with a Hill coefficient of 3.5). The increased sensitivity is due to multiple binding sites and cooperativity. Further, in presence of autoregulation, bistability is observed at a lesser degree of autoregulation (i.e., even at $q = 10$).

Fig. 5 shows the case when a repressor is autoregulated and binds as a dimer to the upstream repression sequences (URSs) of a gene with cooperativity. In absence of autoregulation, the value of the sensitivity of the response as calculated from the Hill equation is 3.5 and is also similar to that obtained by an transcriptional activator (curve i in Fig. 4). Further, the response becomes less sensitive in the presence of autoregulation. Further the sensitivity decreases with increase in the degree of autoregulation (i.e., with increase in the value of $q$). In a repressive system autoregulation imparts only amplification to the response, without yielding properties such as sensitivity and bistability.

The different mechanisms analyzed in the above section yield specific transcriptional response and the responses were dependent on the parameter values. It is relevant to study the dependency of the output response to system parameters. This entails representing the output response by a curve. We use Hill equation, which is characterized by the Hill coefficient ($\eta_H$) and half saturation constant ($K_{0.5}$) to represent the output response,
Fig. 6. Variation of Hill coefficient ($\eta_H$) and half saturation constant ($K_{0.5}$) for different mechanisms observed in a genetic switch with variations in key system parameters (a) and (b). Solid line: variation with respect to dimerization constant for a transcriptional activator binding to a gene as a dimer; dotted line: variation with respect to the degree of cooperativity for the transcriptional activator binding as a dimer to two binding sites with cooperativity; (c) variation of Hill coefficient with respect to degree of autoregulation ($q$ in Eq. (1)); (d) variation of half saturation constant with respect to degree of autoregulation. Dotted line represents curve for switching on while solid line represents curve for switching off indicating bistability. Note that bistability was lost at low degree of autoregulation, as both the curves merge.

as given below:

$$O = \frac{\eta_H I}{K_{0.5} + I^m}$$  \hspace{1cm} (26)

where $O$ and $I$ indicate output response and input, respectively. The dependency of $\eta_H$ and $K_{0.5}$ on a parameter signifies the sensitivity of the response to a given change in a parameter value. Fig. 6a shows the variation of Hill coefficient to changes in dimerization constant ($K_d$), when a transcriptional activator binds as a dimer to the operator site (solid line in Fig. 6a). As the dimerization constant increases, the Hill coefficient decreases to 1 from 2. This is due to the fact that the binding of the dimer to the operator site controls the response rather than the dimerization of the activator. The half saturation constant increases with the dimerization constant (see solid line in Fig. 6b). This implies that the output response requires larger amounts of input to switch on the system with increase in the $K_d$ value.

Fig. 6a also shows the variation of Hill coefficient with respect to extent of cooperativity ($m$ in Eq. (21)). Increase in cooperative binding to the second site by a transcriptional activator increases the Hill coefficient, but was bounded between values 1 and 2 (dotted line in Fig. 6a). While $K_{0.5}$ decreases with increase in the value of $m$ indicating that the signal gets amplified (dotted line Fig. 6b).

Autoregulation of the transcriptional activator can yield ultrasensitive response with bistability (see Fig. 4). The Hill coefficient increases with increase in the degree of autoregulation (parameter $q$ in Eq. (1) and shown in Fig. 6c). The half saturation constant decreases with increase in the value of $q$ indicating amplification (see Fig. 6d). Bistability was observed at a very high degree of autoregulation resulting in a different value of $K_{0.5}$ for switching on (dotted line in Fig. 6d) and switching off (solid line in Fig. 6d) the genetic switch. A bistable response typically has a very high Hill coefficient with a steep switch like output and can take values higher than two. The value of Hill coefficient was thus not bounded in this case (values of 50–100 can also be obtained).
Protein binding as a monomer feedback in tandem with other mechanisms Examples of genetic networks exhibiting autoregulation with positive regulation operate in many regulatory networks. Autoregulation operating in tandem with other mechanisms like dimerization, multiple binding sites and cooperativity can impart distinct properties to regulatory networks. Therefore, autoregulation as a fundamental mechanism is observed in many genetic regulatory systems ranging from bacterial systems to higher organisms. Various systems wherein transcriptional activation and repression with autoregulation have been reported in literature and are listed in Tables 1 and 2, respectively. While autoregulation as a phenomenon has been demonstrated in many disparate systems, its role in the operation of genetic networks has been peripherally discussed. Our steady state analysis clearly demonstrates that autoregulation is a fundamental mechanism in genetic networks and is highly context dependent. Further, autoregulation in combination with other known mechanisms like cooperativity can impart system level properties like multiple steady state, ultrasensitivity and amplification.

Steady state analysis indicates that positive autoregulation yields a sensitive response with amplification.

### Table 1: Examples of genetic networks exhibiting autoregulation with positive feedback in tandem with other mechanisms

<table>
<thead>
<tr>
<th>Protein binding as a monomer</th>
<th>Negative feedback in tandem with other mechanisms</th>
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<tbody>
<tr>
<td>MASH1 gene in <em>Saccharomyces cerevisiae</em></td>
<td>Meredith and Johnson (2000)</td>
</tr>
<tr>
<td>SOCS-3 gene in human</td>
<td>Auerhammer et al. (1999)</td>
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<tr>
<td>luxCAD operon in <em>Escherichia coli</em></td>
<td>Gen et al. (2001)</td>
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<td>GLE gene in human cytomegalovirus</td>
<td>Macias and Simkic (1993)</td>
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<tr>
<td>FTZ gene in <em>Drosophila</em></td>
<td>Yu et al. (1999)</td>
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<tr>
<td>PDFC1 genes in <em>Saccharomyces cerevisiae</em></td>
<td>Muller et al. (1999)</td>
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<td>AsCF gene in <em>Apoptophysina nobilis</em></td>
<td>Strüff et al. (2001)</td>
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<tr>
<td>CYR-8 gene in <em>Salmonella typhimurium</em></td>
<td>Ostorowski and Kerdilich (1986)</td>
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<tr>
<td>TyR gene in <em>Escherichia coli</em></td>
<td>Camakaris and Pittard (1982)</td>
</tr>
<tr>
<td>MET-T gene in <em>Salmonella typhimurium</em></td>
<td>Urbanowski and Stangler (1986)</td>
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<tr>
<td>SIG-A gene in <em>Escherichia coli</em></td>
<td>Camerero et al. (2002)</td>
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<tr>
<td>EF-50 gene in <em>Candida albicans</em></td>
<td>Tehrani et al. (2003)</td>
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<tr>
<td>RAF-1 gene in <em>Xenopus laevis</em></td>
<td>Cutler et al. (1998)</td>
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<tr>
<td>Protein binding as a dimer without cooperativity</td>
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<td>PEF and TFM genes expression in fruit fly</td>
<td>Melcher and Xie (2001)</td>
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<tr>
<td>GAL80 gene in <em>Saccharomyces cerevisiae</em></td>
<td>Haber and Adhya (1987)</td>
</tr>
<tr>
<td>REP-E gene in <em>nori</em></td>
<td>Ishii et al. (1994)</td>
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<tr>
<td>rpo gene in <em>Escherichia coli</em></td>
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<tr>
<td>Monomer protein binding with cooperativity</td>
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<tr>
<td>Doc H66y protein in bacteriophage P1</td>
<td>Doudi et al. (2002)</td>
</tr>
<tr>
<td>TOR gene in <em>Saccharomyces cerevisiae</em></td>
<td>Accardi et al. (2000)</td>
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### Table 2: Examples of genetic networks exhibiting autoregulation with negative feedback in tandem with other mechanisms

Amplification offers an advantage in the form of requiring lower amount of regulatory proteins to operate the genetic network. Positive autoregulation is also capable of yielding a bistable response indicating multiple steady states. Bistability is typically observed when a non-linearity caused by sensitive response is superimposed by a positive feedback loop. Thus, dimerization and cooperativity by themselves can yield ultrasensitivity resulting in non-linearity, while autoregulation constitutes a positive feedback loop. In a bistable system, for a given activator concentration at the onset of transcription, the switch resides in two steady states, one leaky off state and another in the on state. This makes the expression completely switch like, as it cannot reside in any other intermediary steps. Such bistable response
is essential for a system, which require a yes or no type of decision. Whereas, in a system with positive autoregulation of activator, which binds as a monomer to the upstream activation sequence, the response does not show bistability due to insufficient non-linearity in the system.

Existence of bistability has been shown experimentally in cellular differentiation and cell cycle progression in Xenopus laevis egg (Shai et al., 2003). However such bistable response is not reported in transcriptional regulation. The network structure associated with bistability (i.e. non-linearity with positive feed back loop) also exits in transcriptional regulatory systems. Theoretical analysis reported here indicates that systems with dimerization and cooperativity in the presence of positive autoregulation can manifest bistable response. Such structures have been reported for TRA gene in F plasmid (Fekete and Frost, 2002) and CI gene expression in bacteriophage-lamda (Ackers et al., 1982). Autoregulation and dimerization of CI protein help in deciding bacteriophage-lamda to reside in the lytic or the lysogenic states, which are two distinct phenotypic states. However, there are many other systems where positive autoregulation would not yield bistability due to the non-existence of non-linearity caused by dimerization and multiple binding sites. Such systems would offer an advantage of a sensitive response and amplification as demonstrated here.

Unlike in a bistable system, systems with negative autoregulation show a smooth transition from one state to another, implying that many intermediary steady states can be achieved depending on the input activator concentration. Thus, this makes the system less ultrasensitive, but a distinct amplification can be achieved in such a system and may be essential for systems, which do not require a yes or no type of status. Although non-linearity in the form of multiple binding sites and cooperativity can exist in such systems, bistability is not observed due to the presence of negative feedback loop (i.e., negative autoregulation).

Parametric sensitivity analysis was also performed on the different mechanisms operating in the genetic switch. The response was represented by Hill equation and the sensitivity to variations in parameter values were obtained by evaluating Hill coefficient and half saturation constant. It was clear from the analysis that irrespective of the mechanism studied (that is dimerization, cooperativity or autoregulation), the response was highly dependent on the parameters. This indicated that these basic mechanisms, operating in the genetic switch, would not yield robustness to parametric variations. Robustness can be achieved through other upstream interactions (like cascades) through a higher degree of complexity.

As demonstrated here, mathematical models can be used to analyze regulatory networks containing autoregulatory loops to provide insights into the properties of the inherent structure. Steady state analysis requires only equilibrium binding characteristics, which are easy to obtain. A dynamic model involves detailed kinetic analyses that are tedious. As demonstrated here, steady state analysis can also be used to enumerate system level properties. It is difficult and tedious to demonstrate some of these properties only through experimental tools. Experiments can provide details about the existing structure and prevailing mechanisms in the network. Thus, molecular biology has established the existence of dimerization, cooperativity and autoregulation with protein–protein and protein–DNA interactions as basic mechanisms prevalent in genetic regulatory networks.

While, theoretical analysis provides insights into system properties such as sensitivity of response, amplification of signal, bistability and multiple steady states. These properties can manifest depending on the connectivity of various molecular mechanisms prevalent in a particular genetic network.

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