A Hill type equation reveals the regulation principle of target protein expression led by p53 pulsing

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Abstract
The central dogma indicates the basic direction of gene expression pathways. For activated gene expression, the relationship between various links from the binding of transcription factor (TF) DNA to protein synthesis remains unclear. There is consensus that at a steady state, protein levels are largely determined by the mRNA level. How can we determine this steady state? Taking p53 as an example, based on the previously discovered Hill-type equation that characterizes mRNA expression under p53 pulsing, I proved that the same equation can be used to describe the average steady state of target protein expression. Therefore, at the steady state, the average fold changes of mRNA and protein expression under TF pulsing are the same. This consensus has been successfully demonstrated. For the gene *BAX* of the p53 target, the observed fold changes in mRNA and protein were 1.72, 1.28, respectively; the mRNA and protein expressions calculated using the Hill-type equation were 1.35. Therefore, using this equation, we are not only able to fine-tune gene expression, but also to predict the proteome from the transcriptome. Furthermore, by introducing two quantitative indicators, we can understand the degree of accumulation and stability of protein expression.

Keywords: Hill equation, p53 target protein expression, steady state, stability of gene expression, protein accumulation, transcription factor pulsing

Introduction

According to the central dogma of molecular biology, the gene expression pathway is from DNA to RNA to protein (1, 2), which is the process of transcribing gene-encoded information into mRNA and translating it into functional proteins. For gene expression, mRNAs and proteins provide useful readouts that connect genes and phenotypes (3). Generally, there are two different gene expression pathways. One is the basal pathway, which regulates homeostatic expression, and the other is the activated or repressed pathway, which dynamically regulates expression in response to environmental stimuli (4-8).

For basal gene expression, steady-state protein levels depend on the transcription rate, mRNA half-life, translation rate constant, and protein half-life (9, 10). Moreover, situations with high transcription rates and low translation rate constants should be ruled out (10). Under environmental stimuli, for gene expression activated by transcription factors (TF), studies on transcripteomics and proteomics indicate that the protein level at the steady state is largely contributed by the mRNA level (3, 11, 12), however, there is little relationship between the protein level and synthesis or protein half-life.

Here, steady state can be defined as a relatively long-term process experienced by cells (11). Therefore, both cell proliferation and differentiation can be considered steady states (11). Apoptosis and senescence also occur in a steady state (13, 14). The steady state achieved after stimulation differs from that before perturbation (15).

For the steady state before stimulation in dendritic cells, mRNA levels explain 68% of the change in protein expression, and for the approximate steady state after stimulation with lipopolysaccharide, mRNA levels explain 90% of the change in protein expression (12, 16). In addition, during the development of *C elegans*, the fold changes in mRNA and protein expression were almost identical. The transcript fold change was 2.02, and the proteins was 2.05 (17). Therefore, we can speculate that the fold-changes in mRNA and protein expression in the steady state are equal. Could we theoretically determine this special steady state of protein expression? Which factors determine protein expression levels in steady state?

Gene expression under environmental stimuli is driven by TF, which maps the corresponding stimulus. The TF dynamics encode specific stimuli (18). The binding components of TF and DNA constitute the decoder of the TF dynamics. This initiates the expression of the corresponding target gene and fulfills the relevant function. Some TFs exhibit oscillatory dynamic behaviors, in which the duration, frequency, and amplitude can all serve as encoding components of gene expression, thereby leading to different cell fates (19, 20).

The tumor suppressor p53 is the most extensively studied TF. In response to DNA damage, p53 concentrations exhibit a series of pulses of fixed duration, frequency, and amplitude, whereas the number of p53 pulses increases with the degree of DNA damage caused by γ irradiation (21). Changing p53 dynamics from pulsed to sustained behavior leads to an altered cell fate from DNA repair to senescence (22). For target gene expression driven by p53 pulsing, there is an interesting phenomenon that the levels of mRNA and protein expression are very close. For example, for the gene *MDM2*, it was observed 10 h or 24 h after stimulation that the fold change in mRNA was 2.2 (10 h)(23) or 2.0 (24 h)(24), while the protein expression was 1.8 (24 h), which can be regarded as the average steady state over the cell population (23). Therefore, without loss of generality, I will take target gene expression under p53 pulsing as an example to seek the principle governing target protein expression at a

steady state.

Following the genetic information flow specified by the central dogma, we obtained a modified Hill equation to characterize the average p53 DNA-binding probability (25). I also found a Hill-type equation that could predict the average fold-change in target mRNA expression under p53 pulsing (26).

$$\bar{m} = 1 + \frac{\Delta}{T}\frac{\beta A^n}{K_A^n + A^n}, \tag{1}$$

where Δ denotes the duration, T is the period, A is the amplitude, β is the maximal fold change in mRNA expression, and K_A is the dissociation constant.

Here, I will complete the last step in describing the central dogma, from mRNA to protein. In response to pulsed p53 input dynamics, p21 mRNA dynamics show pulsing expression; however, p21 protein dynamics exhibit rising expression (27). The half–life of mRNA and protein determines the stability of expression dynamics (23, 24, 28). However, the relationship between steady-state mRNA and protein expression levels remains unclear. Therefore, I tried to prove that the average steady-state fold changes in mRNA and protein expression under p53 pulsing are equal, *i.e.*, the average fold change of protein at steady state equals $\bar{m}$.

Methods

Mathematical model of p53 target protein expression dynamics and its analytical solution

To achieve this goal, we must develop a useful and accurate mathematical model for gene expression under pulsed TF dynamics (16, 29). The ordinary differential equation for the mRNA dynamics is (26)

$$\frac{dmRNA(t)}{dt} = \beta_0' + \beta' \frac{S^n(t)}{K_A^n + S^n(t)} - \alpha mRNA(t), \tag{2}$$

where

$$S(t) = \begin{cases} A, & (i-1)T \le t < (i-1)T + \Delta \\ 0, & (i-1)T + \Delta \le t \le iT \end{cases} \quad i = 1,2,\ldots,$$

$mRNA(t)$ denotes the target mRNA concentration, $S(t)$ is the piecewise constant function of TF p53 pulses, β_0' is the basal transcription rate, β' is the maximal transcription rate activated by p53, α is the mRNA decay rate constant, and n is the Hill coefficient. The initial condition is $mRNA(0) = m_0$, where m_0 denotes the basal mRNA concentration.

The equation for the protein expression dynamics is (9, 10, 23)

$$\frac{dprotein(t)}{dt} = \sigma mRNA(t) - \mu protein(t) \tag{3}$$

where $protein(t)$ represents the target protein concentration, σ and μ denotes the rate constants of translation and degradation, respectively. The initial protein concentration was determined using $protein(0) = p_0$.

For activated expression dynamics, considering $m_0 = \beta_0'/\alpha$, $p_0 = \sigma m_0/\mu$, let $m(t) = mRNA(t)/m_0$, $P(t) = protein(t)/p_0$, Equation 2 and Equation 3 become

$$\frac{dm(t)}{dt} = \alpha\left(1 + \beta\frac{[S(t)]^n}{K_A^n + [S(t)]^n} - m(t)\right), \quad m(0) = 1, \tag{4}$$

$$\frac{dP(t)}{dt} = \mu(m(t) - P(t)), \qquad P(0) = 1 \ . \tag{5}$$

where $m(t)$ and $P(t)$ represent the fold changes in mRNA and protein expression, respectively, and $\beta = \beta' / \beta_0'$ denotes the maximal fold change in transcription. Because Equation 4 has been solved, the solution from Reference (26) is rewritten as

$$m_i(\xi_i) = 1 + m_d \left(1 - \frac{e^{-\alpha\xi_i}}{1 - e^{-\alpha T}} \left((1 - e^{-\alpha(T-\Delta)}) + e^{-i\alpha T}(e^{\alpha\Delta} - 1) \right) \right), \quad \xi_i \in [0, \Delta),$$

$$m_i(\xi_i) = 1 + m_d \frac{(e^{\alpha\Delta} - 1)(1 - e^{-i\alpha T})}{1 - e^{-\alpha T}} e^{-\alpha\xi_i}, \qquad \xi_i \in [\Delta, T]. \tag{6}$$

$$\xi_i = t - (i-1)T$$

where

$$m_d = \frac{\beta A^n}{K_A^n + A^n} \ .$$

$m_i(\xi_i)$ represents the mRNA fold change during the i-th TF pulse.

Solving Equation 5) is challenging. Assuming $\alpha \neq \mu$, we can obtain analytical solutions for target protein expression in response to p53 pulsing as follows:

$$P_i(\xi_i) = 1 + m_d + A_i e^{-\mu\xi_i} - \frac{\mu m_d C(i)}{\mu - \alpha} e^{-\alpha\xi_i}, \xi_i \in [0, \Delta),$$

$$P_i(\xi_i) = 1 + B_i e^{-\mu\xi_i} + \frac{\mu m_d D(i)}{\mu - \alpha} e^{-\alpha\xi_i}, \qquad \xi_i \in [\Delta, T], \tag{7}$$

$$\xi_i = t - (i-1)T$$

where

$$A_i = \frac{\alpha m_d}{\mu - \alpha} (e^{\mu\Delta} - \frac{(e^{\mu\Delta} - 1)(1 - e^{-i\mu T})}{1 - e^{-\mu T}}),$$

$$B_i = -\frac{\alpha m_d}{\mu - \alpha} \frac{(e^{\mu\Delta} - 1)(1 - e^{-i\mu T})}{1 - e^{-\mu T}},$$

$$C(i) = \frac{1 - e^{-\alpha(T-\Delta)} + e^{-i\alpha T}(e^{\alpha\Delta} - 1)}{1 - e^{-\alpha T}},$$

$$D(i) = \frac{(e^{\alpha\Delta} - 1)(1 - e^{-i\alpha T})}{1 - e^{-\alpha T}}.$$

$P_i(\xi_i)$ represents the protein fold-change under the ith p53 pulse. Here, a detailed derivation of A_i and B_i is presented. From Equation. (7), we consider the following:

$$P_{i-1}(T) = P_i(0), \quad P_i(\Delta+) = P_i(\Delta-), \tag{8}$$

thus there are

$$A_i - B_i = \frac{\alpha m_d}{\mu - \alpha} e^{\mu\Delta}, \quad B_{i-1} e^{-\mu T} - B_i = \frac{\alpha m_d}{\mu - \alpha} (e^{\mu\Delta} - 1). \tag{9}$$

Since $P_1(0) = 1$, we can derive:

$$A_1 = \frac{\alpha m_d}{\mu - \alpha}, \quad B_1 = -\frac{\alpha m_a}{\mu - \alpha} (e^{\mu\Delta} - 1). \tag{10}$$

Then from the difference equation 41, we can obtain:

$$B_i = B_1 e^{-(i-1)\mu T} - \frac{\alpha m_d}{\mu-\alpha}\frac{(e^{\mu\Delta}-1)(1-e^{-(i-1)\mu T})}{1-e^{-\mu T}}. \tag{11}$$

Substituting B_i into Equation 9 yields A_i.

Results

Target protein expression at steady state exhibits oscillations

The steady state is the relatively long-term behavior of the cells. By inspecting Equation 7, for sufficient p53 input pulses, let $i \to \infty$, we can obtain the steady state of the target protein expression dynamics:

$$\begin{aligned} P_{ss}(\xi) &= 1 + m_d + \frac{\alpha m_d}{\mu-\alpha}\frac{1-e^{-\mu(T-\Delta)}}{1-e^{-\mu T}}e^{-\mu\xi} - \frac{\mu m_d}{\mu-\alpha}\frac{1-e^{-\alpha(T-\Delta)}}{1-e^{-\alpha T}}e^{-\alpha\xi}, \xi \in [0,\Delta), \\ P_{ss}(\xi) &= 1 - \frac{\alpha m_d}{\mu-\alpha}\frac{e^{\mu\Delta}-1}{1-e^{-\mu T}}e^{-\mu\xi} + \frac{\mu m_d}{\mu-\alpha}\frac{e^{\alpha\Delta}-1}{1-e^{-\alpha T}}e^{-\alpha\xi}, \qquad \xi \in [\Delta, T]. \end{aligned} \tag{12}$$

$P_{ss}(\xi)$ reached its maximum and minimum at Δ and 0 or T, respectively. Therefore, the steady-state of the target protein expression dynamics is a repetitive and invariant oscillation. The maximal fold change or peak of oscillations was

$$P_{ss,\max} = P_{ss}(\Delta) = 1 + m_d + \frac{\alpha m_d}{\mu-\alpha}\frac{e^{-\mu\Delta}-e^{-\mu T}}{1-e^{-\mu T}} - \frac{\mu m_d}{\mu-\alpha}\frac{e^{-\alpha\Delta}-e^{-\alpha T}}{1-e^{-\alpha T}} \tag{13}$$

And the minimal fold change or valley is

$$P_{ss,\min} = P_{ss}(0) = 1 + m_d + \frac{\alpha m_d}{\mu-\alpha}\frac{1-e^{-\mu(T-\Delta)}}{1-e^{-\mu T}} - \frac{\mu m_d}{\mu-\alpha}\frac{1-e^{-\alpha(T-\Delta)}}{1-e^{-\alpha T}} \tag{14}$$

For a given target protein, β and K_A are relatively fixed, and the amplitude depends on the p53 dynamic parameters and half-life of mRNA and protein. Apparently, the smaller the amplitude, the more stable the steady state.

A Hill type equation can characterize the constant steady state

Let us now examine the characteristics of the steady state within the limits of α and μ. From Equation 9 and 10, when $\alpha T \ll 1, \mu T \ll 1$ was applied,

$$P_{ss,\max} = P_{ss,\min} = 1 + \gamma m_d = 1 + \frac{\Delta}{T}\frac{\beta A^n}{K_A^n + A^n} = \bar{m} \tag{15}$$

Equation 15 is the same as Equation 1, which is exactly the Hill-type equation found previously (26).

Similarly, when $\alpha T \ll 1, \mu\Delta \gg 1$, *i.e.* $\mu T \gg 1$, there are

$$P_{ss,\max} = P_{ss,\min} = 1 + \gamma m_d.$$

And when $\mu T \ll 1$, $\alpha\Delta \gg 1$, *i.e.* $\alpha T \gg 1$, there are

$$P_{ss,\max} = P_{ss,\min} = 1 + \gamma m_d.$$

When $\alpha\Delta \gg 1, \mu\Delta \gg 1$, *i.e.* $\alpha T \gg 1, \mu T \gg 1$, there are

$$P_{ss,\max} = P_{ss,\min} = 1 + m_d \tag{16}$$

Equation 16 is the same as the classical Hill equation, which governs the steady-state mRNA fold-change under sustained p53 dynamics (26). Therefore, in the limit of a very long or short half-life for mRNA and protein, oscillations contract into a constant line that is very stable.

For several target genes of p53, Table 1 lists the observations for α, μ, β, and K_A. Only 3 genes had complete data. α and μ determine the trajectory of target protein expression dynamics (23). The four scenarios discussed above correspond to the four sets of protein expression dynamics defined in reference(23).

As shown in Table 1, the mRNA decay rate constants of *PUMA*, *MDM2*, and *p21* are from (28), and the mRNA half-life of *BAX* is 38.77 h (30), thus, the mRNA decay rate is $\alpha = \ln 2/38.77 \approx 0.018\ \mathrm{h}^{-1}$ (23). The protein degradation rate constants of *PUMA*, *MDM2*, *p21*, and *BAX* are obtained from (23). The dissociation constants of *PUMA*, *MDM2*, *p21*, *BAX* ,and *GADD45A* are from (31). For maximal mRNA expressions of *p21*(*CDKN1A*), *GADD45A*, *MDM2*, and *BAX* , $\log_2$ (maximal mRNA fold chan is 3.9008(3 h), 3.1949(3 h), 2.7861(3 h), and 1.2373(9 h)(24), thus the maximal fold changes are 14.937, 9.157, 6.898, and 2.358, respectively.

Table 1. The observed values of α, μ, β and K_A corresponding to genes

Gene	Function	mRNA decay rate constant	Protein degradation rate constant	Dissociation constant	Maximal mRNA fold change
PUMA	Apoptosis	0.73 h^{-1}	0.056 h^{-1}	260 nM	Unknown
MDM2	Feedback inhibition	0.27 h^{-1}	0.7916 h^{-1}	12.3 nM	$2^{2.7861} = 6.898$
p21	Cell cycle arrest	0.265 h^{-1}	0.2546 h^{-1}	4.9 nM	$2^{3.9008} = 14.937$
BAX	Apoptosis	0.018 h^{-1}	0.0262 h^{-1}	73 nM	$2^{1.2373} = 2.358$
GADD45A	DNA repair	Unknown	Unknown	7.7 nM	$2^{3.1949} = 9.157$

The average fold change of mRNA and protein expression are the same at steady state

The average fold change of the target protein over the ith period can be calculated by:

$$\bar{P}_i = \frac{1}{T}\int_0^T P_i(\xi_i)d\xi_i = 1 + \frac{\Delta}{T}m_d + \frac{\alpha m_d}{(\mu-\alpha)\mu T}(e^{\mu\Delta}-1)e^{-i\mu T} - \frac{\mu m_d}{(\mu-\alpha)\alpha T}(e^{\alpha\Delta}-1)e^{-i\alpha T}. \tag{17}$$

Let $i \to \infty$, there is the average steady state

$$\bar{P} = 1 + \frac{\Delta}{T}m_d = 1 + \frac{\Delta}{T}\frac{\beta A^n}{K_A^n + A^n} \tag{18}$$

As we expect

$$\bar{P} = \bar{m} \tag{19}$$

Therefore, we proved that at steady state, the average fold changes in mRNA and protein were the same. This result also holds for the average levels of different proteins and their corresponding mRNA within a single cell, which is consistent with the observed results (17). Similarly, for the average of the same proteins over the cell population, the average expression levels of mRNA and protein were also the same.

Next, we considered the target protein BAX as an example to examine the predictability of the Hill-type equation for protein expression. The observed fold-change of the BAX protein 24 h after stimulation was 1.28 (23), and the BAX mRNA was $2^{0.7802} = 1.72$ (24). For any cell, assuming that Δ/T remains unchanged, the average BAX expression over the cell population can be calculated using Hill-type equation (26):

$$\bar{P}_{BAX} = \bar{m}_{BAX} = 1 + 2^{1.23} \cdot 0.37 \cdot \frac{60^{1.8}}{73^{1.8} + 60^{1.8}} = 1.35 \tag{20}$$

The values for β and K_A are listed in Table 1. Hill coefficient $n = 1.8$ (31). $A = 60$ nM (25). The duty cycle of p53 pulsing γ can be written as

$$\gamma = \frac{\text{average pulsed p53 concentration over a period}}{\text{average sustained p53 concentration over a period}}$$

$$= \frac{\frac{1}{T}\int_0^T p53(t)dt}{\frac{1}{T}\int_0^T A dt} = \frac{\int_0^T p53(t)dt}{AT} = \frac{\text{AUC}\{ p53(t)|_0^T \}}{\text{AUC}\{ A|_0^T \}},$$

where $\text{AUC}\{ p53(t)|_0^T \}$ represents the area under the curve $p53(t)$. For general periodic function, $p53(t)$, during $[0, T]$ can be approximated by a square wave function $S(t)$, as long as

$$\text{AUC}\{ p53(t)|_0^T \} = \text{AUC}\{ S(t)|_0^T \}.$$

Thus

$$\gamma = \frac{\int_0^T S(t)dt}{AT} = \frac{A\Delta}{AT} = \frac{\Delta}{T}.$$

Here, γ is taken as 0.37 to minimize the error between the predicted p21 mRNA fold change and the observed fold change(26).

Longer mRNA or protein half-life determine the relaxation time of target protein expression dynamics

We have previously obtained the relaxation time for mRNA dynamics (26). Similarly, the time of protein dynamic trajectory relaxation to the average steady state can be calculated by (25, 26, 32)

$$\tau_{pulsed} = \frac{T\sum_{i=1}^{\infty}\left|\bar{P}_i - \bar{P}\right|}{\bar{P} - 1} = \frac{T}{\Delta|\mu - \alpha|}\left|\frac{\alpha}{\mu}\frac{e^{\mu\Delta} - 1}{e^{\mu T} - 1} - \frac{\mu}{\alpha}\frac{e^{\alpha\Delta} - 1}{e^{\alpha T} - 1}\right|. \tag{21}$$

When $\alpha T \ll 1$, $\mu T \ll 1$, τ_{pulsed} can be expanded in the Taylor series:

$$\tau_{pulsed} \approx \frac{1}{\alpha} + \frac{1}{\mu} - \frac{T}{2}(1 - \gamma). \tag{22}$$

Thus, the number of p53 pulses required to reach the average steady state is

$$\frac{\tau_{pulsed}}{T} \approx \frac{1}{\alpha T} + \frac{1}{\mu T} - \frac{1}{2}(1 - \gamma) \tag{23}$$

For sustained p53 input dynamics, $\Delta = T$, thereby

$$\tau_{sus} \approx \frac{1}{\alpha} + \frac{1}{\mu} \tag{24}$$

Therefore, compared to the sustained input, pulsed p53 dynamics cause the target protein dynamics to reach the maximum more quickly. In other words, if the half-lives of mRNA and protein and $T(1-\gamma)/2$ have the same order of magnitude, oscillatory p53 input enhances the sensitivity of protein expression. On the other hand, as shown in Table 1, the gene *BAX* with a longer half-life of mRNA and protein requires multiple p53 pulse inputs to reach a steady state, which not only provides sufficient

time for DNA repair but also accumulates sufficient expression levels required for triggering apoptosis.

The index of target protein accumulation under multiply p53 pulses

To understand the degree of accumulation of the target protein expression in response to multiple p53 input pulses, the index of protein accumulation can be defined as

$$\lambda = \frac{P_{ss,\max}}{P_{1,\max}}, \tag{25}$$

which is the ratio of the maximal protein fold change at steady state to the maximal protein fold change during 1-th pulse.

$P_{ss,\max}$ is given by Equation 9, and

$$P_{1,\max} = P_1(\Delta) = 1 + m_d + \frac{\alpha m_d}{\mu - \alpha} e^{-\mu\Delta} - \frac{\mu m_d}{\mu - \alpha} e^{-\alpha\Delta}. \tag{26}$$

When $\alpha T \ll 1$, $\mu T \ll 1$, there are

$$P_{ss,\max} = 1 + \gamma m_d, \quad P_{1,\max} = 1.$$

Thus

$$\lambda = 1 + \gamma m_d. \tag{27}$$

Similarly, when $\alpha T \ll 1$, $\mu\Delta \gg 1$, *i.e.* $\mu T \gg 1$, there is

$$\lambda = 1 + \gamma m_d.$$

When $\alpha\Delta \gg 1$, *i.e.* $\alpha T \gg 1$, and $\mu T \ll 1$, there is

$$\lambda = 1 + \gamma m_d.$$

When $\alpha\Delta \gg 1$, i.e. $\alpha T \gg 1$and $\mu\Delta \gg 1$,*i.e.* $\mu T \gg 1$, there are

$$P_{ss,\max} = 1 + m_d, \quad P_{1,\max} = 1 + m_d.$$

Thus $\lambda = 1$ (28)

Therefore, proteins with longer half-lives have a higher accumulation; however, proteins with shorter half-lives have a lower accumulation.

The first three cases discussed above broadly correspond to the rising expression dynamics observed in reference (23), and the last corresponds to the oscillatory expression dynamics. In other words, the rising expressions have higher accumulation, and the pulsing expressions have lower accumulation.

Peak to valley ratio can measure the stability of mRNA and protein expression dynamics under multiply p53 pulses

From Equation 6, it can be seen that the mRNA expression dynamics at steady state also exhibit oscillatory behavior. Let $i \to \infty$, The steady state for mRNA dynamics can be written as

$$\begin{aligned} m_{ss}(\xi) &= 1 + m_d \left(1 - \frac{e^{-\alpha\xi}(1 - e^{-\alpha(T-\Delta)})}{1 - e^{-\alpha T}}\right), && \xi \in [0, \Delta), \\ m_{ss}(\xi) &= 1 + m_d \frac{(e^{\alpha\Delta} - 1)}{1 - e^{-\alpha T}} e^{-\alpha\xi}, && \xi \in [\Delta, T]. \end{aligned} \tag{29}$$

The peak is

$$m_{ss,\max} = m_{ss}(\Delta) = 1 + m_d \frac{1 - e^{-\alpha\Delta}}{1 - e^{-\alpha T}}. \tag{30}$$

And, the valley is

$$m_{ss,\min} = m_{ss}(T) = 1 + m_d \frac{e^{\alpha\Delta} - 1}{e^{\alpha T} - 1}. \quad (31)$$

For a given gene, the peak-to-valley ratio for target mRNA expression can be defined as

$$\rho_m = \frac{m_{ss,\max}}{m_{ss,\min}}. \quad (32)$$

The closer ρ_m is to 1, the more stable the mRNA expression dynamics. In particular, when the $\alpha T \ll 1$

$$m_{ss,\max} = m_{ss,\min} = 1 + \gamma m_d. \quad (33)$$

Thus

$$\rho_m = 1. \quad (34)$$

Therefore, the longer the mRNA half-life, the more stable is the mRNA expression dynamics. Similarly, when $\alpha\Delta \gg 1$, *i.e.* $\alpha T \gg 1$, there are

$$m_{ss,\max} = 1 + m_d, \quad m_{ss,\min} = 1. \quad (35)$$

Thereby

$$\rho_m = 1 + m_d \quad (36)$$

Therefore, target mRNA dynamics with shorter half-life is unstable.

Similarly, we can define the peak-to-valley ratio that characterizes the stability of the target protein expression dynamics as:

$$\rho_p = \frac{P_{ss,\max}}{P_{ss,\min}}. \quad (37)$$

As can be seen from the previous discussion, for the four extreme cases of α and μ, and $\rho_p = 1$. However, because of the short half-life of mRNA, $\rho_m = 1 + m_d > 1$. Therefore, translation of mRNA enhances its stability. By calculating the peak-to-valley ratio of each target gene, we can compare the stability of expression of different genes.

The regulatory principle of protein expression dynamics under p53 pulsing

Let us examine the Hill type equation. For a very high binding affinity, *i.e.* $K_A \gg A$,

$$\bar{P} = 1 + \frac{\Delta}{T} \frac{\beta A^n}{K_A^n + A^n} \approx 1 + \beta \frac{\Delta}{T} \quad . \quad (38)$$

Therefore, target protein expression with high p53 DNA-binding affinity is insensitive to amplitude. Only through duration and frequency can fine-tuning of protein expression be achieved, demonstrating the regulatory ability of duration and frequency beyond saturation. In addition, it is important to note that $\Delta/T < 1$, namely, the frequency must be less than that of the $1/\Delta$. Thus, a situation in which too high a frequency causes the protein expression to decrease is avoided. Furthermore, the results from the experiment showed that gene expression increased proportionally with TF frequency (33), which agrees with the prediction from the equation.

The maximum mRNA expression of β was determined using binding affinity. The higher the affinity, the greater the maximal mRNA level (24, 31). For example, as shown in Table 1, for genes *p21*, *GADD45*, *MDM2*, *BAX*, K_A are 4.9 nM, 7.7 nM, 12.3 nM, 73 nM (31), and the corresponding β are 14.937, 9.157, 6.898, 2.358, respectively (24). Therefore, the maximal gene expression level is mainly determined

by the TF DNA-binding affinity, whereas the stability of gene expression is determined by the half-life of mRNA and protein. Therefore, regulation of binding affinity has become a key issue in gene expression control. Gurdon *et al.* can greatly improve the binding affinity by extending the residence time of the TF target (34), allowing the duration and frequency of TF dynamics to finely tune gene expression.

Discussion

TF is a node of a complex network, which is similar to a bridge connecting cellular signal transduction networks and transcription networks. Taking the famous TF p53 as an example, I proved that the average steady state of mRNA and protein expression fold changes driven by p53 pulsing are the same. The Hill-type equation obtained may also be expected from previous methods (3, 11, 13, 16). Using this equation, we cannot only clearly understand the regulation principle of gene expression, but also put this equation into practice. In addition, we provide two quantitative indicators to determine the degree of accumulation and stability of protein expression under multiple TF input pulses.

For gene expression activated in response to stimulation, this equation does not depend on the rate constants of translation and degradation. However, for basal gene expression, Equation 2 and 3 become

$$\frac{dmRNA(t)}{dt} = \beta_0' - \alpha mRNA(t) \tag{39}$$

$$\frac{dprotein(t)}{dt} = \sigma mRNA(t) - \mu protein(t) \tag{40}$$

It must be noted that $mRNA(t)$ and $protein(t)$ cannot be dimensionless to eliminate the parameters σ and μ, respectively. Therefore, the steady-state equations for basal gene expression are

$$\beta_0' - \alpha mRNA(t) = 0, \tag{41}$$

$$\sigma mRNA(t) - \mu protein(t) = 0. \tag{42}$$

i.e.

$$mRNA_{ss} = \frac{\beta_0'}{\alpha}, \quad protein_{ss} = \frac{\sigma}{\mu} mRNA_{ss}, \tag{43}$$

where $mRNA_{ss}$ and $protein_{ss}$ represent the concentrations of basal mRNA and protein at a steady state, respectively. The symbols and definitions used are listed in Table 2. Therefore, for the basal gene expression system, the absolute levels of protein expression at steady state are determined by the rate constant of translation and degradation and mRNA levels, respectively, which agrees with previous results (9-12, 16).

The gene expression dynamics were random and complex. The Hill-type equation reflects the principle of the deep simplicity of gene expression dynamics. The history of mechanics, physics, and chemistry indicates that the essence of nature is simple. Complex phenomena have evolved on the basis of the principle of simplicity. According to the regulation principle revealed by this equation, we can control gene expression from upstream of the genetic information flow described by the central dogma. Only four parameters need to be adjusted, namely, the duration, frequency, and amplitude of TF and binding affinity, and we can control the steady-state levels of mRNA and protein expression.

Because the levels of mRNA and protein expression at steady state are equal, a large amount of transcriptome data has been accumulated over the years; therefore,

using this equation, we may predict the proteome simply by the transcriptome.

The classical Hill equation is applied when the TF dynamics are constant. When $\Delta = T$ is applied, the equation is reduced to the classical Hill equation: Therefore, this equation broadens the application of classic biochemical theory. Through the derivation process, this generalized equation, which reveals the regulatory mechanism of gene expression, can be applied to any activated gene expression pathway driven by TF dynamics.

Hill wrote: "My object was rather to see whether an equation of this type can satisfy all the observations, than to base any direct physical meaning on n and K (35, 36)." More than 100 years later, we have not forgotten his caveat (36).

Table2. Variable and parameter definitions

Symbol	definition	Units
A	Amplitude of p53 pulses, or sustained constant p53 dynamics	nM
n	Hill coefficient	-
Δ	Duration of p53 pulses	h
T	Period of p53 pulses	h
K_A	Dissociation constant	nM
$P(t)$	Protein fold change	-
$mRNA(t)$	mRNA concentration	nM
$protein(t)$	Protein concentration	nM
$m_i(t)$	mRNA fold change during the i-th pulse	-
$m(t)$	mRNA fold change	-
$m_{ss}(\xi)$	mRNA fold change at steady state	-
$m_{ss,\max}$	Maximal mRNA fold change at steady state	-
$m_{ss,\min}$	Minimal mRNA fold change at steady state	-
m_d	Net mRNA fold change at steady state under sustained p53	-
$P_{ss}(\xi)$	Protein fold change at steady state	-
$P_{ss,\max}$	Maximal protein fold change at steady state	-
$P_{ss,\min}$	Minimal protein fold change at steady state	-
$\overline{P_i}$	Average protein fold change over the i-th pulse	-
$\overline{m}$	Average mRNA fold change at steady state	-
$\overline{P}$	Average protein fold change at steady state	-
m_0	Basal mRNA concentration	nM
p_0	Basal protein concentration	nM
$mRNA_{ss}$	Basal mRNA concentration at steady state	nM
$protein_{ss}$	Basal protein concentration at steady state	nM
β	Maximal mRNA fold change	-

β'	Maximal transcription rate	nMh^{-1}
β'_0	Basal transcription rate	nMh^{-1}
τ_{sus}	Relaxation time to steady state under sustained p53	h
τ_{pulsed}	Relaxation time to steady state under p53 pulsing	h
α	Rate constant of mRNA decay	h^{-1}
σ	Rate constant of protein translation	h^{-1}
μ	Rate constant of protein degradation	h^{-1}
$P_{1,\max}$	Maximal protein fold change during the 1-th pulse	-
λ	Index of protein accumulation	-
ρ	Peak to valley ratio	-
γ	Duty cycle of p53 pulsing	-

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data Availability. Data used in this article come from [17, 23-26, 28, 30, 31] or are included in the text.

Author contributions. X.S.: conceptualization, formal analysis, investigation, methodology, writing—original draft, writing—review, and editing.

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