The Gain Stability and Output Signal-to-noise Ratio Analysis of the Negative Feedback Control in Genetic Circuits

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Original scientific paper
Received: March 15, 2012
Accepted: September 16, 2012

Negative feedback genetic circuits (NFGC) are key regulatory motif of cellular robustness with the capability of reducing noise in genetic interaction network. NFGC have the same control theory frame as negative feedback amplifier circuits (NFAC). NFAC can enhance gain stability and output signal-to-noise ratio (OSNR) of output signal (voltage). Whether NFGC possess these two advantages or not is still unclear. We have investigated the advantages of NFGC through using feedback depth analysis to explore the gain stability, and analyzed OSNR of some typical negative feedback genetic circuits by modeling the corresponding electrical systems. The two methods are both based on the similarity of electrical and biological systems within the control theory framework. We found that NFGC can achieve high gain stability compared with linear cascade. Meanwhile, negative feedback can enhance gain stability by adjusting effective input, thereby making genetic circuits more robust to noise. Moreover, OSNR in NFGC have no enhancement, compared with the respective linear cascades. These findings suggest novel implications in how cellular systems with negative feedback can control signal and noise, and supply some guidance for controlling and synthesizing genetic circuits.

Key words:
noise control, effective input, synthetic biology, modelling

Introduction

Gene expression can be disturbed by noise, which in turn can be considered as including three components: intrinsic noise, global (environmental) noise and transmitted noise.[1] A specific cellular component level will suffer perturbations due to the intrinsic noise related to stochastic effects of low numbers of molecules. The number of RNA polymerases or ribosomes, different cell cycle stage, mRNA degradation machinery, and the cell environment are main sources of global noise, inducing gene expression fluctuation.[2,3] The transmitted noise originates in intrinsic noise and global noise of upstream gene, hence, it will be the predominant noise source for the downstream gene in a linear cascade expression.[2,4]

Previous studies showed that there are direct links between circuit architecture and noise.[5,6] To buffer the noise, some cellular systems evolve by specific mechanisms which can give further guide for designing genetic circuits.[6–10] NFGC[11–13] are being argued as the key regulatory motif for buffering noise,[14–17] which might be extrinsic noise.[18] It has been demonstrated that NFGC provide more stability compared with corresponding linear cascades, and the stability will decrease in the case of a low binding constant for the repressor, low transcription rate, or short half-life repressor.[14,19] And NFGC have been analyzed in many modeling studies.[20–23] In this work, such a capability of attenuating noise, which can maintain a steady gene expression output relative to a specific input signal, is defined as the gain stability. A negative feedback can buffer both noise and signal. Hence, OSNR could be adopted as a measurement of the output fidelity.

Interestingly, the same structure of negative feedback is also found in electrical system. NFAC, which is a well-known analog circuit in electrical systems, shows a similar noise attenuation effect and topology as NFGC. Hence, negative feedback is a usual motif existing in both electrical and biological systems. It has also been proved that electrical and biological systems both obey control theory.[16,24,25] Many cases show that the control theory works well in modeling and analyzing genetic circuits or signaling pathways.[26–29] At the same time, NFAC can improve gain stability and OSNR. Therefore, it is intriguing whether NFGC can improve gain stability and OSNR similar to NFAC.

In this paper, we explored the possibility of adjusting gain stability and OSNR for various genetic

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circuits classified by inhibition types and topologies. To this end, some NFGC modes were set to investigate the key parameters related to enhancing gain stability by use of feedback depth analysis in electronic systems, then OSNR of typical NFGC were analyzed by modeling and analyzing the corresponding electrical systems. The results suggest that biological systems might have evolutionarily acquired negative feedback to buffer noise using different effective input without improving OSNR.

Methods

Gain stability of different inhibition types of NFGC by the feedback depth analysis was analyzed. Also, NFAC models were constructed for analyzing OSNR of four topology types of NFGC. This analysis was based on the similarity of electrical and biological systems, both of which are subject to the same control theory. Input transcription factor X (short as TFX (P_X), Fig. 1), as a product of G_X, activates expression of target G_Y which then produces an output P_Y. In addition, the output P_Y (herein, it is an accumulation of P_Y in a given time τ) works as a new input to inhibit G_X expression. In the former process (P_X to P_Y), the gain factor A_Y as the ratio of output P_y to input P_X is calculated, and in the latter (P_Y to P_X), the feedback factor F_{Y-X} is determined. A_Y and F_{Y-X} are transfer functions of frequency.[16]

Several assumptions are employed in developing the circuit models. Biological pathways function as low-pass filters, which only transmit low-frequency signals whereas high-frequency signals are severely corrupted or completely lost in transmission.[19,30] Hence, signal and noise are assumed as low-frequency so that they can keep good transmission in genetic circuits. Meanwhile, transfer functions A_Y and F_{Y-X} will be frequency-independent as low frequency does not affect them.[16] And A_Y might be influenced by the input concentration, for keeping it constant, we assumed that the concentration of input P_X is low[31] (in this case, the relation between input P_X and output P_Y is direct ratio, so A_Y will be approximately constant and P_Y is not in homeostasis state[32]), then input P_X can influence the amplifier G_Y (input P_X can be amplified A_Y times by amplifier G_Y like amplifier in electronic circuits) linearly; output P_Y here is an accumulation output stimulated by P_X in a given time τ (τ should be as little as possible). When P_Y as input stimulates downstream G_Z, the concentration of input P_Y also should be low. So G_Z can also work in a linear amplification region). Additionally, we ignored output P_Y degradation, because most protein will take hours to hydrolyze. Hence, we assumed that all reactions are at initial stage (the threshold of the time is T_d ($\tau < T_d$) when TF degradation should be considered). Under these assumptions, Feedback depth model and NFAC model for NFGC were developed as follows:

Feedback depth analysis for NFGC

Two types of NFGC (Fig. 2, we simplified our figures of genetic circuits[33] in the paper by ignoring TF and mRNA except Fig. 1) were constructed for feedback depth analysis. The biological process of both simplification circuits is as follows. In type A genetic circuit (Fig. 2A), the signal control G_X, and produce P_X as a new signal to stimulate G_Y expression. The produced P_Y stimulate G_Z expression, and P_Z can inhibit P_X (the inhibition happens not on promoter of G_X). In type B genetic circuit (Fig. 2B),

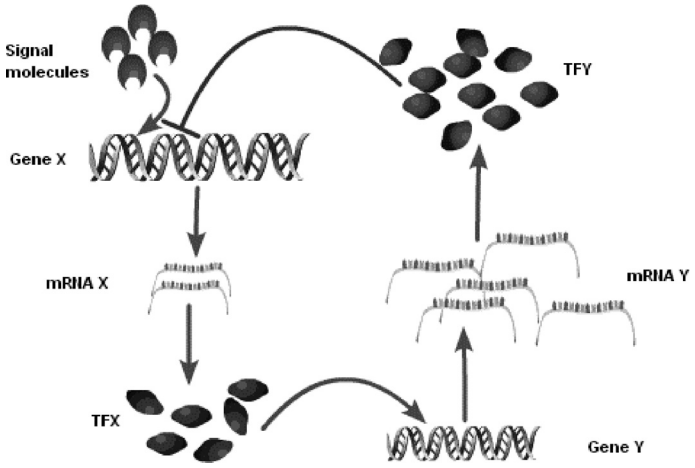


Fig. 1 – *Biological process of a simple NFGC, in which TFX (P_X) stimulates the expression of gene Y(G_Y), and TFY(P_Y) inhibits the expression of gene X(G_X).*

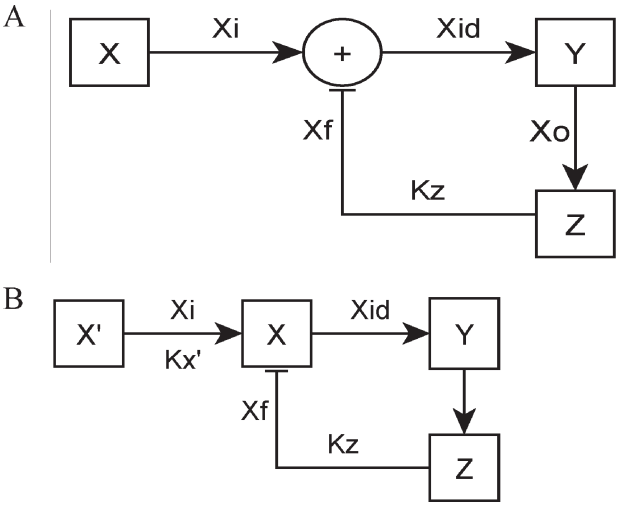


Fig. 2 – *Two types of negative feedback genetic circuit. The boxes represent genes. Arrow edge is activation, and blunt edge is inhibition. $K_{X'}$ and K_Z are dissociation constants. Xi, Xid and Xo are the concentration of input P_X, net input P_X, and output P_Y respectively. And Xf is the reduction of P_X caused by inhibition of P_Z.*

$G_{X'}$ produces $P_{X'}$ as input signal to stimulate G_X expression, then the product P_X stimulates amplifier G_Y. The product P_Y stimulates G_Z expression, then P_Z inhibit G_X expression. Both $P_{X'}$ and P_Z interact on the promoter of G_X. So, G_X in type B genetic circuit is used as an integrator, which processes information received from $G_{X'}$ and G_Z. Hence, both type A and B genetic circuits can be described in a simplification scenario: G_X stimulates G_Y, and G_Y inhibits G_X via G_Z. Herein, we study amplifier G_Y (see its product as output) solely in negative feedback. So, G_X (or $G_{X'}$) and G_Z are both as input, also the amplifier G_Z can be as an intermediate station of G_Y to G_X.

We defined the variable of the two circuit types for analysis. For each type, both the open loop (linear cascade, G_Z was removed) and closed loop were taken into account. In Fig. 2A, P_X reduces from the concentration of *Xi* (maximal expression of G_X without inhibition in a given time τ) to *Xid* (due to inhibition of P_Z) and stimulates G_Y expression. *Xid* is amplified by A_Y times and becomes *Xo.* And *Xf* is the reduction of P_X due to P_Z. The same case is in Fig. 2B, if there is no inhibition of P_Z, the expression amount of G_X activated by $P_{X'}$ is given by *Xi*. With the inhibition of P_Z to G_X, the amount of P_X reduce by *Xf*, and the remaining P_X is *Xid*.

Type A genetic circuit

Open loop: In a given time τ, the maximal concentration of P_X encoded by G_X is *Xi*, and the concentration of net input P_X is *Xid*, where *Xi* = *Xid*. And *Xo* is the output concentration of G_Y expression.

Closed loop:

$$Xid = Xi - Xf$$

The gain factor A_Y of G_Y is given by equation, $A_Y = Xo/Xid$;

Xf is the reduction of P_X inhibited by P_Z, where $Xf = \dfrac{Z^n Xi}{Z^n + K_Z}$ (based on Hill's equation), herein *n* is a Hill coefficient. Hill's equation is adopted because it has been proved very useful in genetic modeling by many experiments.[5,34–37]

Negative feedback factor is F_{Y-X}, where $F_{Y-X} = Xf/Xo$;

The gain factor *Af* of negative feedback is given by the equation, $Af = \dfrac{Xo}{Xi} = \dfrac{A_Y}{1 + A_Y F_{Y-X}}$.

So, the relative variation of *Af* is given by

$$\frac{\mathrm{d}Af}{Af} = \frac{1}{1 + A_Y F_{Y-X}} \frac{\mathrm{d}A_Y}{A_Y} = \frac{Xid}{Xi} \frac{\mathrm{d}A_Y}{A_Y} \qquad (1)$$

The fluctuation of gain factor in negative feedback relative to linear cascade is given by

$$W = \frac{\mathrm{d}Af}{Af} \Big/ \frac{\mathrm{d}A_Y}{A_Y} \qquad (2)$$

It can be derived from above result as:

$$W = \frac{1}{1 + (Z/K_Z)^n} \qquad (3)$$

Type B genetic circuit

Based on inhibition types,[38–40] the regulatory relationships among $P_{X'}$, P_Z and G_X are classified into three types (Fig. 3). (A) Linear mixed inhibition type: the DNA-bound repressor P_Z has no com-

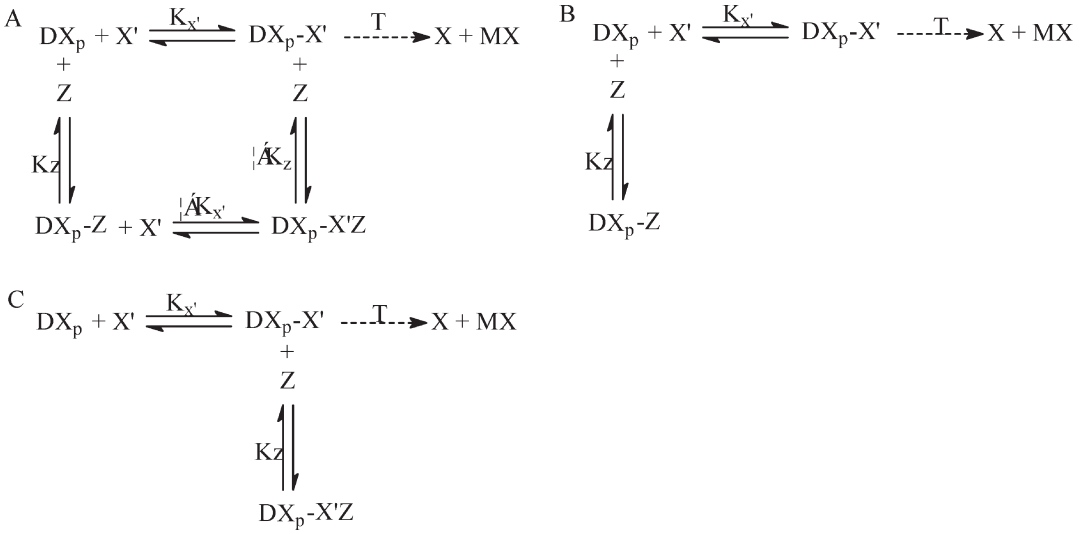


Fig. 3 – *Three inhibition types are linear mixed inhibition type (A), competitive inhibition (B), and uncompetitive inhibition (C), respectively. X', X and Z are TFs. DX_p is gene X combined by RNA polymerase complex. MX is mRNA X transcribed by gene X. T is transcription and translation process. $K_{X'}$, K_Z, $\alpha K_{X'}$, and αK_Z are dissociation constants.*

petition with the activator $P_{X'}$ in binding DX_p, when α =1, it is the case of noncompetitive inhibition; (B) competitive inhibition, inhibitor P_Z interferes with $P_{X'}$ in binding DX_p; (C) uncompetitive inhibition, P_Z can bind on G_X only when $P_{X'}$ binds first.

The three types have the same open loop relations, the expression amounts of G_X activated by $G_{X'}$ is given by:

$$Xi = \frac{X'^n \beta_X}{X'^n + K_{X'}^n} \tag{4}$$

where β_X is the maximal transcriptional and translational concentration of G_X in a given time τ. Here was also set that Hill coefficient equals 1.

Linear mixed inhibition type

Net input:

$$Xid = \frac{\left(\frac{X'}{Kx'}\right)^n \beta_X}{1+\left(\frac{X'}{Kx'}\right)^n + \left(\frac{Z}{Kz}\right)^n + \frac{(ZX')^n}{\alpha(K_Z Kx')^n}} \tag{5}$$

By eqs. (1), (2), (4), (5),

$$W = \frac{\left(\frac{X'}{Kx'}\right)^n + 1}{1+\left(\frac{X'}{Kx'}\right)^n + \left(\frac{Z}{Kz}\right)^n + \frac{(ZX')^n}{\alpha(KzKx')^n}} \tag{6}$$

Competitive inhibition type

Net input:

$$Xid = \frac{\left(\frac{X'}{Kx'}\right)^n \beta_X}{1+\left(\frac{X'}{Kx'}\right)^n + \left(\frac{Z}{Kz}\right)^n} \tag{7}$$

By eqs. (1), (2), (4), (7),

$$W = \frac{\left(\frac{X'}{Kx'}\right)^n + 1}{1+\left(\frac{X'}{Kx'}\right)^n + \left(\frac{Z}{Kz}\right)^n} \tag{8}$$

Uncompetitive inhibition type

Net input:

$$Xid = \frac{\left(\frac{X'}{Kx'}\right)^n \beta_X}{1+\left(\frac{X'}{Kx'}\right)^n + \frac{(ZX')^n}{(KzKx')^n}} \tag{9}$$

By eqs. (1), (2), (4), (9),

$$W = \frac{1+\left/\left(\frac{X'}{Kx'}\right)^n\right.}{1+\left/\left(\frac{X'}{Kx'}\right)^n\right. + \left(\frac{Z}{Kz}\right)^n} \tag{10}$$

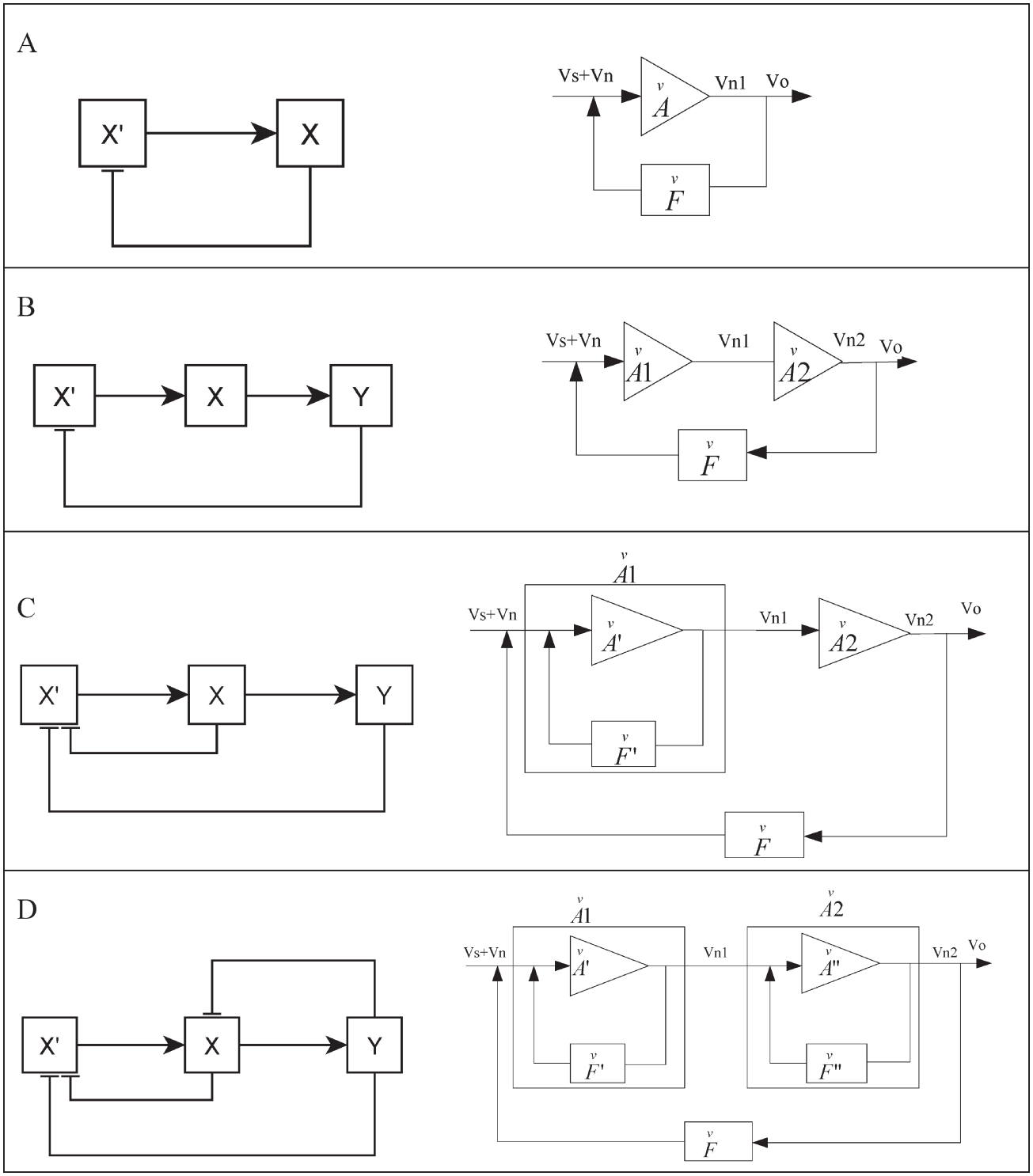
NFAC models for genetic circuits

To investigate OSNR, NFAC models are developed for corresponding genetic circuits which have different topologies. In Fig. 4, four types of negative feedback (A), (B), (C) and (D) were analyzed: (A) Single gain and one negative feedback loop, (B) Double gain and one negative feedback loop, (C) One inner-loop and one outer-loop negative feedback and (D) Double inner-loop and one outer-loop negative feedback. Next, we take the genetic circuit in Fig. 4(B) as an example for studying OSNR. The biological process (Fig. 4(B) left) is as follows: a specific signal stimulates $G_{X'}$, the product $P_{X'}$ as an input to stimulate amplifier G_X, and P_X then stimulates G_Y expression, next, P_Y as the product of G_Y inhibits $G_{X'}$ expression. Negative feedback factor $F_{Y-X'}$ is determined by the amount of P_Y output (an accumulation in a given time τ). Herein, P_Y directly inhibits input $P_{X'}$, not via an intermediate inhibitor like in Fig. 2. In electronic circuit (Fig.4(B) right), both Vs and Vn are amplified by $A1 \times A2$ times, $Vn1$ amplified by $A2$ times, and $Vn2$ not amplified. Then, the output Vo feeds back into the original input port with a negative feedback factor F by negative feedback devices. The biological process and the corresponding electronical mode are equivalent (we have pointed out the theoretical basis in the introduction and method part). The concentration of input signal $P_{X'}$ in genetic circuit is equivalent with voltage Vs in electrical circuit, and the fluctuation of signal in genetic circuit is equivalent with voltage noise (Vn, $Vn1$, $Vn2$). G_X and G_Y are amplifier genes with gain factors A_X and A_Y equivalent with electrical amplifiers with gain factor $A1$ and $A2$ respectively ($A_X = A1$, $A_Y = A2$). Negative feedback factor F in negative feedback devices equals $F_{Y-X'}$ in NFGC.

For obtaining OSNR in genetic circuits, the corresponding electrical circuits were calculated based on their equivalence. The OSNR in closed loop $\left(\frac{S}{N}\right)$ and open loop (linear cascade, $\frac{S'}{N'}$) of electrical circuits were compared as follows:

One negative feedback loop with single gain (Fig. 4A)

Closed loop:



F i g . 4 – *Genetic circuits (left) and their corresponding electronic negative feedback models (right). The expression amount of $G_{X'}$ (it is stimulated by upstream genes, though not depicted in the figure) in the absence of being inhibited corresponds to input signal voltage Vs. The expression amount of G_X in (A) and G_Y in (B)(C)(D) corresponds to output voltage Vo. Vn, Vn1 and Vn2 as input voltage noise, correspond to the sum of global and intrinsic noise in X'→X, X→Y, and Y→X', respectively. The triangle is amplifier ($\breve{A}$, $\breve{A}1$, $\breve{A}2$, $\breve{A}'$ and $\breve{A}''$ are gain factors, therein $\breve{A}1$ in (C) and (D), and $\breve{A}2$ in (D) are gain factors of inner loop) and has an equivalence relation with corresponding gene. The equivalence relations are as follows: G_X corresponds to the amplifier with gain factor $\breve{A}$ in (A), $\breve{A}1$ in (B), and $\breve{A}'$ in (C) and (D); G_Y equals the amplifier with gain factor $\breve{A}2$ in (B) and (C), and $\breve{A}''$ in (D). The inhibition to $G_{X'}$ or G_X is depicted by negative feedback factors $F_{Y-X'}$, $F_{X-X'}$, and F_{Y-X}, which corresponds to $\breve{F}$, $\breve{F}'$, and $\breve{F}''$ in NFAC respectively.*

According to negative feedback theory, we can obtain,

$$(Vs + Vn)\,\breve{A} + Vn1 - Vo\,\breve{F}\breve{A} = Vo,$$

so

$$Vo = (Vs + Vn)\frac{\breve{A}}{1 + \breve{A}\breve{F}} + \frac{Vn1}{1 + \breve{A}\breve{F}}$$

Output signal S, $Vs\breve{A} - S\breve{F}\breve{A} = S$, so $S = \dfrac{Vs\breve{A}}{1+\breve{A}\breve{F}}$

Output noise N, $Vs\breve{A} + Vn1 - N\breve{F}\breve{A} = N$,

so

$$N = \frac{Vn\breve{A} + Vn1}{1+\breve{A}\breve{F}},$$

and hence

$$\frac{S}{N} = \frac{\breve{A}\,Vs}{\breve{A}\,Vn + Vn1}$$

Open loop: When $\breve{F} = 0$, we can obtain:

$$Vo = (Vs + Vn)\,\breve{A} + Vn1;$$

$$\frac{S'}{N'} = \frac{\breve{A}\,Vs}{\breve{A}\,Vn + Vn1}.$$

So is yield:

$$\frac{S}{N} = \frac{S'}{N'}.$$

One negative feedback loop with double gain (Fig. 4B)

Closed loop: According to negative feedback theory, we can obtain,

$$(Vs + Vn)\,\breve{A}1\,\breve{A}2 + Vn1\,\breve{A}2 + Vn2 - Vo\,\breve{F}\breve{A}1\,\breve{A}2 = Vo,$$

so

$$Vo = (Vs + Vn)\frac{\breve{A}1\,\breve{A}2}{1 + \breve{A}1\,\breve{A}2\,\breve{F}} + Vn1\frac{\breve{A}2}{1 + \breve{A}1\,\breve{A}2\,\breve{F}} + + \frac{Vn2}{1 + \breve{A}1\,\breve{A}2\,\breve{F}};$$

Output signal S, $Vs\,\breve{A}1\,\breve{A}2 - S\,\breve{A}1\,\breve{A}2\,\breve{F} = S$,

so

$$S = \frac{Vs\,\breve{A}1\,\breve{A}2}{1 + \breve{A}1\,\breve{A}2\,\breve{F}}$$

Output noise N,

$$Vn\breve{A}1\breve{A}2 + Vn1\breve{A}2 + Vn2 - N\breve{A}1\breve{A}2\breve{F} = N,$$

so

$$N = \frac{Vn\,\breve{A}1\,\breve{A}2 + Vn1\,\breve{A}2 + Vn2}{1 + \breve{A}1\,\breve{A}2\,\breve{F}},$$

and hence

$$\frac{S}{N} = \frac{\breve{A}1\,\breve{A}2\,Vs}{\breve{A}1\,\breve{A}2\,Vn + \breve{A}2\,Vn1 + Vn2}.$$

Open loop: When $\breve{F} = 0$, we can obtain:

$$Vo = (Vs + Vn)\,\breve{A}1\,\breve{A}2 + Vn1\,\breve{A}2 + Vn2;$$

$$\frac{S'}{N'} = \frac{\breve{A}1\,\breve{A}2\,Vs}{\breve{A}1\,\breve{A}2\,Vn + \breve{A}2\,Vn1 + Vn2}.$$

So is obtained:

$$\frac{S}{N} = \frac{S'}{N'}.$$

One inner-loop and one outer-loop negative feedback with double gain (Fig. 4C)

Closed loop: According to negative feedback theory, we can obtain,

$$(Vs + Vn)\,\breve{A}1\,\breve{A}2 + Vn1\,\breve{A}2 + Vn2 - Vo\,\breve{F}\,\breve{A}1\,\breve{A}2 = Vo$$

$$Vo = (Vs + Vn)\frac{\breve{A}1\breve{A}2}{1 + \breve{A}1\breve{A}2\breve{F}} + + Vn1\frac{\breve{A}2}{1 + \breve{A}1\breve{A}2\breve{F}} + \frac{Vn2}{1 + \breve{A}1\breve{A}2\breve{F}};$$

$$\breve{A}1 = \frac{\breve{A}'}{1 + \breve{A}'\breve{F}'}$$

Output signal S, $Vs\,\breve{A}1\,\breve{A}2 - S\,\breve{A}1\,\breve{A}2\,\breve{F} = S$,

so

$$S = \frac{Vs\,\breve{A}1\,\breve{A}2}{1 + \breve{A}1\,\breve{A}2\,\breve{F}}$$

Output noise N,

$$Vn\,\check{A}1\,\check{A}2 + Vn1\,\check{A}2 + Vn2 - N\,\check{A}1\,\check{A}2\,\check{F} = N,$$

so

$$N = \frac{Vn\,\check{A}1\,\check{A}2 + Vn1\check{A}2 + Vn2}{1 + \check{A}1\,\check{A}2\,\check{F}},$$

and hence

$$\frac{S}{N} = \frac{\check{A}'\check{A}2\,Vs}{\check{A}'\,\check{A}2\,Vn + (\check{A}2\,Vn1 + Vn2)(1 + \check{A}'\,\check{F}')}.$$

Open loop: When $\check{F} = 0$ and $\check{F}' = 0$,

we can obtain:

$$Vo = (Vs + Vn)\,\check{A}'\,\check{A}2 + Vn1\,\check{A}2 + Vn2;$$

$$\frac{S'}{N'} = \frac{\check{A}'\check{A}2\,Vs}{\check{A}'\,\check{A}2\,Vn + \check{A}2\,Vn1 + Vn2}.$$

So we can obtain:

$$\frac{S}{N} < \frac{S'}{N'}.$$

Double inner-loop and single outer-loop negative feedback with double gain (Fig. 4D)

Closed loop:

$$Vo = (Vs + Vn)\frac{\check{A}1\check{A}2}{1 + \check{A}1\check{A}2\check{F}} + Vn1\frac{\check{A}2}{1 + \check{A}1\check{A}2\check{F}} + \frac{Vn2}{1 + \check{A}1\check{A}2\check{F}};$$

$$\check{A}1 = \frac{\check{A}'}{1 + \check{A}'\check{F}'};$$

$$\check{A}2 = \frac{\check{A}''}{1 + \check{A}''\check{F}''};$$

So we can obtain:

$$\frac{S}{N} = \frac{\check{A}'\check{A}''\,Vs}{\check{A}'\check{A}''Vn + (\check{A}''Vn1 + Vn2 + \check{A}''\check{F}''Vn2)(1 + \check{A}'\check{F}')}$$

Open loop: When $\check{F} = 0$, $\check{F}' = 0$ and $\check{F}'' = 0$,

we can obtain:

$$Vo = (Vs + Vn)\check{A}'\check{A}'' + Vn1\,\check{A}'' + Vn2.$$

$$\frac{S'}{N'} = \frac{\check{A}'\check{A}''\,Vs}{\check{A}'\check{A}''\,Vn + \check{A}''\,Vn1 + Vn2}.$$

So we can obtain:

$$\frac{S}{N} < \frac{S'}{N'}.$$

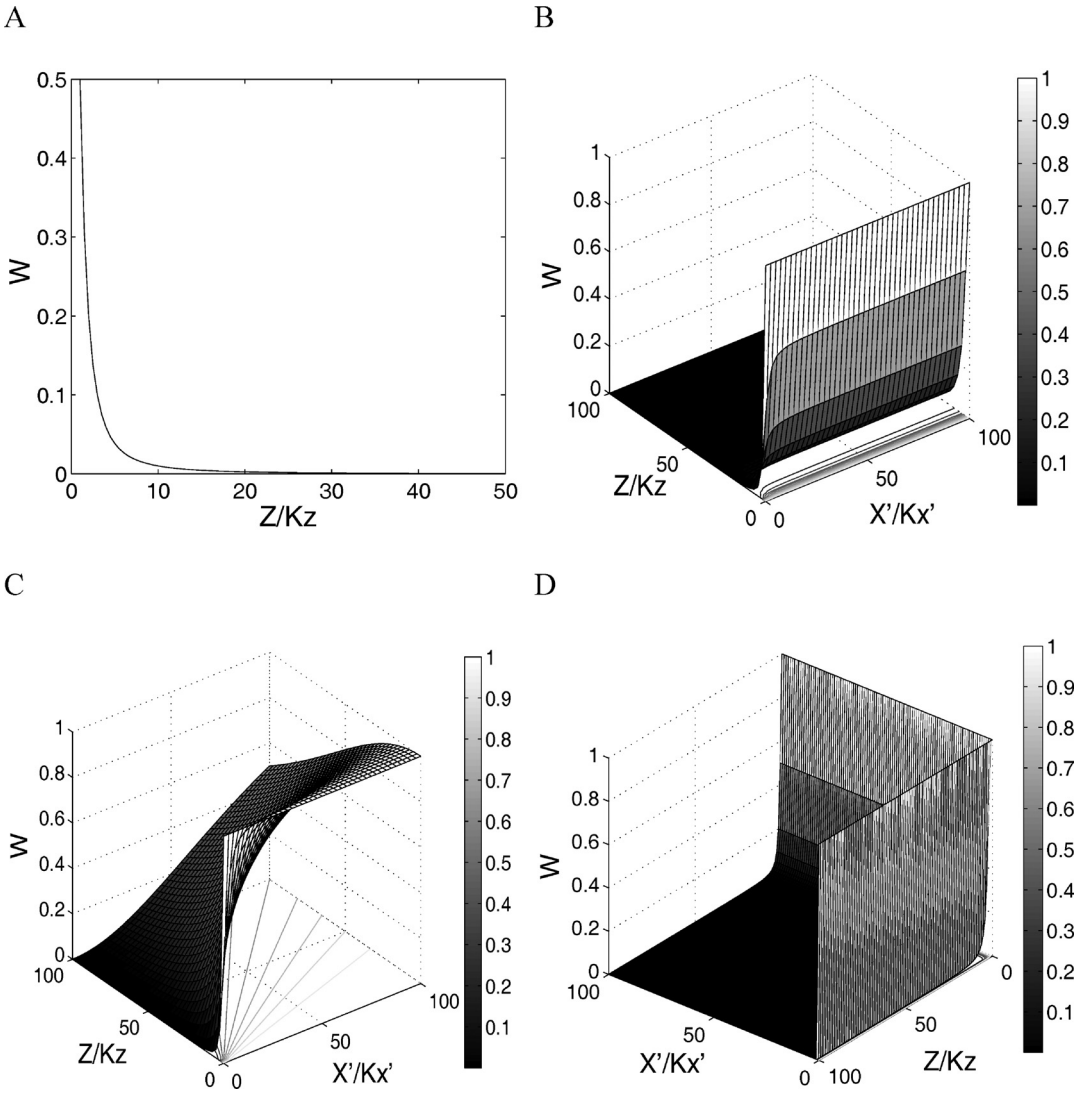
Results

Effective inputs and the stability of NFGC

Gain stability can be applied as a measure of the output stability at a specific input. Therefore, it is an index of robustness for output in negative feedback circuits. W is the fluctuation of gain factor in negative feedback relative to linear cascade. The lower W implies higher gain stability and more robustness to noise for NFGC. W also can be a special form of control coefficient.[41] From eqs. (3), (6), (8) and (10), the fluctuation of gain stability for output of G_Y in negative feedback is lower than in linear cascade(W<1), also W can be changed by adjusting two items, $X'/K_{X'}$ and Z/K_Z. $K_{X'}$ and K_Z are dissociation constants, hence the two items can be viewed as effective activation input and effective inhibition input to G_X in circuit systems (Fig. 2B). From eq. (3), enhancing gain stability of Type A genetic circuit should increase Z/K_Z and the gain stability will be constant when $Z/K_Z > 10$. It is the same case for noncompetitive inhibition type B circuit when $\alpha = 1$ in eq. (6) (Fig. 5A). Gain stability of linear mixed inhibition type mainly depends on Z/K_Z (when it is beyond a certain point, $W = 0$) and is not sensitive to $X'/K_{X'}$ when $\alpha = 10$ (Fig. 5B), also the same result can be obtained when $\alpha = 0.1$, 0.5, 5. Under the condition of higher Z/K_Z with lower $X'/K_{X'}$, it is possible to make the competitive inhibition type more robust (Fig. 5C). The enhanced robustness can be obtained when the two effective input items are not too low for uncompetitive inhibition type (Fig. 5D). Hence, NFGC can have better gain stability than linear cascade and, at the same time, the gain stability in negative feedback can be enhanced by adjusting $X'/K_{X'}$ and Z/K_Z as effective inputs.

OSNR in NFGC compared with linear cascades

NFGC can reduce noise compared with linear cascade. Meanwhile, the output signal will attenuate too. Hence, it is necessary to use OSNR for valuing which attenuates more. Compared with linear cascade, four types of NFGC were developed to analyze the OSNR. We analyzed the electrical circuits modeled for corresponding genetic circuits based on their equivalences. And it turns out that signal



F i g . 5 – *Relative fluctuations of gain stability (W) varying with effective inputs in four negative feedback types (n = 2). (A) In genetic circuit A and noncompetitive inhibition type, W depends on Z/K_Z only. (B), (C) and (D) are the cases in linear mixed inhibition type (when α = 10), competitive inhibition and uncompetitive inhibition type, respectively.*

and noise in negative feedback both have an attenuation compared with linear cascade. However, OSNR in NFGC is lower than linear cascade when the former contains inner-loop. The two will have the same OSNR when negative feedback contains only one loop. This means adding negative feedback to linear cascade can not improve OSNR.

Discussion

Negative feedback is a key regulatory motif for reducing noise in electrical and genetic systems. Under some circumstances, NFAC can also enhance gain stability and improve OSNR. NFAC is equivalent to NFGC because they both obey the same control theory. So based on the feedback depth analysis in electrical systems and the negative feedback electrical models simulating NFGC, NFGC are explored by studying similar properties with electrical circuits, such as an increase of the gain stability and OSNR.

NFGC can achieve high gain stability compared with linear cascade. Moreover, gain stability can be changed by adjusting the two components, $X'/K_{X'}$ and Z/K_Z, named as the effective inputs. In fact, the higher gain stability means the output expression is steadier and the fluctuation of gene expression can be reduced more. Previous study has shown the noise in NFGC can be controlled by inhibitor concentration.[2,14,15] Hence, the high gain stability is equivalent to higher noise attenuation. Hence, the noise in NFGC can be controlled quantitatively by adjusting $X'/K_{X'}$ and Z/K_Z.

Compared with a linear cascade, negative feedback can reduce both signal and noise at the expense of not improving OSNR, which attenuate

more signal than noise. Evidence showed that negative feedback attenuates noise, but also damages signal sensitivity compared with linear cascade, while positive feedback can get higher signal sensitivity than linear cascade in the same noise amplification.[42] Here it is proved that signal sensitivity to noise amplification ratio has the same increasing and decreasing with our OSNR. Hence, the results confirm the findings that OSNR in NFGC can not be enhanced compared with linear cascade.

Some biological systems buffer noise by adjusting some parameters and choosing some regulatory relationships. Based on these findings, if the number of $G_{X'}$ and G_Z copies,[15] the intensity of transcription and translation,[2,14] and the dissociation constant[43] of TF and gene (they can be achieved by changing the promoter intensity to adjust transcription,[44,45] changing translation by adding miRNAs, or using different sized promoters and specific promoters[31] to change dissociation constant) were altered to control effective input, such as $X'/K_{X'}$ and Z/K_Z, the feedback systems can achieve high gain stability of NFGC. Different inhibition type can differ the way they enhance gain stability. Inversely, by the relationships between gain stability and effective input, we can deduce the inhibition type. Meanwhile, negative feedback might not be the best choice for synthesizing high OSNR genetic circuits. Hence, not all genetic circuits are run by negative feedback regulatory loops solely, but also by positive ones,[46] toggle switch,[37,47] network motifs,[48] or oscillator,[49] etc. If we want to send a signal to a target gene with a high fidelity, we can choose a linear cascade path. If we cannot find a pure linear cascade to send the signal, we can adopt some methods to eliminate the effect of possible negative feedback in the circuit, such as adding inhibitor to reduce production of the protein related to negative feedback factor *F*.

This work reflects that negative feedback in electrical circuits and genetic circuits are similar in gain stability, but different in OSNR. The findings show that two systems with different components in nature can co-evolve to some degree, so the principles in electrical systems might be used in genetic systems.[24,50] Hence, by exploring how cellular systems with NFGC control signal and noise, our study supplies some novel implications for controlling cell behaviors and synthesizing genetic circuits.

ACKNOWLEDGEMENTS

This work was supported by grants from National Project '973' of China (2006CB504505). We are very grateful for the friendly and meaningful discussion with Xiao Zhang and Baoquan Ai in South China Normal University.

Nomenclature

$G_{X'}$, G_X, G_Y, G_Z – gene

$P_{X'}$, P_X, P_Y, P_Z – transcription factor

A, A_X, A_Y – gain factor in NFGC

F_{Y-X}, $F_{Y-X'}$, $F_{X-X'}$, F_{Y-X} – negative feedback factor in NFGC

T_d – threshold of the time when TF degradation should be considered

Xi – maximal expression amount of G_X without inhibition in a given time τ

Xid – net concentration of P_X due to inhibition of P_Z

Xf – reduction of P_X due to inhibition of P_Z

Xo – output concentration of G_Y expression

n – Hill coefficient

Af – gain factor in NFGC

W – fluctuation of gain factor in negative feedback relative to linear cascade

DX_p – gene X combined by RNA polymerase complex

Vs – input signal in NFAC

Vn, $Vn1$, $Vn2$ – input noise in NFAC

Vo – output signal in NFGC

$\check{A}$, $\check{A}1$, $\check{A}2$, $\check{A}'$ and $\check{A}''$ – gain factors in NFAC

$\check{A}1\times\check{A}2$ – $\check{A}1$ and $\check{A}2$ – are gain factors of the two amplifiers in (Fig. 4(B) right), so the input Vs and Vn can be amplified by $\check{A}1$ times $\check{A}2$.

$\check{F}$, $\check{F}'$, and $\check{F}''$ – negative feedback factor in NFAC

$\frac{S}{N}$ – OSNR in closed loop

$\frac{S'}{N'}$ – OSNR open loop

$X'/K_{X'}$ – effective activation input

Z/K_Z – effective inhibition input

K_X, $K_{X'}$, K_z, $\alpha K_{X'}$, αK_Z – dissociation constants

MX – mRNA

X′, X, Y and Z – concentration of $P_{X'}$, P_X, P_Y and P_Z respectively

Greek letters

τ – given accumulation time

β_X – maximal transcriptional and translational concentration of G_X in a given time τ

Abbreviations

NFGC – negative feedback genetic circuits

NFAC – negative feedback amplifier circuits

OSNR – output signal-to-noise ratio

TF – transcription factor

T – transcription and translation process

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