Resource competition in three gene motif causes emergence of Feed-forward response

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Abstract. One of the major reason behind failure of the experimental synthetic circuit implementation is ignoring the hidden couplings in gene regulatory dynamics and its consequences. Dependency of the components of a genetic circuit upon its host, due to requirement for resources like ribosome, ATP, transcription factors, tRNA etc., and related effects are of utmost importance. Several times these dependencies are capable of giving rise to unexpected emergent responses. In a resource-limited environment, two apparently unconnected genes can compete for resources for their respective expression and may exhibit indirect regulatory connection; an emergent response thus arises in the system completely because of resource competition. In this work, we have shown how the responses of Feed-Forward loop (FFL), a well-studied regulatory genetic motif, can get recreated considering the resource competition in a three-gene pathway. The chosen motifs show many of the characteristic features of the conventional FFL structure, like response delay and pulse generation. This study pinpoints towards a larger area of research and exploration in synthetic and cellular systems which will reveal novel controlling ideas and unique behavioral changes in the system for its context dependencies.

Keywords: Gene regulation, Resource-driven competition, Feed-Forward loop, Quantitative modeling.

1. Introduction

Living cells carry some re-occurring genetic patterns called motifs which are responsible for some key functions of cell physiology [1] [2]. Focusing on these motifs, synthetic biologists have designed models, first mathematically and then experimentally, to understand the nature of the dynamics in whole-cell or organism scenario. The synthetically constructed gene arrangements, often referred as the circuits, are implemented in living cell, or the host for operating with particular feature tasks. Interestingly the dynamics of the host and circuits often get coupled, most of the time in a nonlinear fashion. Functionality of the implemented synthetic circuit depends
Resource competition in three gene motif causes emergence of Feed-forward response

solely upon its host’s physiology, host’s growth and replication, operating temperature, pH, binding specificities and several other factors. Recent studies, provides sufficient information that host’s physiology can affect, modulate or modify the circuit response not only in a low scale but also in a high scale. The output response can be moderated in a substantial scale or deviated from expected output in a low scale change, and in a high scale modulation, the output response is completely out of sink, giving rise to a new response or even a complete failure of the experiment [3, 4, 5]. Limited knowledge about inter-cellular dynamics may restrict us in explaining these emergent responses, arising mainly because of the nonlinear couplings between the host and the circuit. From last decade, researchers have started focusing on this host-circuit coupling dynamics and gaining insightful results which in long term helps in designing robust and complex synthetic circuit also.

One of the most important reasons of this influence of cellular context, is the dependency of the circuit on the host for the resources required for its gene expression. Here, by resource, we mean the cellular ingredients like RNAP, ribosome, ATP, protein degradation machinery [6, 7], transcription factors etc. that are supplied by the host cell for the gene expression process. Any gene circuit, endogenous or synthetic, uses the resources from the host cell for their respective gene expression. Experiments prove that these resources are present in a limited manner inside cell [8, 9]. Recent experiments establish that the amount of available functional RNAP in the cell limits the transcription process majorly [10]. The effect of transcription factor sharing and its copy number in gene expression process is shown in [11] and it has been established that in isogenic population of cells, this resource sharing enhances noise in the process of mRNA distribution [12]. These works indicate that as long as the demand of resource is low and supply exceeds the demand, a gene circuit functions as expected. But for a complex network where the resource demand is high, or the circuit is functioning at a higher production regime, the resource supply might be insufficient compared to the demand. Under this circumstances, the circuit can develop unprecedented competition with other genes that are gathering resources from the same local pool.

Recently, to capture cellular resource sharing mathematically, models have been developed by researchers considering different approaches (e.g., resources as variable source, resource availability as a function of cell growth rate etc.) [13, 14, 8, 15]. Georgy et al. in their study provides an experimental evidence of cellular economy and proteins showing isocost like expression, while operating in a tight budget of ribosomes [16]. Recent study in this field reported major changes in toggle functionality as a consequence of resource competition [17, 18]. Theoretical models on competition of canonical and alternative sigma factors for RNAP in the steps of transcription initiation [19] and transcription elongation [20] shows bacterial responses on environmental fluctuations. Resource competition, specially ribosome competition effects in the protein production curve and insightful results in amplification and sensitivity modulation of proteins has been also reported [21] using mathematical models. The effect of sharing degradation machinery and protease class of proteins is shown in [7] where the system shows emergent responses as a consequence of competition.

In this work, we attempt to establish that resource distribution and competition has the capability to regulate the local and global dynamics of the system. When not taken into consideration, the outcome seems unpredictable and drastically different from the expected response. We consider a motif that apparently, does not bear any resemblance with the well-known motif, Feed-Forward Loop (FFL); however, due to
Resource competition in three gene motif causes emergence of Feed-forward response

resource competition interesting responses can be observed similar to the FFL motif. Here, we show that the inherent structure of the FFL circuit are compensated by a limited pool of availability, the resource competition plays similar role like an indirect repression. We report that the selected motifs behave like conventional FFL motifs and the unique responses of FFL motifs like response delay, pulse formation etc. are found to be carried out by resource driven motifs as well. In section 2 we have briefly discussed about conventional basic structure of FFL motifs. In section 3 we have discussed about the selected three gene motifs regulated by resource competition. In section 4 we discuss the output responses giving rise to FFL-like behavior. Finally we conclude with some relevant discussion in section 5 respectively.

2. Feed-forward loop motif.

In bacterial physiology, presence of the three gene motif known as Feed-Forward Loop (FFL) is found to modulate cellular dynamics of Yeast and E.coli very prominently. FFL motif is one of the most abundantly found motif in nature where three genes having their unique pattern of regulation (activation or repression) gives rise to coherent and incoherent motifs as shown in Fig. 1. In a three gene motif (say \(X, Y\) and \(Z\)), one regulating the next in series (i.e \(X\) regulating \(Y\), \(Y\) is regulating \(Z\)) and also the first gene (say \(X\)) is regulating the third gene (say \(Z\)) in a direct fashion. Distinctive resultant behaviour in protein synthesis, like response acceleration, response delay, pulse formation etc. can be achieved by these regulatory motifs.

Depending upon these mode of regulations (activation or repression) FFL motifs are conventionally classified in two groups, each containing four motifs, namely coherent FFL and incoherent FFL motifs as shown in Fig. 1. In coherent type FFL motifs, the direct regulation hand of \(X \rightarrow Z\) is in harmony with the indirect regulation hand \((X\) regulating \(Z\) via \(Y\)); these two are of opposite regulation in case of incoherent motifs. Some of these motifs arise abundantly in bacterial physiology while some of these are arising less frequently. Presence of further AND gate logic and OR gate logic specifies either both the direct and indirect regulation in a combined way regulates \(Z\) production (AND logic) or any one of this regulation is sufficient to initiate \(Z\) regulation (OR logic).

In convention, an activator say \(S_x\) and \(S_y\) activates the proteins. Presence of activator, that is when \(S_x = 1\), the first protein is in active state \(X\), and in absence of activator, \(S_x = 0\), implies \(X = 0\) and similar for \(Y\) (In the experimentally verified ara system, \(X = CRP, Y = araC, Z = araBAD, S_x = cAMP\) and \(S_y = L-arabinose\) [22].

Concentration of \(Y\) and \(Z\) can be represented by the set of equation, in a constitutive production of \(X\):

\[
\frac{dY}{dt} = B_y + \beta_y f(X, K_{xy}) - Y \delta_y
\]

\[
\frac{dZ}{dt} = B_z + \beta_z G(X, K_{xz}, Y, K_{yz}) - Z \delta_z
\]

(1)

Where \(\beta_i, (i \in \{y, z\})\) is the maximum production rate and \(K_{ij}\) \((i, j \in \{x, y, z\}, i \neq j)\) is the activation or repression coefficient, signifies the activation on gene \(i\) by transcription factor \(j\). The AND gate function is represented by,

\[
G = f(X, K_{xz}) \ f(Y, K_{yz})
\]
where the activator function is given by \( f(u, k) = \frac{(\frac{u}{k})^n}{1 + (\frac{u}{k})^n} \) and repression function is represented by \( f(u, k) = \frac{1}{1 + (\frac{u}{k})^n} \). \( B_y \) and \( \delta_y \) are the basal transcription rate and total degradation rate of \( Y \) respectively, which includes the total dilution and degradation rates of \( Y \) in cell. \( B_z \) and \( \delta_z \) represents same for \( Z \). \( n \) is co-operativity which accounts for the multimer formation of proteins.

Existence of this motif in yeast [2], *C. elegans* [22], *B. subtilis* [23, 24], Sea urchin [23], *E. coli* [23, 22], fruit fly [23], human [25] and in many more diverse organisms are already seen. Instead of having this diversity, all two gene input circuits are not a FFL motif. As example, in *E. coli* nearly 40% two input operons are found to participate as FFL [2]. In search of the reason for this, it is observed that the mutation in the binding sites of promoters can change the regulation arrows, even to the extent of removing it completely, resulting no more regulatory linkage in them. Thus \( X \) to \( Y \) regulation arrow is absent in some motifs which are referred as simple motifs (Fig. 2c) [26], and compared with FFL motif (Fig. 1) to explain its unique regulatory behavior. Conventionally \( X \rightarrow Z \) and \( Y \rightarrow Z \) these two regulations are considered as essential to maintain AND gate regulation and it can be said that the presence or absence of \( X \rightarrow Y \) is the key factor that differentiates FFL like motif and simple regulation motif. Mutation in gene dynamics is sometime biased by the preferences of bio-chemical reactions but majorly it is a random process [27]. It is possible that in some mutation the \( X \rightarrow Z \) disappears leaving the rest of the wiring pattern intact. In this scenario, if the two proteins develop a resource competition with asymmetric resource affinity, an effective repression will come into picture. In this paper, we have carefully chosen some three gene motifs where certain conventional direct regulation, more precisely repression (i.e. Hill function type repression which includes co-operativity with \( n = 2 \) via dimer formation, as shown in Fig. 2a), can be replaced by this resource competition scenario as shown in Fig. 2b. We have also considered resource driven simple regulation model in Fig. 2d which will be further used for evaluating the performance of resource driven FFL motif.

3. Model Formulation

In our resource sharing model we focus especially on ribosome competition in the step of translation process. To illustrate this process, we can take the example of *yeast* where approximately 60000 mRNA molecule starts translating parallelly [28, 29], while available ribosome (which is limited, nearly 240000 in *yeast*) possibly scans the same transcript simultaneously. Now, if any one mRNA starts accommodating ribosome with higher binding affinity, others translation initiation will be delayed (as the total supplier pool is getting affected) and suppressed as a result. Considering this circumstances, let us consider a simple pathway where \( X \rightarrow Y \rightarrow Z \) (\( X \) represses \( Y \), \( Y \) activates \( Z \)). Suppose mRNAs for \( X \) and \( Z \) are involved in such a resource competition here. If \( X \) recruits ribosomes more efficiently for its production, \( Z \) will suffer a deficiency, resulting into a repression in terms of resource. The intention behind choosing this particular architecture is to erase the \( X \rightarrow Z \) direct regulation (repression, for coherent type 2 FFL) and introduce resource competition instead. This will complete the resource driven FFL (rFFL) motif where \( X \) represses \( Z \) via resource competition. Before implementing mathematical equations, let us elaborate our considerations below:
Resource competition in three gene motif causes emergence of Feed-forward response

Figure 1. Feed-Forward Loop model motifs. Arrow head symbols represents activation, hammer-head symbol represents repression. There are 4 coherent motifs and 4 incoherent motifs conventionally.

- We consider ribosome is distributed over small several cytoplasmic compartments in cell. The limited presence of this translational resource in protein production is verified experimentally in some recent work [10]. We focus in the local resource pool here, present in the immediate vicinity of circuit of interest, which captures the circuit dynamics in a realistic way. Let \( T \) represents the pool of ribosome, available for translation for its neighbourhood genes.

- The pool of mRNA, as a result of transcription are respectively \( g_x \) and \( g_z \), which are ready to be translated into proteins \( X \) and \( Z \). Being expressed in the local field of cytoplasm, we consider both \( X \) and \( Z \) are collecting resource ribosomes from the same pool \( T \).
Resource competition in three gene motif causes emergence of Feed-forward response

Figure 2. (a). Selected FFL motif among all motifs where X to Z regulation is of repression type. X to Z dotted line represents these repressions can be replaced by resource competition for emergent resource driven repression in these motifs. Arrowhead symbols represents activation and hammerhead symbols represents repression. Activations will be replaced by repression according to motif requirement in (b), (c), (d) figures. (b). Representing schematic diagram of proposed resource competition model. X and Z are collecting resource from the same pool T, with affinities respectively \( res_x \) and \( res_z \). (c). Simple regulation motif of conventional FFL motif structure. X and Y are regulating Z but there is no regulation of X to Y. (d). Resource competition regulated simple motif structure.
- The mRNA copies, which are ready for translation makes a ribosome bound complex in a step and get translated in next step. The small sub-unit of ribosome binds to three initiation factors IF1, IF2, IF3 along with a methionine-carrying tRNA first, then binds to mRNA and forms the complex. Let, \( c_x \) and \( c_z \) represents ribosome bound complex of \( X \) and \( Z \) respectively. Now from available total free pool \( T \), \( c_x \) and \( c_z \) represents the bound complex, further free ribosome thus available for translation is given by \((T - c_x - c_z)\).

- mRNA binds with ribosomes with certain affinity. Let us consider \( \text{res}_x \) and \( \text{res}_z \) represents the resource affinity for \( X \) and \( Z \) mRNA respectively. This affinity for resource allocation depends upon various factors. The accessibility of the ribosome binding site on the mRNA significantly determines the basal translation level [30, 31], while Polycistronic mRNA pool contains a multiple ribosome binding sites (RBS) in most of the bacterial organisms [32]. The recruitment of ribosome to this RBS are temperature dependent. Temperature fluctuation induces re-folding of the mRNA which interacts with proteins and regulates the synthesis level in selective cases [33]. The nature of the environmental legands also modulates this ribosome recruitment. Thus resource affinity \( \text{res}_x \) and \( \text{res}_z \) can be taken as different taking care of all these biological factors.

- Protein is produced from respective complex at a certain rate, \( \epsilon_x \) and \( \epsilon_z \) respectively for \( X \) and \( Z \).

- \( \delta_x, \delta_y, \delta_z, \delta c_x, \delta c_z \) represents the overall degradation rates which accounts for the dilution and degradation inside cell for respectively protein \( X, Y, Z \) and complex \( c_x \) and \( c_z \).

- We achieve conventional AND gate logic of FFL, where both the direct regulation of \( X \) to \( Z \) hand, along with the indirect regulation hand of \( Z \) regulation via \( Y \) acts combinely as electronic AND gate logic, by multiplying \( c_z \epsilon_z \) ( \( Z \) production term from its respective complex) with \( Y \) regulatory term.

- In [26] a step like behavior of \( X \) induced by \( S_x \) was considered. The same is achieved by allowing \( X \) to produce from its complex for a time period say \( t = 0 \) to \( 10 \) when \( S_x = 1 \). For our model, we consider \( S_x = 0 \) blocks the complex formation \( c_x \) at \( t = 10 \), thus the protein \( X \) is allowed to decay sharply, giving a nearly step like production of \( X \) wrt. time \( t \).

- Respective resource driven simple regulation model is represented by the same equation of \( Z \) with \( Y \) constitutively expressed, \( Y = 1 \). As mentioned before a schematic diagram of resource driven simple regulation model is shown in Fig. [24].

Mathematical modelling for the type 2 coherent FFL motif by our proposed resource competition model where \( X \) to \( Z \) repression arises due to resource competition is given by Eq. 2

\[
\begin{align*}
\frac{dc_x}{dt} &= \text{res}_x \left( T - c_x - c_z \right) g_x - c_x \delta c_x \\
\frac{dc_z}{dt} &= \text{res}_z \left( T - c_x - c_z \right) g_z - c_z \delta c_z \\
\frac{dX}{dt} &= c_x \epsilon_x - X \delta_x \\
\frac{dY}{dt} &= B_y + \beta_y \frac{1}{1 + \left( \frac{X}{X_{yy}} \right)^n} - Y \delta_y
\end{align*}
\]
Resource competition in three gene motif causes emergence of Feed-forward response

\[ \frac{dZ}{dt} = B_z + \beta_z c_z \left( \frac{Y}{\gamma_z} \right)^n - Z \delta_z \]

Following similar arguments, we re-create resource driven FFL (rFFL) capable of mimicking coherent type 3, incoherent type 2 and incoherent type 3 FFL motif. The reason behind choosing these four motifs is that in all these architectures \( X \) to \( Z \) competition is a repression.

4. Results

4.1. Behavior of Coherent type 2 rFFL motif:

We analyse the response of \( Z \) upon step-like addition of inducer \( S_x \), in presence of \( Y \) in our AND logic rFFL coherent type 2 motif. At time \( t = 0 \), \( X \) starts producing from its complex \( c_x \) by collecting resource ribosome with affinity \( res_x \) from the pool \( T \). At the same time \( Z \) also started to produce its complex \( c_z \) and thus allocating resource at a rate of \( res_z \). With the higher value of \( res_x \) (\( res_x > res_z \)), \( X \) is allocating more resources from fixed pool \( T \) than \( Z \). Thus availability of resource for \( Z \) production decreases, causing lower production of \( Z \). This puts an effective repression on \( Z \) as \( X \) gets produced at the cost of \( Z \). Moreover, \( X \) also repress \( Y \), the activator of \( Z \) production. Thus \( Z \) is low throughout the active \( X \) state. At \( t = 10 \), following the conventional FFL motifs we make \( S_x \), the inducer of \( X \) production zero. This blocks the complex of \( X \) production, \( c_x = 0 \). As soon as \( c_x \) production stops \( X \) drastically falls to zero and we investigate the pattern of \( Z \) formation here. For reference, we put the nature of AND logic conventional FFL (cFFL) of coherent type 2 behavior in Fig. 3c.

Now we compare rFFL coherent type 2 with corresponding simple motif (Fig. 2d). With respect to the resource driven simple motif, where \( X \) to \( Z \) resource competition and \( Y \) to \( Z \) regulation works separately, we find the rFFL like structure shows delay in reaching \( Z \) steady state as shown in Fig. 3a. The response time of protein \( Z \), defined as the time to reach 50% of its final concentration [34, 35] is greater in rFFL motif than that of corresponding simple motif. Also the variation in resource allocation rates, which determines the efficiency of collecting resource for the production of the protein significantly modulates the delay here as shown in Fig. 3b. The red line of \( X \) shows the behavior of protein production kinetics with respect to \( S_x \) on step at \( t = 0 \), and \( S_x \) off state at \( t = 10 \). \( res_x = 1, res_z = 0.05 \) the green representative of protein \( Z \) is delayed wrt. \( res_x = res_z = 1 \) the blue line. Similar to the cFFL motifs [20] \( Z \) goes on with \( X \) off, that is output is inverted in nature. This is easily explainable as \( X \) goes off, repression upon \( Z \) via resource and repression upon activator of \( Z \), that is repression on \( Y \) both vanishes. Thus \( Y \) activates \( Z \) and \( Z \) production increases making it on for \( S_x \) off state.

Steady state logic of \( Z \) is both sensitive to \( S_x \) and \( S_y \) as similar to cFFL motif. Putting \( Y \) in off state by \( S_y = 0 \), \( Z \) steady state goes off.

4.2. Key characteristics features of conventional FFL demonstrated by rFFL:

- **\( S_x \) off state delay in Coherent rFFL motif**: \( Z \) steady state state is delayed in coherent type 2 and type 3 rFFL motif wrt. the corresponding resource driven simple regulation motif in \( S_x \) off state. The behavior is similar to the conventional motif responses [26]. Additionally, here the variation in resource affinity value
Resource competition in three gene motif causes emergence of Feed-forward response

Figure 3. Comparative analysis of Kinetic behavior of coherent type 2 rFFL motif and cFFL motif in AND gate logic. (a). Resource driven motif. Red line represents X. The green and the blue line represents Z in rFFL and resource driven simple logic respectively. Note that wrt. blue line (the resource driven simple logic), the green curve (rFFL logic) shows delay in reaching the steady state. (b). Variation in resource affinity affects the delay in reaching the steady state of Z. Red line is for X. Blue line is for Z, res_x = 1, res_z = 1. Green line is for Z, when res_x = 1, res_z = 0. (c). Kinetics of coherent type 2 cFFL AND gate motif. k_{xy} = 0.1, k_{yz} = 1, k_{xz} = 1, n = 2. Red line is for X. Green line shows nature of Z in FFL motif response, blue line is for corresponding simple regulation model. \beta_y = \beta_z = 1, \delta_x = \delta_y = \delta_z = 1, \delta_{cx} = 1, \delta_{cz} = 1, \epsilon_x = 1, \epsilon_z = 1, g_x = 5, g_z = 5, T = 10, B_y = B_z = 0 for both (a) and (b).

regulates the delay response significantly (Fig. 3b, Fig. 4b).

As explained earlier, from \( t = 0 \) to \( t = 10 \) the resource driven repression of Z via X is on; along with for coherent type 2, X repress the activator (Y) of Z (for coherent type 3, X activates the repressor of Z). So with respect to resource driven simple motif where X to Z is repression and Y activates Z, in rFFL motif Y level is low due to presence of repression on Y via X. At \( t = 10 \), when the repression is withdrawn from Z, the level rises and becomes steady in a higher level. Also a delay is seen in reaching the steady state of Z for coherent type 2 rFFL logic as the activator Y level is low here thus further Z production is delayed (similar logic applicable for rFFL coherent type 3 motif) when compared with resource driven simple motif. These behaviors are similar to the cFFL responses.

- **Steady state logic of Z is dependent on S_x in Coherent rFFL**: Steady state logic of Z is inverted with \( S_x \) in coherent type 2 and coherent type 3 rFFL motif, that is Z goes on in \( S_x \) off step. As mentioned earlier, \( S_x \) off state releases the repression on Z both in terms of resource and indirect regulatory repression via Y in both coherent type 2 and 3 rFFL motif, thus Z production increases making Z response inverted with \( S_x \).

- **Steady state of Z is dependent on S_y in Coherent type 2 rFFL motif but not in Coherent type 3 rFFL**: The steady state of Z responds strongly to \( S_y \) in case of coherent type 2 rFFL and the inverted output nature is lost when \( S_y = 0 \) (at \( S_x = 0, S_y = 1 \) the Z steady state is inverted in nature). But the steady state of Z is not dependent on \( S_y \) in case of coherent type 3 rFFL motif. Both these behaviors are similar to the conventional motif responses.

- **Pulse generation in S_x off state of Incoherent rFFL motifs**: Incoherent type 2 and 3 cFFL model motif shows pulse formation in \( S_x \) off state for AND gate logic. Our AND logic rFFL model shows similar results in output as shown in Fig. 5a and 5c: X and Z are collecting resource from the pool \( T \), and thus X is putting
Resource competition in three gene motif causes emergence of Feed-forward response

Figure 4. Comparative analysis of Kinetic behavior of coherent type 3 rFFL motif and cFFL motif in AND gate logic. (a). Resource driven motif. Red line represents X, green line is for rFFL Z response and blue line is resource driven simple logic. Note that wrt. blue line (the resource driven simple logic), green curve (the rFFL logic) shows delay in reaching the steady state. (b). Variation in resource affinity affects the delay in reaching the steady state of Z. Red line is for X. Blue line is for Z, res_x = 1, res_z = 1. Green line is for Z, when res_x = 1, res_z = 0.1. (c). Kinetics of coherent type 3 cFFL AND gate motif [20]. Red line is for X. Green line shows nature of Z in FFL motif response, blue line is for corresponding simple regulation model. Parameter values are k_{xy} = k_{yz} = k_{xz} = 1, n = 2. For (a), (b) and (c) rest parameters are β_y = β_z = 1, δ_x = δ_y = δ_z = 1 B_y = B_z = 0.

a repression in Z production, strength depending upon resource affinity. Now for incoherent type 3 motif, at t = 10, the complex formation of X, that is in a straightforward way the production of X is blocked and no resource demands for X is valid as well. Thus the available resource pool is now open for Z and the repression in terms of resource is no more. Thus Z production suddenly increases at S_x off state. The wiring pattern shows X activates Y, which is also an activator of Z, thus as X = 0 now this X can’t activate Y, eventually Y production decreases and further Z production decreases as Y is not produced enough so it can’t activate Z, thus Z decays eventually. Thus Z shows a pulse in output as the sudden increase in production and eventually dies out. Similarly the pulse formation in Incoherent type 2 rFFL motif can be explained.

• No pulse is created in S_x on state of Incoherent rFFL motifs: The incoherent type 2 and 3 cFFL do not generate pulse in response to S_x on step. The resource driven motifs are showing similar results (Fig. 5a and 5c).

• Steady state behavior of Incoherent rFFL with no basal activity, S_y effect: Steady state logic of incoherent type 2 rFFL motif is found to depend on S_y. In presence of S_y, Z creates a pulse and then comes down to low state eventually, while in absence of S_y the steady state is high and no pulse is created. But type 3 incoherent rFFL motif has a constant steady state 0, which do not depends upon S_y. These behaviors are similar to the cFFL behaviors as well.

• S_y effect in pulse generation in rFFL Incoherent motifs: Similar to the cFFL motif our resource driven motif also gives similar result in case of incoherent type 2 rFFL motif. Here, Z shows pulse in output when S_y is on, but Z is high and steady when S_y is off. In incoherent type 3 rFFL motif, Z shows no pulse in S_y off state.
Resource competition in three gene motif causes emergence of Feed-forward response

Figure 5. Pulse formation in AND logic rFFL incoherent motifs and comparative cFFL motif output response. Red line is for X, blue is for Z in simple regulation system, green is for Z in FFL motif response output. (a) Resource driven incoherent type 3 motif. \( k_{xy} = 0.1, k_{yz} = 0.1, \text{res}_x = 1, \text{res}_z = 0.5 \). A pulse is seen in the S\(_x\) off state for Z response. (b) Incoherent type 3 cFFL motif. \( k_{xy} = 1, k_{yz} = 0.5, k_{xz} = 0.5 \). (c) Pulse formation in Incoherent type 2 rFFL motif. Parameter values are \( k_{xy} = 0.1, k_{yz} = 0.1, \text{res}_x = 1, \text{res}_z = 0.5 \). For both (a) and (c) \( n = 2, T = 10, \delta c_x = 1, \delta c_z = 1, \epsilon_x = 1, \epsilon_z = 1, g_x = 5, g_z = 5, B_y = B_z = 0, \beta_y = \beta_z = 1 \).

5. Conclusion and Future directions

Emergent responses in biological circuits as a consequence of context dependency is drawing the attention of research community in recent past \[4, 36, 17, 21\]. Among these several context dependencies, ribosome limitation is a major controlling factor in gene expression dynamics. Different processes, which are apparently not connected, gets coupled implicitly due to limited presence of this essential translational resource in the neighboring cellular environment. In this work, we have taken some commonly occurring motifs, specifically three gene patterns, which are quite different from conventional FFL structures as a repressive regulation is absent. This absences of this regulatory arm can arise from mutation in the system, which is a very random stochastic yet unavoidable change in genome structure. In human, average 175 mutations per diploid genome per generation (i.e., average mutation rate of \( 2.5 \times 10^{-8} \)) is noted \[37\], while in E. coli the average mutation rate is \( 2.1 \times 10^{-7} \) per gene per generation \[38\]. The selected motifs shows FFL-like response when driven by resource competition, in a resource-limited cellular environment. An emergent repression arising from the context dependency, more precisely two mRNAs competing for translational ribosome, fulfills the repression condition in the chosen motifs, and the fundamental functional responses of conventional FFLs, like response delay, pulse generation, dependency of steady sates upon inducers etc., are achieved in proposed rFFL architectures. Acceleration or delay in response is depicted in FFL motif due to its unique construction, and the same is achieved in rFFL, solely caused by sharing resource from common ribosome pool. This nonlinear coupling between the host and the circuit can modulate the dynamics of the entire system significantly. Our work not only depicts the possibilities of vast modification in gene circuit response due to resource limitation, but also proposes an emergent architecture for one of the most common genetic motifs, Feed-forward loops.

It is important to note that our considerations are only valid for a resource limited, low growth system. Growth of the system is directly linked with the number of active ribosomes participated in translation and thus with the biomass of the system.
Resource competition in three gene motif causes emergence of Feed-forward response

Cellular macromolecular composition could be highly correlated with cell growth [39], and further investigations can be planned considering this factor in a future work. Moreover, biological processes mostly take place in a noisy environment. In a recent work on the noise characteristics of FFL [40], the relation between functionality and abundance has been suggested, keeping the noise factor in mind. Deterministic and stochastic characteristics of functionality, dynamics and response of the proposed rFFL motifs can also elaborately studied, to develop further understanding on complex synthetic circuit operation in diverse host cells.

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