Disentangling the cost of gene expression

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Gene expression is essential for biological functions but also incurs a fitness cost, measured by the relative reduction in growth rate. However, the limiting factors generating the fitness cost remain elusive due to the complexity of cell growth. In this work, we disentangle the fitness cost into components generated by different limiting resources based on a resource competition model. We derive analytical formulas connecting the fitness cost to the copy numbers of the ribosome, RNA polymerase, and transcription factor. Comparing our model predictions with experimental data of *Saccharomyces cerevisiae*, we demonstrate that the competition for ribosomes dominates the translation cost and the competition for transcription factors dominates the transcription cost. The model also systematically connects the fitness cost to genetic and environmental properties, making predictions quantitatively consistent with various experimental observations. Our work builds a systematic framework for gene expression cost, guiding synthetic biology to optimize genetic design.

INTRODUCTION

Gene expression is the most fundamental cellular process, where genes are transcribed into mRNAs, and mRNAs are translated into proteins critical for a cell's viability. However, gene expression also exerts a fitness cost, e.g., the expression of an unnecessary exogenous gene slows down the growth of the host cell [1–8]. Despite its fundamental importance in the evolution of gene expression regulation, the dominant source of gene expression cost is still elusive. One potential source is the competition for metabolites, e.g., ATP, nucleotides, and amino acids [6, 9, 10]. Nevertheless, due to the complexity of going from numerous biochemical processes to cell growth, theoretical predictions based on metabolic consumption are challenging; furthermore, it cannot explain why the cost associated with mRNA production is comparable to that of protein production [5] considering that the ATP consumption rate of mRNA production is much lower than that of protein production [6, 9]. Another possible source of gene expression cost is the dilution effects, where unnecessary proteins dilute the concentrations of key biomolecules, such as ribosomes and RNA polymerases (RNAPs) [4, 11]. However, the dilution theory cannot relate the cost to the specific properties of the studied gene, which is crucial for synthetic biology. In the meantime, it disagrees with the experimental observations that gene expression cost is generated in the process instead of the products; particularly, the fitness cost is independent of the degradation rate of the exogenous protein [5, 12]. Besides, it is still unclear how the fitness cost can be decomposed into a sum of transcription and translation costs [5].

In this work, we calculate the fitness cost, i.e., the relative growth rate reduction, based on the resource competition scenario. In the simplest scenario, we assume that genes compete for RNA polymerases for transcription and mRNAs compete for ribosomes for translation. We successfully disentangle the fitness cost into two components generated from the competition for RNAPs and ribosomes. Intriguingly, the ribosome cost is simply the relative fraction of ribosomes used by the unnecessary gene. The RNAP cost is the relative fraction of RNAPs used by the unnecessary gene multiplied by the fraction of active ribosomes. Intriguingly, our predicted costs due to ribosome-competition and RNAP-competition agree quantitatively with experimental data of *S. cerevisiae* [5]. Surprisingly, the total measured cost generated by transcription is much higher than the prediction based solely on RNAP competition. By extending our model to include transcription factors (TFs), we show that the cost generated by competition for TFs dominates over the RNAP cost, and the predicted TF cost agrees quantitatively with experiments [5]. Our model quantitatively explains why the cost of mRNA production can be comparable to that of protein production.

RESULTS

Resource competition model of gene expression

We introduce a gene expression model at the genome-wide level, in which genes compete for RNA polymerase (RNAP) to transcribe mRNAs, and mRNAs compete for ribosome to translate proteins (Figure 1) [13, 14]. In eukaryotes, "RNAP" in this work represents RNA polymerase II, which is responsible for eukaryotic mRNA transcription. To simplify without losing the essential results, we treat the endogenous genes as an ensemble of "average" genes so that they have the same binding affinity to RNAPs, and their mRNAs have the same binding affinity to ribosomes. Here, we temporarily neglect the exogenous genes to simplify the equations, which

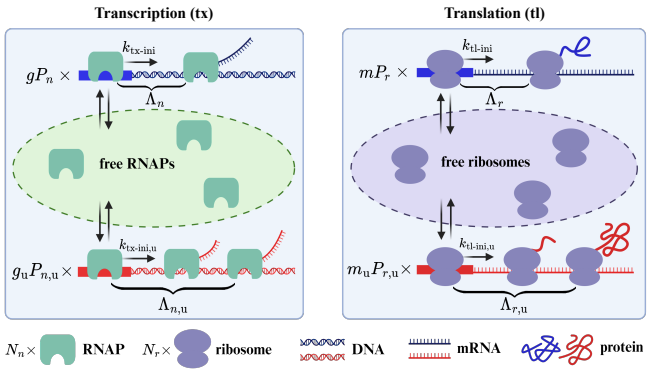

FIG. 1. **Schematic of the resource competition model** The endogenous and exogenous genes compete for the limiting resources of RNAPs and ribosomes. RNAPs can be in three states: initiating (bound to promoters), elongating, and freely diffusing. Their numbers sum up to a fixed total copy number of RNAPs. Endogenous genes (blue) and exogenous genes (red) compete for RNAPs where g and g_{u} are their copy numbers, and P_n and $P_{n,\text{u}}$ are their probabilities for promoters to be bound by RNAPs, respectively. Similarly, endogenous mRNAs (blue) and exogenous mRNAs (red) compete for ribosomes where m and m_{u} are their copy numbers, and P_r and $P_{r,\text{u}}$ are probabilities for their RBSs to be bound by ribosomes, respectively.

can have different binding affinities from the endogenous ones.

At the transcription level, RNAPs can be in three states: initiating RNAPs bound to promoters, elongating RNAPs along the DNA, and freely diffusing RNAPs in the nucleus. The conservation of the total RNAP copy number leads to

$$gP_n\Lambda_n + N_n f_n = N_n. \tag{1}$$

Here, g is the genome size, i.e., the total copy number of endogenous genes, P_n is the probability of a promoter bound by an RNAP, N_n is the total number of total RNAPs, and f_n is the fraction of free RNAPs. Λ_n is the gene capacity of RNAPs, i.e., the maximum number of RNAPs on a single gene copy, including initiating and elongating RNAPs. We model P_n as a Michaelis-Menten (MM) function of the concentration of free RNAPs [15–17]

$$P_n = \frac{[n]_{\text{f}}}{[n]_{\text{f}} + K_n}. \tag{2}$$

Here, $[n]_{\text{f}} = N_n f_n / V_{\text{n}}$ is the concentration of free RNAPs in the nucleus, V_{n} is the nuclear volume, and K_n is the corresponding dissociation constant [15–17]. Combining Eqs. [1, 2], one can find the unique f_n that satisfies the conservation equation.

A similar conservation equation applies to ribosomes:

$$mP_r\Lambda_r + N_r f_r = N_r. \tag{3}$$

Here, m is the copy number of endogenous mRNA molecules, P_r is the probability for the ribosome-binding-site (RBS) of an mRNA to be bound by a ribosome, and Λ_r is the mRNA capacity of ribosomes, i.e., the maximum number of ribosomes on a single mRNA copy. N_r is the total number of ribosomes, and f_r is the fraction of free ribosomes. P_r is a MM function of the free ribosome concentration [14, 18, 19],

$$P_r = \frac{[r]_{\text{f}}}{[r]_{\text{f}} + K_r}. \tag{4}$$

Here, $[r]_{\text{f}} = N_r f_r / V_{\text{c}}$ is the concentration of free ribosomes in the cytoplasm, V_{c} is the cytoplasmic volume, and K_r is the corresponding dissociation constant [13, 14].

The equilibrium of production and degradation sets the total mRNA copy number:

$$m = g\beta_m\tau. \tag{5}$$

Here, $\beta_m = k_{\text{tx-ini}}P_n$ is the mRNA production rate per gene, where $k_{\text{tx-ini}}$ is the transcription initiation rate for an RNAP-bound promoter (number of initiations per unit time), and τ is the mRNA lifetime. The time derivative of endogenous protein mass M is set by production and degradation:

$$\dot{M} = m\beta_p a - dM, \tag{6}$$

where a is the mass of the "average" endogenous protein, and d is the average degradation rate. Here, $\beta_p = k_{\text{tl-ini}}P_r$ is the protein production rate per mRNA, where $k_{\text{tl-ini}}$ is the translation initiation rate for a ribosome-bound RBS. We calculate the growth rate as the time derivative of endogenous protein mass normalized by the endogenous protein mass, $\mu = \dot{M}/M$ so that

$$\mu = \frac{g\beta_m\tau\beta_p a}{M} - d \tag{7}$$

The fitness cost of exogenous unnecessary gene expression

We introduce an exogenous and unnecessary gene to a host cell and seek to compute the fitness cost, defined as the reduction in the growth rate due to the expression of the exogenous gene:

$$C = -\frac{\Delta\mu}{\mu}, \tag{8}$$

In this work, we mostly consider a simplified scenario in which the copy numbers of endogenous proteins and cell volume are unaffected by the produced exogenous proteins. We point out that this scenario may be a good approximation if the produced proteins have a high degradation rate. For non-degradable proteins, the cell volume

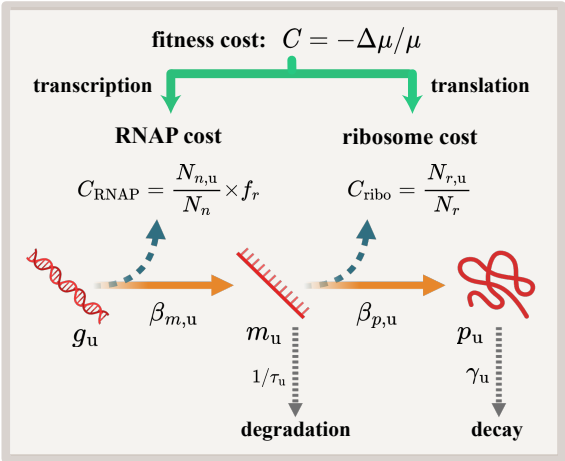

FIG. 2. **The RNAP and ribosome costs of gene expression.** The fitness cost can be disentangled into a ribosome cost and an RNAP cost. The cost due to competition for ribosomes is the fraction of ribosomes used by the exogenous mRNAs. The cost due to competition for RNAPs is the fraction of RNAPs used by the exogenous genes multiplied by the fraction of free ribosomes.

can increase upon the expression of exogenous proteins [4, 5, 20], which may lead to the dilution of key factors such as ribosome and RNAP. On the other hand, cells also make more endogenous proteins to compensate for the dilution effects [5]. Our simplified model postulates that these two opposite effects cancel out and can be neglected, which is justified by the experimental observations that changing the degradation rate of exogenous protein does not affect its fitness cost [5, 12]. We show later that this assumption is supported by experimental data using a more complex model with explicit changes in endogenous proteins and cell volumes.

Intriguingly, the resource competition model predicts that the cost of exogenous gene expression can be explicitly disentangled into a cost due to RNAP competition, i.e., RNAP cost, and a cost due to ribosome competition, i.e., ribosome cost (Figure 2 and the details of the derivation are included in Methods A):

$$C = \underbrace{\frac{N_{n,\mathrm{u}}}{N_n} f_r}_{C_{\mathrm{RNAP}}-\text{RNAP cost}} + \underbrace{\frac{N_{r,\mathrm{u}}}{N_r}}_{C_{\mathrm{ribo}}-\text{ribosome cost}} . \tag{9}$$

Here, $N_{n,\mathrm{u}}$ and $N_{r,\mathrm{u}}$ are the copy numbers of RNAP and ribosome working on the exogenous gene. The above equation demonstrates that the cost of expressing an unnecessary gene is determined by the number of RNAPs and ribosomes they consume. In particular, the RNAP cost is damped by a downstream factor f_r, the fraction of free ribosomes in the total ribosome pool.

Since the mRNA and protein production rates of the exogenous gene are proportional to $N_{n,\mathrm{u}}$, $N_{r,\mathrm{u}}$, respectively, we also express the cost as a function of the produced mRNA and protein copy number, which is more transparent to compare with experimental data

$$C = c_m m_{\mathrm{u}} + c_p p_{\mathrm{u}}, \tag{10}$$

where

$$c_m = \frac{f_r T_{\mathrm{tx,u}}}{N_n \tau_{\mathrm{u}}} \text{ and } c_p = \frac{T_{\mathrm{tl,u}} \gamma_{\mathrm{u}}}{N_r} \tag{11}$$

are the RNAP cost per mRNA and the ribosome cost per protein, respectively. Here, $T_{\mathrm{tx,u}}$ is the total duration for an RNAP to transcribe an exogenous gene, including the duration in the initiation state. $T_{\mathrm{tl,u}}$ is the total duration for a ribosome to translate an exogenous mRNA, including the duration in the initiation state. τ_{u} is the lifetime of the exogenous mRNA, and $\gamma_{\mathrm{u}} = \mu + d_{\mathrm{u}}$ is the decay rate of the exogenous protein. Eq. [10] suggests that cells can lower the gene expression cost by utilizing fewer mRNA copy numbers to produce a given amount of proteins, in agreement with experiments [21, 22].

Limiting transcription factors dominate transcription cost in *Saccharomyces cerevisiae*

In the experiments by Kafri et al., the authors integrated exogenous mCherry genes into *S. cerevisiae* and measured the relative growth rate reduction [5]. To compare with their experiments, we estimate the cost coefficients based on the resource competition model using the relevant parameters of *S. cerevisiae*. Intriguingly, the predicted ribosome cost per gene copy is very close to the measured translation cost ($C_{\mathrm{tl}}/g_{\mathrm{u}}$ in Table I), meaning that ribosome competition is likely the dominant source of translation cost. However, the experimentally measured transcription cost per gene, $C_{\mathrm{tx}}/g_{\mathrm{u}} = 6 \times 10^{-3}$ (Methods B), is much higher than the RNAP cost per gene copy $c_m m_{\mathrm{u}}/g_{\mathrm{u}} = 2 \times 10^{-4}$ (Table I). These results suggest that other limiting factors must exist at the transcription level besides RNAP.

Experiments have shown that protein-burdened cells resemble mutants deleting transcription coactivators, including Mediator, SAGA, and SWI-SNF, both transcriptionally and phenotypically [40]. These results suggest that transcription-initiation-associated proteins may also be limiting factors for transcription. Therefore, we extend our model to include the resource competition for transcription-initiation-associated proteins, including general transcription factors, mediators, and chromatin remodeling proteins [41–43]. For simplicity, we combine all these proteins as a coarse-grained type of TF, and the transcription initiation requires the binding of TF near the promoter (Figure 3a). Therefore, the mRNA production rate also depends on the TF concentration: $\beta_m = k_{\text{tx-ini}} P_t P_n$ where $P_t = \frac{[t]_{\mathrm{f}}}{[t]_{\mathrm{f}} + K_t}$ is the TF binding probability, and K_t is the TF dissociation constant.

TABLE I. **Comparisons between the costs estimated from the experimental data [5] and the resource competition model for an mCherry gene in** *S. cerevisiae***.** Here, we analyze the data of TDH3 promoter in the standard culture. In this table, all variables correspond to one gene copy ($g_\mathrm{u} = 1$) for simplicity. Numerical values are rounded to one significant figure, considering the uncertainties in experiments and estimations. Details of the experimental values are in Methods B. Details of the theoretical estimations are the following:
(1) To estimate $C_\mathrm{ribo} = c_p p_\mathrm{u} = \frac{\gamma_\mathrm{u} T_\mathrm{tl,u}}{N_r} p_u$ (Eqs. [9-11]), we take the following parameters: the protein decay rate $\gamma_\mathrm{u} \approx \mu = 0.4/\mathrm{h}$ [5], the ribosome copy number $N_r = 3 \times 10^5$ [23, 24], and the mCherry protein copy numer for each gene copy $p_\mathrm{u} = 1.5 \times 10^6$ (Methods B). The protein decay rate is dominated by growth rate ($\gamma_\mathrm{u} \approx \mu$) because degradation is much slower compared to cell growth [25–27]. The duration for a ribosome to translate an mCherry mRNA is estimated as $T_\mathrm{tl,u} \approx L_\mathrm{tl,u}/v_\mathrm{tl,u} = \frac{236\ \mathrm{aa}}{10\ \mathrm{aa/s}} = 24$ s, where $L_\mathrm{tl,u}$ is the length of the exogenous mRNA translated in amino acids [28], and $v_\mathrm{tl,u}$ is the translation elongation speed of ribosome [29, 30].
(2) To estimate $C_\mathrm{RNAP} = c_m m_\mathrm{u} = \frac{f_r T_\mathrm{tx,u}}{N_n \tau_\mathrm{u}} m_\mathrm{u}$ (Eqs. [9-11]), we take the following parameters: the fraction of free ribosomes $f_r = 0.3$ [31], the copy number of RNAP $N_n = 3 \times 10^4$ [32], the mRNA lifetime $\tau_\mathrm{u} = 16$ min [33], and the mCherry mRNA copy numer for each gene copy $m_\mathrm{u} = 6 \times 10^2$ (Methods B). The duration for an RNAP to transribe an mCherry gene is estimated as $T_\mathrm{tx,u} \approx L_\mathrm{tx,u}/v_\mathrm{tx,u} = \frac{1000\ \mathrm{nt}}{30\ \mathrm{nt/s}} = 30$ s, where $L_\mathrm{tx,u}$ is the length of the exogenous gene transcribed in nucleotides [5], and $v_\mathrm{tx,u}$ is the transcription elongation speed of RNAP [34–36]. In both (1) and (2), we neglect the duration of initiation, which is typically much shorter than the duration of elongation.
(3) An mCherry gene driven by TDH3 promoter accounts for 3.4% of the total protein number, 200 times more than the contribution of an average gene, which we estimate as $1/g$ with $g = 6000$ [37, 38]. Assuming the primary difference comes from the promoter strength, we take $\beta_{m,\mathrm{u}}/\beta_m = 200$. Since the actual fraction of free transcription factors is unknown, we take $f_t = 0.5$, which does not affect the order of magnitude. We use Eq. [13] to estimate c_g, which is equal to C_TF given $g_\mathrm{u} = 1$. Other parameters are taken as $f_n = 0.9$ [39] and $f_r = 0.3$ [31].
(4) The translation cost C_tl (theory) is equal to the ribosome cost C_ribo.
(5) The transcription cost C_tx (theory) is the sum of the RNAP cost and the TF cost: $C_\mathrm{tx} = C_\mathrm{RNAP} + C_\mathrm{TF}$.

Variable (per gene copy)	C_ribo	C_RNAP	C_TF	C_tl	C_tx
Theory	1×10^{-2}	2×10^{-4}	5×10^{-3}	1×10^{-2}	5×10^{-3}
Experiment	——	——	——	9×10^{-3}	6×10^{-3}

Intriguingly, we find the fitness cost can be explicitly decomposed as (Methods C):

$$C = \underbrace{\frac{N_{t,\mathrm{u}}}{N_t} f_n f_r}_{C_\mathrm{TF}} + \underbrace{\frac{N_{n,\mathrm{u}}}{N_n} f_r}_{C_\mathrm{RNAP}} + \underbrace{\frac{N_{r,\mathrm{u}}}{N_r}}_{C_\mathrm{ribo}} = c_g g_\mathrm{u} + c_m m_\mathrm{u} + c_p p_\mathrm{u}. \tag{12}$$

The transcription cost now consists of two components generated by competition for TF and RNAP: $C_\mathrm{tx} = C_\mathrm{TF} + C_\mathrm{RNAP}$ (Figure 3b). Here, $N_{t,\mathrm{u}}$ is the copy number of TF working on the exogenous gene, and N_t is the total copy number of TF. One should note that the TF cost is damped by the two downstream factors f_n and f_r. We introduce the TF cost per gene as $c_g = \Lambda_{t,\mathrm{u}} P_{t,\mathrm{u}} f_n f_r / N_t$ where $\Lambda_{t,\mathrm{u}}$ is the maximum TF number recruited by an exogenous gene and $P_{t,\mathrm{u}}$ is the corresponding TF binding probability. The competition for TFs means that integrating exogenous genes alone can already impose a cost due to TF competition, even without downstream mRNA production.

Because it is difficult to directly estimate $N_{t,\mathrm{u}}$ and N_t, we take an indirect approach assuming that the TF binding limits the mRNA production rates, that is, the ratio between the mRNA production rates of the exogenous gene and one endogenous gene can be approximated by the corresponding ratio of the copy numbers of TFs working on them, $\frac{\beta_{m,u}}{\beta_m} = \frac{N_{t,u}/g_u}{N_t(1-f_t)/g}$ where f_t is the fraction of free TFs. Given this approximation, we estimate c_g as (Methods C)

$$c_g = \frac{\beta_{m,u}}{\beta_m g}(1 - f_t) f_n f_r. \tag{13}$$

Surprisingly, the estimated TF cost is close to the measured transcription cost (Table I), meaning that the TF cost dominates the total transcription cost. To summarize, our theory successfully decomposes the total fitness cost into different components depending on the limiting factors. In particular, the translation cost is dominated by competition for ribosomes, and the transcription cost is dominated by competition for TFs (Figure 3b).

Effects of gene constructs and growth conditions

We rewrite Eq. [12] as

$$\frac{C}{g_\mathrm{u}} = c_g + \beta_{m,\mathrm{u}} \Big(\frac{T_\mathrm{tx,u}}{N_n} f_r + \frac{T_\mathrm{tl,u}}{N_r} \tau_\mathrm{u} \beta_{p,\mathrm{u}} \Big) \tag{14}$$

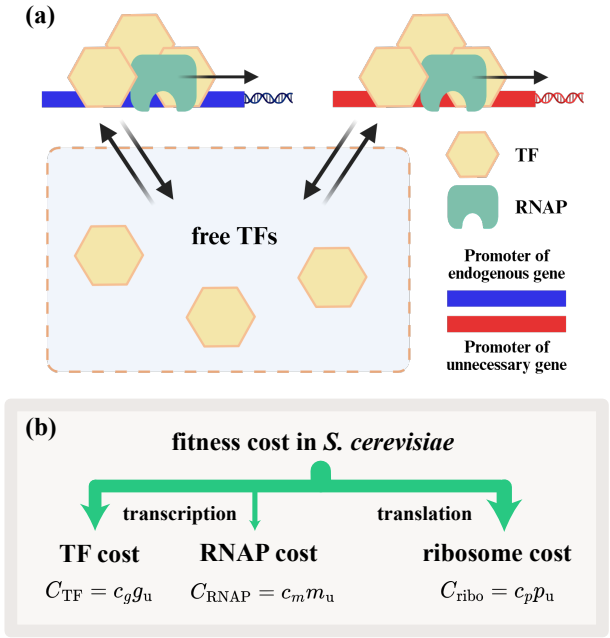

FIG. 3. **The extended model incorporating the TF cost.** (a) Competition for the limiting TFs generates an additional component of transcription cost. (b) The fitness cost in *S. cerevisiae* can be decomposed into a TF cost, an RNAP cost, and a ribosome cost, each proportional to the gene copy number, mRNA copy number, and protein copy number, respectively. The arrow widths represent their relative contributions to the total fitness cost (Table I).

where $\beta_{m,\mathrm{u}}$ is the mRNA production rate per exogenous gene copy and $\beta_{p,\mathrm{u}}$ is the protein production rate per exogenous mRNA copy. Eq. [14] predicts that the cost per gene positively correlates with the mRNA production rate $\beta_{m,\mathrm{u}}$, in agreement with the lower cost for the weaker promoter PGK1 compared with TDH3 (Figure 4a). Eq. [14] predicts that if the produced mRNA has a shorter lifetime, the cost per gene should also be smaller, and this prediction agrees with the lower cost of the DAmP mutant of mCherry mRNA, whose lifetime is about 1/10 of the wild type (Figure 4a). Since the protein decay rate does not enter Eq. [14], we predict the cost per gene is independent of protein stability, validated by experiments showing that the CLN2 variant with much higher protein degradation leads to a similar cost per gene compared with TDH3.

We also calculate the cost per proteome fraction (Supplementary Information Section B), a typical metric often used in experiments for both *S. cerevisiae* and *E. coli* (Table S3):

$$\frac{C}{\phi_{\mathrm{u}}} = \frac{\gamma_{\mathrm{u}} M_{\mathrm{tot}}}{a_{\mathrm{u}}} \left[\frac{1}{\tau_{\mathrm{u}} \beta_{p,\mathrm{u}}} \left(\frac{c_g}{\beta_{m,\mathrm{u}}} + \frac{T_{\mathrm{tx,u}}}{N_n} f_r \right) + \frac{T_{\mathrm{tl,u}}}{N_r} \right], \quad (15)$$

where M_{tot} is the total protein mass, including endogenous and exogenous proteins. ϕ_{u} is the proteome fraction of the unnecessary proteins produced. Interestingly, our model predicts that the gene constructs of lower cost per gene above (smaller $\beta_{m,\mathrm{u}}$ and τ_{u}) have a higher cost per proteome fraction. Meanwhile, the gene construct with unstable proteins should have a higher cost per proteome fraction. All these predictions agree with experiments (Figure 4b).

When the growth condition changes, all the parameters in Eqs. [14-15] should presumably change. Nevertheless, cells are richer in transcriptional resources in the low N condition [31]. Therefore, we predict a smaller c_g and a larger N_n, corresponding to a lower cost per gene and per proteome, in agreement with experiments (Figure 4a-b). Similarly, for the poor medium YPEG, the transcription and translation elongation speeds are presumably slower, leading to a longer $T_{\mathrm{tx,u}}$ and $T_{\mathrm{tl,u}}$ and a higher cost both per gene and per proteome fraction, in agreement with experiments (Figure 4a-b). On the contrary, for the rich medium YPD, the cost per gene and proteome fraction are both lower (Figure 4a-b). We calculate the fold change (FC) of different gene constructs and conditions compared to the reference (TDH3 construct in standard culture), which clearly illustrates that the cost per gene and the cost per proteome move in opposite directions for different gene constructs but move in the same direction when the medium changes (Figure 4c).

Cost generated by the products is insignificant to the fitness

So far, we have considered fixed endogenous protein numbers and cell volume upon the expression of exogenous unnecessary genes, which can be a good approximation if the produced unnecessary protein is quickly degraded. We extend our model to a more general scenario where the endogenous protein copy numbers and cell volume are changeable and obtain the additional fitness cost of the product (C') due to the produced unnecessary protein (Supplementary Information Section C):

$$C' = -\frac{\Delta\phi'_r}{\phi'_r} - 2 f_r \frac{\Delta\phi_{\mathrm{tx}}}{\phi_{\mathrm{tx}}} + f_r \frac{\Delta\eta_{\mathrm{nc}}}{\eta_{\mathrm{nc}}} \quad (16)$$

Here, $\phi'_r = N_r a_r / M$, the ribosome proteome fraction in the endogenous proteins, different from $\phi_r = N_r a_r / M_{\mathrm{tot}}$, the ribosome proteome fraction in the total proteome. ϕ_{tx} is the proteome fraction encoding for proteins contributing to transcription (e.g., RNAP and transcription factors). $\eta_{\mathrm{nc}} = V_{\mathrm{n}}/V_{\mathrm{c}}$ is the volume ratio of nucleus to cytoplasm (N/C ratio). $\Delta\phi'_r$, $\Delta\phi_{\mathrm{tx}}$, and $\Delta\eta_{\mathrm{nc}}$ in Eq. [16] represent the active/passive regulation of translation and transcription resources, and N/C ratio. Interestingly, Eq. [16] illustrates that increasing translation and transcription resources can relieve the cost, but the rise of the nucleus-to-cell volume ratio aggravates the cost.

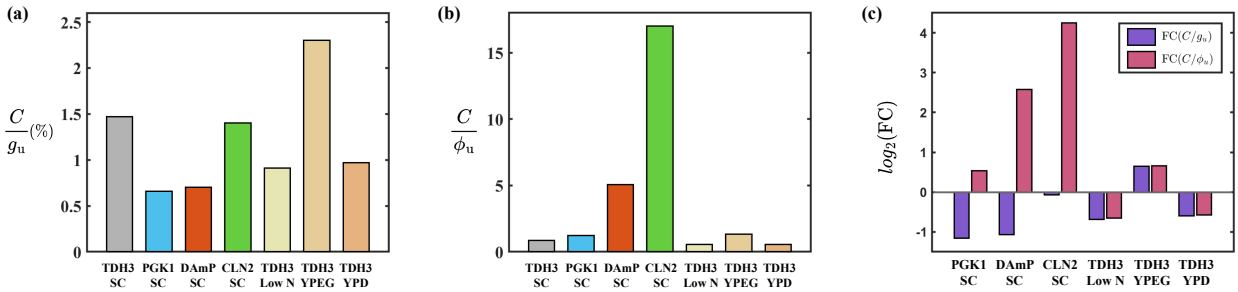

FIG. 4. **The fitness cost of** *S. cerevisiae* **for different gene constructs and growth conditions.** (a) The experimentally measured fitness cost per gene copy number (g_u). PGK1 is a weaker promoter compared to TDH3. DAmP construct has unstable mRNA with a shorter mRNA lifetime. CLN2 construct produces a protein with a high degradation rate. Low N represents a medium lack of nitrogen, where the amount of transcriptional resources is measured to be higher [31]. YPEG medium lacks carbon sources, while YPD is a rich medium with a complete nutrient supply. (b) The experimentally measured fitness cost per proteome fraction. (c) The fold changes (FC) of the cost per gene and per proteome compared to the reference state (TDH3, SC).

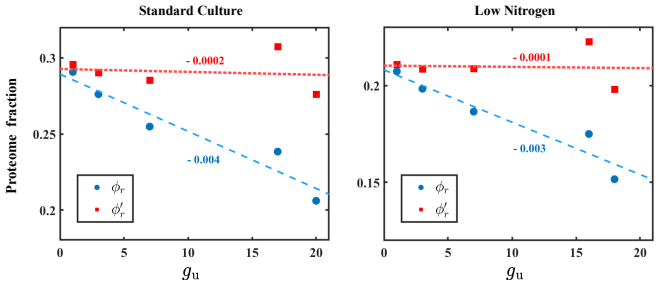

FIG. 5. **The ribosomal proteome fraction in the total proteome (ϕ_r) and the ribosomal proteome fraction in the endogenous proteome (ϕ_r').** With the expression of mCherry proteins in *S. cerevisiae*, ϕ_r decreases, whereas ϕ_r' stays almost constant. Results under two conditions are shown, and the data is from Ref. [31].

Intriguingly, experimental data of *S. cerevisiae* shows that although ϕ_r is diluted by the exogenous proteins, ϕ_r' stays invariant (Figure 5). Furthermore, the proteome fraction encoding for transcriptional proteins is also approximately constant [31], which implies that the volume expansion is compensated by making more transcriptional proteins. We also expect that the N/C ratio is also approximately constant if the synthesized mCherry proteins are evenly distributed in the cell [44]. Therefore, according to Eq. [16], the cost of the products appears insignificant to the overall fitness cost, rationalizing previous experimental observations of *E. coli* [12] and *S. cerevisiae* [5]. Here, we point out that the insignificant cost of the products applies to proteins without extra toxicity when overexpressed. In certain cases where proteins can change the nutrient uptake (e.g., LacY [45]) or be toxic due to misfolding [46–48], the products do generate a considerable fitness cost, which should be isolated as a protein-specific cost.

DISCUSSION

Many experiments have measured the growth rate reduction to quantify the fitness cost of gene expression [1, 2, 4, 5]. It has been widely presumed that the cost lies in both transcription and translation [5, 10, 49]. However, the mechanism behind this postulation remains elusive. In this work, we use a simple resource allocation model and successfully disentangle the fitness cost into different components generated by TF, RNAP, and ribosome competition. In particular, we show that the ribosome cost likely dominates the translation cost and the transcription cost in *S. cerevisiae* is dominated by the TF cost. We remark that the coarse-grained TF in our model may correspond to the general transcription factors, which help to position RNAP correctly at the promoters, aid in pulling apart the two strands of DNA, and release RNAP from the promoters to start elongation, including TFIIB, TFIID, etc. The proteins are needed at nearly all promoters used by RNA polymerase II [42, 43]. Other crucial proteins may also be candidates for the limiting factors, including mediator [41], transcription activator, and chromatin remodeling proteins [43]. Our framework provides valuable guidance on designing gene circuits with minimum interference with the host cells [50, 51]. We also discuss the differences between our model and previous ones regarding the fitness cost [4, 10, 11, 49] in Supplementary Information Section A and Table S1-S2.

We propose more systematic experiments in the future to obtain a deeper understanding of the fitness cost of gene expression. First, measurements of the simultaneous changes in the cell volume, ribosome copy number, RNAP copy number, etc., upon overexpression of exogenous genes will be invaluable for us to understand how cells adapt to limited resources for gene expression. Second, more accurate and high-throughput measurements

of fitness cost are required to enable quantitative modeling with more mechanistic insights.

METHODS

A. Derivation of the fitness cost

In this section, we do not consider the competition for TFs, and the discussion regarding the TF cost is in Methods C. When an exogenous unnecessary gene (denoted by "u") is introduced to a host cell, Eq. [1] is rewritten as:

$$gP_n\Lambda_n + N_{n,\mathrm{u}} + N_n f_n = N_n, \tag{17}$$

where $N_{n,\mathrm{u}} = g_\mathrm{u} P_{n,\mathrm{u}}\Lambda_{n,\mathrm{u}}$ is the number of RNAPs on the exogenous genes. In this work, we always take the differentiation against g_u at $g_\mathrm{u} \to 0$ unless otherwise mentioned. Differentiating Eq. [17] with respect to g_u, we obtain $g\Lambda_n \frac{\mathrm{d}P_n}{\mathrm{d}g_\mathrm{u}} + \frac{\mathrm{d}N_{n,\mathrm{u}}}{\mathrm{d}g_\mathrm{u}} + N_n \frac{\mathrm{d}f_n}{\mathrm{d}g_\mathrm{u}} = 0$. Differentiating Eq. [2] leads to $\frac{\mathrm{d}P_n}{\mathrm{d}g_\mathrm{u}} = \frac{P_n(1-P_n)}{f_n}\frac{\mathrm{d}f_n}{\mathrm{d}g_\mathrm{u}}$. Combining the above two equations with Eq. [17] with $N_\mathrm{u} = 0$, we find that

$$Z_{f_n} = \frac{1}{f_n}\frac{\mathrm{d}f_n}{\mathrm{d}g_\mathrm{u}} = -\frac{1}{1-P_n(1-f_n)}\frac{\mathrm{d}N_{n,\mathrm{u}}}{N_n \mathrm{d}g_\mathrm{u}}. \tag{18}$$

Here, we introduce Z_y as an abbreviation for $\frac{1}{y}\frac{\mathrm{d}y}{\mathrm{d}g_\mathrm{u}}$ where y is any variable of interest. Similarly, Eq. [3] is rewritten as:

$$mP_r\Lambda_r + N_{r,\mathrm{u}} + N_r f_r = N_r, \tag{19}$$

where $N_{r,\mathrm{u}} = m_\mathrm{u} P_{r,\mathrm{u}}\Lambda_{r,\mathrm{u}}$ is the number of ribosomes on the mRNA produced by the exogenous gene. Differentiating the equation with respect to g_u and considering the fact that $m = gk_{\text{tx-ini}}P_n\tau$ (Eq. [5]) is also a function of g_u lead to

$$Z_{f_r} = -\frac{1}{1-P_r(1-f_r)}\left[\frac{\mathrm{d}N_{r,\mathrm{u}}}{N_r \mathrm{d}g_\mathrm{u}} + (1-f_r)(1-P_n)Z_{f_n}\right]. \tag{20}$$

Differentiating Eq. [7], we get

$$Z_\gamma = (1-P_n)Z_{f_n} + (1-P_r)Z_{f_r}, \tag{21}$$

where $\gamma = \mu + d$ is the protein decay rate. Given the definition of cost,

$$C = -\int_0^{g_\mathrm{u}} Z_\mu \mathrm{d}g_\mathrm{u} = -\frac{\gamma}{\mu}\int_0^{g_\mathrm{u}} Z_\gamma \mathrm{d}g_\mathrm{u}, \tag{22}$$

and combining Eqs. [18] and [20-22], we get

$$C = \frac{\gamma}{\mu}\left(\frac{N_{n,\mathrm{u}}}{N_n}s_n\theta_r + \frac{N_{r,\mathrm{u}}}{N_r}s_r\right). \tag{23}$$

Here, we introduce s_n and s_r as the sensitivity factors

$$\begin{aligned} s_n &= \frac{1-P_n}{1-P_n+P_n f_n}, \\ s_r &= \frac{1-P_r}{1-P_r+P_r f_r}. \end{aligned} \tag{24}$$

In the limit $P_n \to 0$, transcription activity is rare, and s_n reaches its maximum value 1. In the limit $P_n \to 1$, cells have plenty of RNAPs and $s_n = 0$. A similar discussion applies to s_r. We also introduce the downstream factor

$$\theta_r = \frac{f_r}{1-P_r(1-f_r)}, \tag{25}$$

which represents the dampening effects of downstream processes on the RNAP cost: a small fraction of free ribosomes f_r dampens the RNAP cost.

In typical biological scenarios applied to microorganisms, Eq. [23] is simplified to Eq. [9] under the following conditions: 1. $\gamma \approx \mu$ because endogenous protein degradation is negligible compared to the growth rate [25–27]; 2. P_n is significantly lower than one such that $s_n \approx 1$; 3. P_r is significantly lower than one such that $s_r \approx 1$ and $\theta_r \approx f_r$. We remark that the conditions $P_n \ll 1$ and $P_r \ll 1$ are only the properties of the "average" endogenous gene, which is typically weakly expressed [17, 19, 49, 52].

Combining $N_{n,\mathrm{u}} = g_\mathrm{u}P_{n,\mathrm{u}}\Lambda_{n,\mathrm{u}}$, $\Lambda_{n,\mathrm{u}} = k_{\text{tx-ini,u}}T_{\text{tx,u}}$ and $\beta_{m,\mathrm{u}} = k_{\text{tx-ini,u}}P_{n,\mathrm{u}}$, we have

$$N_{n,\mathrm{u}} = g_\mathrm{u}\beta_{m,\mathrm{u}}T_{\text{tx,u}} \tag{26}$$

where $\beta_{m,\mathrm{u}}$ is the mRNA production rate of the exogenous gene. Eq. [26] is simply the result of the balance between the on and off rates of RNAPs on the exogenous gene in the steady state. Similarly, we have

$$N_{r,\mathrm{u}} = m_\mathrm{u}\beta_{p,\mathrm{u}}T_{\text{tl,u}}. \tag{27}$$

where $\beta_{p,\mathrm{u}}$ is the protein production rate of the exogenous mRNA. Replacing the $N_{n,\mathrm{u}}$ and $N_{r,\mathrm{u}}$ in Eq. [9] by Eqs. [26-27], we finally get Eq. [10].

B. Estimating the transcription and translation cost per gene/mRNA/protein from experiments in *S. cerevisiae*

The fitness costs of two gene constructs expressing mCherry proteins were measured, denoted as "wt" and "DAmP", respectively [5]. Both constructs were driven by the TDH3 promoter, while the DAmP is lack of terminator which leads to a shorter mRNA lifetime (τ_{DAmP}) than the wt mRNA lifetime (τ_{wt}). According to Eq. [12] and Eq. [14], the transcription cost of the two constructs is the same, but the translation costs differ due to different mRNA lifetimes. Therefore, we have the following linear equations:

$$\begin{bmatrix} C^{\mathrm{wt}} \\ C^{\mathrm{DAmP}} \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ 1 & \frac{\tau_{\mathrm{DAmP}}}{\tau_{\mathrm{wt}}} \end{bmatrix} \begin{bmatrix} C_{\mathrm{tx}} \\ C_{\mathrm{tl}} \end{bmatrix}. \tag{28}$$

where C^{wt} and C^{DAmP} are experimentally measured fitness cost per gene copy of the *wt* and *DAmP* constructs, respectively. Here C_{tx} and C_{tl} refer to the costs of the wild-type construct. Solving the simultanoues linear equations with $C^{\mathrm{wt}}/g_{\mathrm{u}} = 1.50 \times 10^{-2}$, $C^{\mathrm{DAmP}}/g_{\mathrm{u}} = 7.0 \times 10^{-3}$, and $\tau_{\mathrm{DAmP}}/\tau_{\mathrm{wt}} = 0.1$, we get $C_{\mathrm{tl}}/g_{\mathrm{u}} = 8.9 \times 10^{-3}$ and $C_{\mathrm{tx}}/g_{\mathrm{u}} = 6.1 \times 10^{-3}$.

Each gene copy contributes about 1.7% to the proteome for the TDH3 construct in the standard culture [5], which corresponds to a protein copy number of $p_{\mathrm{u}}/g_{\mathrm{u}} = 1.7\% \times M/a_{\mathrm{u}} = 1.5 \times 10^6$ with $M = 4.0 \times 10^{-12}$ g [53, 54] and $a_{\mathrm{u}} = 27$ kDa. Assuming that the protein production rate per mRNA and the mRNA lifetime of the unnecessary gene are the same as the average endogenous genes ($\beta_{p,\mathrm{u}} = 10^3$/h [55], $\tau_{\mathrm{u}} = 16$ min), we estimate $m_{\mathrm{u}}/g_{\mathrm{u}} = \gamma_{\mathrm{u}} p_{\mathrm{u}}/\beta_{p,\mathrm{u}} g_u = 6 \times 10^2$ with $\gamma_{\mathrm{u}} = 0.4$/h [5].

C. The fitness cost due to transcription factors

To include the effects of TFs, we rewrite the conservation equation for transcription factors:

$$gP_t\Lambda_t + N_{t,\mathrm{u}} + N_t f_t = N_t \tag{29}$$

where $N_{t,\mathrm{u}} = g_{\mathrm{u}} P_{t,\mathrm{u}} \Lambda_{t,\mathrm{u}}$ is the number of TF on the exogenous promoters. Here, Λ_t is the maximum number of TF on an endogenous promoter, and P_t is the probability for an endogenous promoter to be bound by TF, which is TF-concentration dependent: $P_t = \frac{[t]_{\mathrm{f}}}{[t]_{\mathrm{f}} + K_t}$, with K_t the coarse-grained dissociation constant. $\Lambda_{t,\mathrm{u}}$, $P_{t,\mathrm{u}}$ and $K_{t,\mathrm{u}}$ are the corresponding values for the exogenous promoter. The copy number of RNAPs on each gene is $\Lambda_n P_t P_n$, and the endogenous mRNA production rate is $\beta_m = k_{\text{tx-ini}} P_t P_n$. Similar results apply to the exogenous gene. Following the same method of derivation as Methods A, we find the fitness cost as

$$C = \frac{N_{t,\mathrm{u}}}{N_t} s_t \theta_n \theta_r + \frac{N_{n,\mathrm{u}}}{N_n} s_n \theta_r + \frac{N_{r,\mathrm{u}}}{N_r} s_r. \tag{30}$$

Here, the sensitivity factor of TF is $s_t = \frac{1-P_t}{1-P_t+P_t f_t}$, and the downstream factor $\theta_n = \frac{f_n}{1-P_n(1-f_n)}$. Surprisingly, the total fitness cost can be precisely decomposed into the contributions of each limiting resource. The corresponding downstream resources, including RNAP and ribosome, dampen the cost due to a finite number of TF. Approximating $s_t = s_n = s_r = 1$, $\theta_n = f_n$ and $\theta_r = f_r$, we obtain Eq. [12].

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