A general model of the dynamics of genes with dual $\sigma$ factor preference

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Abstract

*Escherichia coli* uses the ability of $\sigma$ factors to recognize specific DNA sequences in order to quickly control large gene cohorts. While most genes respond to only one $\sigma$
factor, approximately 5% have dual $\sigma$ factor preference. The ones in significant
numbers are ‘$\sigma^{70+38}$ genes’, responsive to $\sigma^{70}$, which controls housekeeping genes, as
well as to $\sigma^{38}$, which activates genes during stationary growth and stresses. We show
that $\sigma^{70+38}$ genes are almost as upregulated in stationary growth as genes responsive
to $\sigma^{38}$ alone. Also, their response strengths to $\sigma^{38}$ are predictable from their promoter
sequences. Next, we propose a sequence- and $\sigma^{38}$ level-dependent, analytical model
of $\sigma^{70+38}$ genes applicable in the exponential, stationary, and in the transition period
between the two growth phases. Finally, we propose a general model, applicable to
other $\sigma$ factors as well. This model can guide the design of synthetic circuits with
sequence-dependent sensitivity and plasticity to transitions between the exponential
and stationary growth phases.

Author Summary

Present challenges in Synthetic Biology include the design of genetic circuits that are
robust to growth phase transitions and whose responsiveness is sequence-
dependent, and, thus predictable prior to design. We present and validate an
empirical-based, sequence-dependent analytical model of $E. coli$ genes with dual
responsiveness to the regulators $\sigma^{70}$ and $\sigma^{38}$. These genes, supported by our
sequence-dependent model, could become building blocks for synthetic genetic
circuits functional in both the exponential and the stationary growth phases.

1. Introduction
In *Escherichia coli* (*E. coli*), genes are expressed by RNA polymerase (RNAP), which has 5 subunits (α₂ββ′ω) and can synthesize RNA by starting and ending at sequence-specific DNA locations, with the former being known as ‘promoter’ regions [1]. Transcription is constantly regulated, mostly at the promoter regions, which typically harbor transcription factor (TF) binding sites (TFBS) and other regulatory sequence motifs [2–7]. Such regulation is an essential survival skill of adaptability to transitions in both internal as well as external conditions [8,9].

One mechanism of large-scale, relatively synchronous gene expression regulation is executed by σ factors [4,10–13]. *E. coli* has seven different σ factors [14]. During exponential growth in optimal conditions, RNAP mostly transcribes genes with preference for σ⁷₀, responsible for basic cell functions [15]. Other σ factors are expressed under specific stresses [16], and activate a specific gene cohort. For example, after growing exponentially at the cost of environment components, *E. coli* usually switches to stationary growth. This is triggered, among other, by RpoS (σ³⁸), whose appearance activates ~10% of the genome [17,18], which leads to key phenotypic modifications [18–24].

During this adaptation, the concentration of RNAP remains relatively constant [25] and, since the number of RNAP core enzymes is limited, σ factors compete for them [10,12,14,20,26,27]. Thus, when σ³⁸ numbers increase, not only are the genes responsive to σ³⁸ activated, but also other genes, previously active, are indirectly negatively regulated, due to the reduction in RNAPs carrying σ⁷₀ [10,26,28].

For this regulatory system to be efficient, promoters need to have high specificity to one and only one σ factor. Nevertheless, there is a small fraction of promoters that can recognize more than one σ factor [6,29,30]. The most common (84%) are the
promoters responsive to both $\sigma^{70}$ and $\sigma^{38}$ [31]. Other cases are too rare to obtain sufficient statistics on their kinetics (Table S3 in S3 Appendix).

Here we investigate how the sequences of promoters recognized by both $\sigma^{70}$ and $\sigma^{38}$ relate to the dynamics of genes that they control (here named ‘$\sigma^{70+38}$ genes’). From flow cytometry and RNA-seq measurements, along with sequence analysis, we establish a relationship between RNA and protein levels in each growth phase with the promoter sequence (logos of positions -41 to -1 shown in Fig S1 in S2 Appendix).

From this, we propose a model of the dynamics $\sigma^{70+38}$ genes that fits their behavior prior, during, and following the transition to stationary growth. The model is dependent on both the promoter-sequence as well as the levels of $\sigma$ factors in the cell.

2. Results

2.1. RNA fold changes when shifting to stationary growth

To study how $\sigma^{70+38}$ genes respond to stationary growth, we first identified when are cells in exponential and stationary growth. We used both wild type (WT, control) cells and a MGmCherry strain [19] carrying fluorescently tagged $\sigma^{38}$ to measure its levels prior to, during, and after shifting from exponential to stationary growth (Section 4.2).

Cells at 150 min after being placed in fresh medium are used as representatives of cells in mid-exponential growth (Fig 1A). Meanwhile, at 500 min and onwards, cells exhibit stationary growth (Fig 1A).

From the measurements, $\sigma^{38}$ levels are small at 150 min, while from 500 min onwards they are stably high (Fig 1B). Considering that the cell areas are ~15% smaller in the
stationary phase (Figs S3A and S3B in S2 Appendix), at 700 min the absolute concentration of $\sigma^{38}$ is $\sim$32 times higher than at 150 min (Fig 1C and Fig S3C in S2 Appendix). Meanwhile, RNAP concentration is largely stable (only $\sim$7% higher during stationary growth) (Fig 1C and Fig S3D in S2 Appendix).

Next, we measured changes in RNA levels, by RNA-seq, between moments 150 min and 500 minutes by calculating the log$_2$ fold changes in RNA levels (LFC$_{RNA}$) (Sections 4.2 and 4.4). Gaussian fits to the LFC$_{RNA}$ distributions (Fig 1D) of $\sigma^{70+38}$ genes, $\sigma^{70}$ genes, and $\sigma^{38}$ genes, as well as all other genes, have high coefficient of determination ($R^2$) values of the fits suggesting that they capture well the shapes of the empirical distributions.

In general, $\sigma^{70+38}$ genes are nearly as upregulated as $\sigma^{38}$ genes (Fig 1D). On the other hand, $\sigma^{70}$ genes are weakly downregulated, likely due to indirect negative regulation caused by the increase in $\sigma^{38}$ levels combined with the limited pool of RNAP core enzymes [10,12,14,20,26,27]. Finally, most other genes (~2737 out of the 4308 genes) are relatively unresponsive.
Fig 1. Cell growth, RNAP, RpoS and genome-wide RNA levels when changing from (A) Optical densities ‘O.D.₆₀₀’ of wild-type MG1655 (control) and MGmCherry (rpoS::mcherry) strains (Section 4.1). (B) Mean population fluorescence of WT and MGmCherry strains. Black vertical bars show the timing of measurements in exponential (150) and stationary (500 minutes onwards) growth. At 0 minutes, cells were moved to fresh medium (Section 4.2). (C) Fold-change between the exponential and stationary growth phases of mean cellular fluorescence relative to cell area (FC(µ/Cell Area)) due to changes in RNAP-GFP and in RpoS-mCherry, respectively. Error bars are the standard error of the mean (SEM). (D) Gaussian fits to the distributions of LFCRNA of gene cohorts. Vertical lines mark the mean.

2.2. Propagation of shifts in RNA numbers to protein numbers

We used a YFP strain library to measure single-cell protein levels (Section 4.5) of 9 out of the 64 σ70+38 genes (only 15 of the 64 are represented in this library and some exhibited too weak signals to be detected by the flow cytometer). These 9 genes, according to their LFCRNA, cover well the state space of response strengths of σ70+38 genes (Section S1.2 in S1 Appendix).

From the single-cell distributions of protein levels in exponential and stationary growth, we extracted means, µP, and squared coefficient of variations, CV²P. We also calculated the log₂ fold changes in µP, LFCP. Since LFCP and the corresponding LFCRNA are linearly positively correlated, with a high R² (Fig 2A), we conclude that changes in RNA levels of σ70+38 genes during the growth phase transition propagate to their protein levels.

We then analyzed the noise in the dynamics of these genes, prior and after the growth phase transition. In Figs 2B1 and 2B2, CV²P decreases quickly with µP for small µP,
but only weakly for high $\mu_P$. This is well described by a function of the form:

$$CV_P^2 = C \times \mu_P^{-1} + N \ [32]$$

(where $N$ is the noise floor, a lower bound on the cell-to-cell variability of protein levels in clonal populations due to extrinsic factors [33]), in agreement with past reports [32,34,35].

**Fig 2.** Single-cell protein levels of $\sigma^{70+38}$ genes in the exponential and stationary growth phases. (A) Log$_2$ fold-change of protein levels, $LFC_P$, plotted against the corresponding $LFC_{RNA}$, during the shifting from exponential to stationary growth. (B1) and (B2) $CV_P^2$ plotted against $\mu_P$ of $\sigma^{70+38}$ genes in the exponential and stationary growth phases, respectively. Error bars (small) are the standard error of mean. All figures show the best fitting curves and their 95% prediction bounds (shadow areas). Horizontal dashed lines are the noise floors.

### 2.3. Full sequence-dependent model of the shift in the transcription dynamics of $\sigma^{70+38}$ genes

Here, we present a sequence-dependent model of transcription of $\sigma^{70+38}$ genes. Based on past works, we assume that only $\sigma^{70}$ is present in high numbers (in holoenzyme form) in the exponential phase and that only $\sigma^{38}$ increases significantly in numbers when shifting to stationary growth [10,14,20,26,36–38]. The model therefore accounts for the competition between $\sigma^{70}$ and $\sigma^{38}$ for binding to RNAP core enzymes, since these exist in limited numbers in *E. coli*.
[10,12,14,20,26,27] and since σ factor numbers differ between the two growth phases.

For this, we set reactions for binding and unbinding of σ⁷₀ and σ³⁸ to free floating RNAP core enzymes, (R1a) and (R1b), where $K_{σ^{70}}$ and $K_{σ^{38}}$ are the ratios between the association and dissociation rate constants:

\[ \text{RNAP} + σ^{70} \rightleftharpoons \text{RNAP}.σ^{70} \] \hspace{1cm} (R1a)

\[ \text{RNAP} + σ^{38} \rightleftharpoons \text{RNAP}.σ^{38} \] \hspace{1cm} (R1b)

Given (R1a) and (R1b), RNA production dynamics depends on the ratio between $σ^{70}$ and $σ^{38}$ numbers if, and only if, $K_{σ^{70}}$ and $K_{σ^{38}}$ differ. Meanwhile, the limited pool of RNAPs is accounted for by setting:

\[ [\text{RNAP}_{\text{total}}] = [\text{RNAP}.σ^{70}] + [\text{RNAP}.σ^{38}] \] \hspace{1cm} (1)

Next, to introduce the dual responsiveness to $σ^{70}$ and $σ^{38}$, we define two competing, sequence-dependent reactions of transcription, (R2a) and (R2b), differing in which holoenzyme binds to the promoter. Further, to also introduce sequence dependence, we define two sequence-based distances: $D_{σ^{38}}$ and $D_{σ^{70}}$. $D_{σ^{38}}$ is the distance, in number of nucleotides, of a promoter sequence differing from the consensus (average) sequence of promoters with $σ^{38}$ dependency. $D_{σ^{70}}$ is the distance in number of nucleotides differing from the consensus sequence of promoters with $σ^{70}$ dependency (Section 4.8.3):

\[ \text{Pro} + \text{RNAP}.σ^{70} \xrightleftharpoons[k^{-1}_{σ^{70}}(D_{σ^{70}})]{k_{σ^{70}}(D_{σ^{70}})} \text{Pro} + \text{RNA} + \text{RNAP} + σ^{70} \] \hspace{1cm} (R2a)

\[ \text{Pro} + \text{RNAP}.σ^{38} \xrightleftharpoons[k^{-1}_{σ^{38}}(D_{σ^{38}})]{k_{σ^{38}}(D_{σ^{38}})} \text{Pro} + \text{RNA} + \text{RNAP} + σ^{38} \] \hspace{1cm} (R2b)
The rate constants of these reactions, which control the affinities to the holoenzymes, are expected to differ between genes, as they depend on the promoter sequences (and potentially which transcription factors are acting on the promoters, here not represented for simplicity). Nevertheless, here, also for simplicity, \( k^{\sigma_{70}} \) and \( k^{\sigma_{38}} \) are assumed to be a function of only \( D_{\sigma_{70}} \) and \( D_{\sigma_{38}} \) respectively.

Together, the set of reactions (R1a), (R1b), (R2a) and (R2b), model the transcription kinetics of \( \sigma_{70+38} \) genes before, during and, after shifting from exponential to stationary growth. The rates \( k^{\sigma_{70}}(D_{\sigma_{70}}) \) and \( k^{\sigma_{38}}(D_{\sigma_{38}}) \) are dissected below.

### 2.4. Reduced model of the shift in transcription kinetics

To reduce the model, we assume that the numbers of RNAP.\( \sigma_{70} \) and RNAP.\( \sigma_{38} \) in the cells are significantly larger than 1, which is expected. Also, we assume that the rate-limiting steps in transcription initiation (during which a promoter is occupied and thus new events cannot start [39,40]) are shorter in time-length than the intervals between transcription events. If so, for any \( \sigma_{70+38} \) gene:

\[
\text{Pro} \xrightarrow{[\text{RNAP.}\sigma_{70}] k^{\sigma_{70}}(D_{\sigma_{70}})} \text{Pro + RNA + RNAP + } \sigma_{70} \quad (\text{R3a})
\]

\[
\text{Pro} \xrightarrow{[\text{RNAP.}\sigma_{38}] k^{\sigma_{38}}(D_{\sigma_{38}})} \text{Pro + RNA + RNAP + } \sigma_{38} \quad (\text{R3b})
\]

We then merge (R3a) and (R3b) into a single transcription process:

\[
\text{Pro} \xrightarrow{[\text{RNAP.}\sigma_{70}] k^{\sigma_{70}}(D_{\sigma_{70}}) + [\text{RNAP.}\sigma_{38}] k^{\sigma_{38}}(D_{\sigma_{38}})} \text{Pro + RNA} \quad (\text{R4})
\]

Finally, to complete the model, we included a reaction for translation of RNAs into proteins (R5), as well as events of RNA (R6) and protein (R7) decay due to degradation and dilution by cell division:
189 RNA + Rib $\xrightarrow{k_{56}}$ RNA + Rib + P \hspace{1cm} (R5)

190 RNA $\xrightarrow{k_{RNA}}$ $\emptyset$ \hspace{1cm} (R6)

191 P $\xrightarrow{k_{P}}$ $\emptyset$ \hspace{1cm} (R7)

2.5. Analytical solution of the reduced model

Based on the reduced model, we obtained an analytical solution for the expected fold change in RNA numbers of a gene whose promoter has preference for both $\sigma^70$ as well as $\sigma^{38}$. From (R4), (R5), (R6), and (R7) we derived the expected RNA numbers produced by a $\sigma^{70+38}$ gene:

$$RNA = \left[ RNAP_{\sigma^{70}} \cdot k_i^{\sigma^{70}} (D_{\sigma^{70}}) \right] + \left[ RNAP_{\sigma^{38}} \cdot k_i^{\sigma^{38}} (D_{\sigma^{38}}) \right] \cdot \gamma_{RNA}$$ \hspace{1cm} (2)

For simplicity, the rate constants are not expected to differ between the growth phases (as they depend on biophysical parameters that should not change significantly, such as binding affinities, etc.). Consequently, the fold-change in RNA numbers between the two growth phases should equal:

$$FC_{RNA} = \frac{RNA_{\text{sta}}}{RNA_{\text{exp}}} = \left[ RNAP_{\sigma^{70}} \cdot k_i^{\sigma^{70}} (D_{\sigma^{70}}) \right] _{\text{sta}} + \left[ RNAP_{\sigma^{38}} \cdot k_i^{\sigma^{38}} (D_{\sigma^{38}}) \right] _{\text{sta}} \cdot \gamma_{RNA} = \left[ RNAP_{\sigma^{70}} \cdot k_i^{\sigma^{70}} (D_{\sigma^{70}}) \right] _{\text{exp}} + \left[ RNAP_{\sigma^{38}} \cdot k_i^{\sigma^{38}} (D_{\sigma^{38}}) \right] _{\text{exp}} \cdot \Gamma$$ \hspace{1cm} (3)

where $\gamma$ is the ratio between the RNA decay rates in the two growth phases:

$$\Gamma = \frac{\gamma_{RNA_{\text{exp}}}}{\gamma_{RNA_{\text{sta}}}}$$ \hspace{1cm} (4)

Since $[RNAP_{\text{total}}]$ is similar in the two growth phases (Fig 1C):
\[ FC_{RNA} = \frac{\left[ \frac{RNAP.\sigma^{70}}{RNA_{total}} \right]_{sta} \cdot k^{70}_{t} \left( D_{\sigma^{70}} \right) + \left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right]_{sta} \cdot k^{38}_{t} \left( D_{\sigma^{38}} \right)}{\left[ \frac{RNAP.\sigma^{70}}{RNA_{total}} \right]_{exp} \cdot k^{70}_{t} \left( D_{\sigma^{70}} \right) + \left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right]_{exp} \cdot k^{38}_{t} \left( D_{\sigma^{38}} \right)} \cdot \Gamma} \] (5)

Also, considering equation (1), then:

\[ \left[ \frac{RNAP.\sigma^{70}}{RNA_{total}} \right] + \left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right] \approx 1 \] (6)

For simplicity, let \( \rho_{exp} \) and \( \rho_{sta} \) be:

\[ \rho_{exp} = \frac{\left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right]_{exp}}{\left[ \frac{RNAP.\sigma^{70}}{RNA_{total}} \right] + \left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right]_{exp} \cdot K^{\sigma^{38}}} \] (7a)

\[ \rho_{sta} = \frac{\left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right]_{sta}}{\left[ \frac{RNAP.\sigma^{70}}{RNA_{total}} \right] + \left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right]_{sta} \cdot K^{\sigma^{38}}} \] (7b)

From (5):

\[ FC_{RNA} = \frac{(1 - \rho_{sta}) \cdot k^{70}_{t} \left( D_{\sigma^{70}} \right) + \rho_{sta} \cdot k^{38}_{t} \left( D_{\sigma^{38}} \right) \cdot \Gamma}{(1 - \rho_{exp}) \cdot k^{70}_{t} \left( D_{\sigma^{70}} \right) + \rho_{exp} \cdot k^{38}_{t} \left( D_{\sigma^{38}} \right) \cdot \Gamma} \] (8)

Finally, since, in the exponential growth phase, \( \left[ \frac{RNAP.\sigma^{70}}{RNA_{total}} \right] \gg \left[ \frac{RNAP.\sigma^{38}}{RNA_{total}} \right] \), then

\[ \rho_{exp} \approx 0. \] Thus, (8) can be simplified:

\[ FC_{RNA} = \gamma \cdot (1 - \rho_{sta}) + \rho_{sta} \cdot \frac{k^{38}_{t} \left( D_{\sigma^{38}} \right)}{k^{70}_{t} \left( D_{\sigma^{70}} \right)} \] (9)
All parameters in (9) can be measured directly, except \( k^{\sigma_{70}}(D_{\sigma_{70}}) \) and \( k^{\sigma_{38}}(D_{\sigma_{38}}) \).

Meanwhile, in the stationary phase: 
\[
\left[ \sigma^{38} \right]_{\text{sta}} = 0.3 \cdot \left[ \sigma^{70} \right]_{\text{sta}}
\]
[20,26,37]. Also, \( K^{\sigma_{70}} = 5K^{\sigma_{38}} \) [41]. Thus:

\[
\rho^{\text{sta}} = \frac{\left[ \text{RNAP} \cdot \sigma^{38} \right]_{\text{sta}}}{\left[ \text{RNAP}_{\text{total}} \right]} \approx \frac{0.3 \cdot \left[ \sigma^{70} \right]_{\text{sta}} \cdot K^{\sigma_{70}}}{\left[ \sigma^{70} \right]_{\text{sta}} \cdot K^{\sigma_{70}} + 0.3 \cdot \left[ \sigma^{70} \right]_{\text{sta}} \cdot K^{\sigma_{38}} \cdot K^{\sigma_{70}}} = 0.0566
\]

(10)

2.6. Promoter sequence affects the expression of \( \sigma^{70+38} \) genes

To model the influence of the promoter sequence (i.e., \( D_{\sigma_{70}} \) and \( D_{\sigma_{38}} \)) on the response strength of \( \sigma^{70+38} \) genes to \( \sigma^{38} \), we studied three models. In all, we assumed the basal levels of transcription rates, \( 'k_{0}^{\sigma_{70}} \) and \( 'k_{0}^{\sigma_{38}} \). The overall transcription rates are tuned by a function \( f \) (Table 1) of the influence of \( D_{\sigma_{70}} \) and \( D_{\sigma_{38}} \) on the basal transcription rate:

\[
k^{\sigma_{70}}(D_{\sigma_{70}}) = k_{0}^{\sigma_{70}} \cdot f(D_{\sigma_{70}}) \quad (11a)
\]

\[
k^{\sigma_{38}}(D_{\sigma_{38}}) = k_{0}^{\sigma_{38}} \cdot f(D_{\sigma_{38}}) \quad (11b)
\]

Consider that, in general, as \( D_{\sigma_{i}} \) of a promoter increases, the promoter should become more distinct from the ‘average’ promoter with preference for \( \sigma^{i} \). Consequently, its affinity to \( \sigma^{i} \) is expected to decrease. If this holds true, then the promoter consensus sequence of genes with preference for a given \( \sigma \) factor should have strong affinity to that \( \sigma \) factor. We hypothesize, for simplicity, it has the strongest affinity. If true, it follows that as \( D_{\sigma_{38}} \) increases, the transcription rate by RNAP.\( \sigma^{38} \) should decrease.

Having this in account, to model how a promoter sequence controls the transcription kinetics of promoters recognized by \( \sigma^{70} \) and by \( \sigma^{38} \), we considered a linear model
(model I in Table 1), a rational model (model II), where the transcription rate is inversely proportional to $D_{\sigma i}$, and an exponential model (model II), similar to the one in [6,29,30] for $\sigma^{70}$ genes.

**Table 1.** Models of how a sequence’s p-distance, $D_m$, affects a promoter’s response to changes in the concentration of its preferred $\sigma$ factor. Here, $m$ is an empirical-based constant.

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Equation</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Linear model</td>
<td>$f(D_{\sigma i}) = 1 - m_i \cdot D_{\sigma i}$, $m_i &gt; 0$ &amp; $m_i \times D_{\sigma i} &lt; 1$, $i = 38$ or $70$</td>
<td></td>
</tr>
<tr>
<td>II. Rational model</td>
<td>$f(D_{\sigma i}) = \frac{1}{1 + m_i \cdot D_{\sigma i}}$, $m_i &gt; 0$, $i = 38$ or $70$</td>
<td></td>
</tr>
<tr>
<td>III. Exponential model</td>
<td>$f(D_{\sigma i}) = e^{-m_i \cdot D_{\sigma i}}$, $m_i &gt; 0$, $i = 38$ or $70$</td>
<td></td>
</tr>
</tbody>
</table>

We fitted the models (Fig 3) to the empirical fold changes (FC) of $\sigma^{70+38}$ genes ($\text{FC}_{RNA}$) with known input TFs (Table S4 and Table S6) (FC rather than LFC were used, because this allowed for simpler equations for the fitting surfaces). From the $R^2$ values of the best fitting models (Table 2), we find that the model III best describes how $D_{\sigma 70}$ and $D_{\sigma 38}$ affect $k_{\sigma 70}$ and $k_{\sigma 38}$.

Next, we validated the fitted models by quantifying how well they predict the dynamics (Fig 3) of $\sigma^{70+38}$ genes without input TFs (Table S4 and Table S6). Given the $R^2$ values, we conclude that the tuned model III predicts well their dynamics. Further, the fact that the model obtained from genes with TF regulation fits well (with a higher $R^2$) the genes without TF regulation is suggestive that, in our measurement conditions, such TF regulation is not exerting a significant role.
Table 2. Goodness of fit, measured by the $R^2$ of the surface fitting of $FC_{RNA}$ as a function of $D_{038}$ and $D_{070}$. Shown are the models and best fitting parameter values, where $K_0 = \frac{k_{038}}{k_{070}}$. The surfaces were fitted to $\sigma_{70+38}$ genes without input TFs and validated on genes $\sigma_{70+38}$ genes with input TFs.

<table>
<thead>
<tr>
<th>Surface Equation</th>
<th>Coefficients</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$FC_{RNA} = \gamma \cdot (1 - \rho_{sta}) + \gamma \cdot \rho_{sta} \cdot K_0 \cdot \left( \frac{1 - m_{38} \times D_{\sigma_{38}}}{1 - m_{70} \times D_{\sigma_{70}}} \right)$</td>
<td>$m_{38}$</td>
<td>-834.80</td>
</tr>
<tr>
<td></td>
<td>$m_{70}$</td>
<td>-1.01$\times$10$^4$</td>
</tr>
<tr>
<td></td>
<td>$\Gamma$</td>
<td>85.60</td>
</tr>
<tr>
<td></td>
<td>$K_0$</td>
<td>2.39$\times$10$^{-4}$</td>
</tr>
<tr>
<td></td>
<td>$\rho_{sta}$</td>
<td>0.06 (Equation (10))</td>
</tr>
<tr>
<td>$\sigma_{70+38}$ genes with input TFs</td>
<td>&lt;0</td>
<td></td>
</tr>
<tr>
<td>$\sigma_{70+38}$ genes without input TFs</td>
<td>&lt;0</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Surface Equation</th>
<th>Coefficients</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$FC_{RNA} = \gamma \cdot (1 - \rho_{sta}) + \gamma \cdot \rho_{sta} \cdot K_0 \cdot \left( \frac{1 + m_{70} \cdot D_{\sigma_{70}}}{1 + m_{38} \cdot D_{\sigma_{38}}} \right)$</td>
<td>$m_{38}$</td>
<td>-0.38</td>
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<td></td>
<td>$m_{70}$</td>
<td>26.51</td>
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<td></td>
<td>$\Gamma$</td>
<td>0.21</td>
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<td></td>
<td>$K_0$</td>
<td>241.70</td>
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<tr>
<td></td>
<td>$\rho_{sta}$</td>
<td>0.06 (Equation (10))</td>
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<tr>
<td>$\sigma_{70+38}$ genes with input TFs</td>
<td>0.04</td>
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### III. Exponential model

<table>
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<th>Surface Equation</th>
<th>III. Exponential model</th>
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<tbody>
<tr>
<td>( FC_{RNA} = g(t - \rho_{sta}) + g \cdot \rho_{sta} \cdot K_0 \cdot e^{-(m_{38}D_{\sigma38} - m_{70}D_{\sigma70})} ) (12c)</td>
<td>Coefficients</td>
</tr>
<tr>
<td>( m_{38} )</td>
<td>30.78</td>
</tr>
<tr>
<td>( m_{70} )</td>
<td>56.90</td>
</tr>
<tr>
<td>( \Gamma )</td>
<td>28.20</td>
</tr>
<tr>
<td>( K_0 )</td>
<td>3.841 ( \times 10^{-9} )</td>
</tr>
<tr>
<td>( \rho_{sta} )</td>
<td>0.06 (Equation (10))</td>
</tr>
</tbody>
</table>

| \( R^2 \) | \( \sigma^{70+38} \) genes with input TFs | 0.55 |
| \( \sigma^{70+38} \) genes without input TFs | 0.98 |

Fig 3. Fold changes in RNA levels of \( \sigma^{70+38} \) genes plotted against their promoter sequence \( p \)-distances to the consensus sequence of \( \sigma^{70} \) dependent promoters (\( D_{\sigma70} \)) and of \( \sigma^{38} \) dependent promoters (\( D_{\sigma38} \)). (A1-A3) show the best fitting surfaces to \( FC(\mu_{RNA}) \)
assuming (A1) a linear function, (A2) a rational function, and (A3) an exponentially decreasing function. Only $\sigma^{70+38}$ genes with input TFs and FDR < 0.05 are included. Light red points are above the surface, while dark ones are below. (B1-B3) Same plots but the surfaces are those obtained from (A1-A3) and applied to $\sigma^{70+38}$ genes without input TFs and FDR < 0.05. The dashed black lines depict the vertical distances between the estimated and measured FC($\mu_{RNA}$).

Finally, from Fig 2A, the LFC of protein numbers (LFC$_P$) correlates linearly with LFC$_{RNA}$. The same is predictable from the model (reactions R5 to R7) (Section S1.3 in S1 Appendix). Here, to obtain the best fitting model for protein log$_2$ fold changes, we fitted a line to the data in Fig 2A, and extracted from it a scaling factor, $\alpha$, between LFC$_{RNA}$ and LFC$_P$. From this:

$$LFC_P = \log_2 \left[ \Gamma \cdot (1 - \rho_{sta}) + \Gamma \cdot \rho_{sta} \cdot K_0 \cdot e^{-(m_{sta} - m_{y}) D} \right] \cdot \alpha + \beta$$

(13)

where $\beta$ equals -0.67 and is the intercept between the y axis and the best fitting line, while $\alpha$ is the scaling factor between RNA and protein log$_2$ fold changes and equals 0.1 (Fig 2A).

2.7. $\sigma^{70+38}$ genes without known input transcription factors

Next, we fitted the exponential model III directly to $\sigma^{70+38}$ genes without input TFs (not to mistake with when they were used to validate the surface created from $\sigma^{70+38}$ genes with TF inputs). The best fitting model ($R^2$ of 1) has similar $m_{38}$ and $m_{70}$ (49.04 and 52.48, respectively). Thus, for this restricted set of genes, since $\Delta = D_{a38} - D_{a70}$ (Section 4.8.3), the surface equation (12c) in Table 2 can be approximated to:

$$FC_{RNA} \approx \Gamma \cdot (1 - \rho_{sta}) + \Gamma \cdot \rho_{sta} \cdot K_0 \cdot e^{-rD} \cdot \frac{m_{sta} + m_{y}}{2}$$
Applying the $\log_2$ to both sides, one has:

$$LFC_{RNA} \approx \log_2 \left( \Gamma \cdot (1 - \rho_{sta}) + \Gamma \cdot \rho_{sta} \cdot K_0 \cdot e^{-r_D} \right) = C_1 + \log_2 \left( \Gamma \cdot \rho_{sta} \cdot K_0 \right) + \log_2 \left( e^{-r_D} \right) = C_1 + C_2 + \log_2 \left( e^{-r_D} \right)$$

If $(C_1 + C_2)$ is much smaller than $\log_2 \left( e^{-r_D} \right)$, we expect that

$$LFC_{RNA} \propto \log_2 \left( e^{-r_D} \right) \approx -r \cdot D \over \ln(2)$$

(14)

Therefore, and even though $D_{\sigma38}$ and $D_{\sigma70}$ are not significantly correlated (Fig 4A), one finds a linear correlation between $LFC_{RNA}$ and $\Delta$ (Fig 4B).

**Fig 4.** Log$_2$ fold changes in RNA levels of $\sigma^{70+38}$ genes and their promoter $p$-distances to the consensus sequence of $\sigma^{70}$ dependent promoters ($D_{\sigma70}$) and of $\sigma^{38}$ dependent promoters ($D_{\sigma38}$). **(A)** Scatter plot of $D_{\sigma38}$ plotted against $D_{\sigma70}$. **(B)** Scatter plot of $\Delta = D_{\sigma38} - D_{\sigma70}$ plotted against $LFC_{RNA}$. The shadows of the best fitting lines are the 95% prediction bounds.

For comparison, we produced similar figures in search for linear correlations between $\Delta$ and $LFC_{RNA}$ in the cohorts of genes whose promoters have preference for $\sigma^{70}$, $\sigma^{38}$, $\sigma^{24}$, $\sigma^{28}$, $\sigma^{32}$, and $\sigma^{54}$ (Figs 5A-5F). No significant correlation with a high value of $R^2$ was found. We further studied the two small cohorts of genes whose promoters have preference for two $\sigma$ factors other than $\sigma^{70+38}$. Here, correlations are found, but they
are not statistically significant, likely due to the small numbers of genes (Figs 5G and 5H).

**Fig 5. Fold changes in RNA levels and Δ.** Genes whose promoters have preference for (A) $\sigma^{70}$ (B) $\sigma^{38}$ (C) $\sigma^{24}$ (D) $\sigma^{32}$ (E) $\sigma^{54}$ (F) $\sigma^{70+24}$ and (H) $\sigma^{70+32}$ are plotted against $\Delta = D_{38} - D_{70}$. The shadows of the best fitting lines are the 95% prediction bounds.

### 2.8. Expanding the model to the growth phase transition period

We expanded the model to include the transition period between the growth phases. For this, we collected temporal data on the protein numbers of three $\sigma^{70+38}$ genes, specifically pstS, aidB and asr. These genes are used as representative of $\sigma^{70+38}$...
genes with, strong, mild, and weak response strengths to the phase transition, respectively (Fig 6A). All data was well fitted by Hill functions (Fig 1B and Fig 6A). Moreover, in all three genes, there is a linear relationship between the fold changes in protein levels and in $\sigma^{38}$ levels over time (Fig 6B).

**Fig 6. Temporal changes in the fold-change of protein levels as $\sigma^{38}$ changes.** (a) Protein levels of three $\sigma^{70-38}$ genes prior to, during, and after the transition from exponential to stationary growth phases. The balls are the empirical mean fold changes (FC$_P$) in protein expression levels relative to the first moment. The lines are the corresponding best fitting Hill functions (parameter values in Table S5 in S3 Appendix). (b) Scatter plot of FC$_P$ against the corresponding $\sigma^{38}$ levels (data from Fig 1B) over time. The shadows are the 95% prediction bounds.

The dependency on $\sigma^{38}$ levels is accounted for in model ($\rho_{sta}$ is a function of $\sigma^{38}$ levels in equation (7b), which are time dependent). Consequently, given the goodness of fit of the Hill functions, we propose the following time-dependent model:

$$FC_P(t) = \left(\frac{FC_{Pmax}^{\sigma_{38}} - 1}{h^s + t^s}\right) t^s + b,$$

(15a)
where:

$$LFC_P^{\text{max}} = \left( \log_e \left[ \frac{\Gamma (1 - \rho_{\text{sta}}^{\text{max}}) + \Gamma \cdot \rho_{\text{sta}}^{\text{max}} \cdot K_0 \cdot e^{-(m_{10} D_{\sigma 38} m_{38} D_{\sigma 38})}}{1 + \rho_{\text{sta}}^{\text{max}} \cdot K_0 \cdot e^{-(m_{10} D_{\sigma 38} m_{38} D_{\sigma 38})}} \right] \right) \cdot \alpha - \beta \quad (15b)$$

$$FC_P^{\text{max}} = \left( \frac{\Gamma (1 - \rho_{\text{sta}}^{\text{max}}) + \Gamma \cdot \rho_{\text{sta}}^{\text{max}} \cdot K_0 \cdot e^{-(m_{10} D_{\sigma 38} m_{38} D_{\sigma 38})}}{2^\beta} \right)^{\sigma} \quad (15c)$$

Here, $\rho_{\text{sta}}^{\text{max}}$ is the final (thus, maximum) concentration of RNAP-$\sigma^{38}$ relative to the total concentration of bound RNAPs. Meanwhile, $FC_P^{\text{max}}$ is the expected fold change in protein numbers of a $\sigma^{70+38}$ gene, which is reached after the transition to stationary growth is complete. Finally, $b$ (the intercept) is the expression level controlled by the promoter of interest, when in exponential growth phase. Meanwhile, $s$ (the slope) is the corresponding response strength, and $h$ (the half-activation coefficient) is a measure of response time to changes in $\sigma^{38}$ levels.

### 2.9. Model generalization

While here we only found a correlation between the promoter sequence and the gene’s response in $\sigma^{70+38}$ genes, it may be that genes whose promoters have different $\sigma$ preferences exhibit similar sequence-dependent behaviors during the stresses that they are responsive to. Thus, we generalized the model to be applicable to any stress and responsive gene cohort. As an example, we set a model for genes responsive to all seven $\sigma$ factors of *E. coli*. For this, first, we expand reactions (R1a) and (R1b), to include all $\sigma$ factors as follows:

$$\text{RNAP} + \sigma^i \xrightleftharpoons{K_i^w} \text{RNAP} \cdot \sigma^i, \text{ where } i = 70, 54, 38, 32, 28, 24 \text{ or } 19 \quad (R8a-R8g)$$

Next, we generalize eq. (1) to account for the $\sigma$ factors in holoenzyme form:
\[ [\text{RNAP}_{\text{total}}] = \sum_i [\text{RNAP}_{i\sigma}] \], where \( i = 70, 54, 38, 32, 28, 24 \) or 19 \hfill (16)

Given this, we generalize (R4) as follows:

\[ \text{Pro} \sum_k \{\text{RNAP}_{i\sigma} \} \times (D_{ik}) \rightarrow \text{Pro} + \text{RNA} \], where \( i = 70, 54, 38, 32, 28, 24 \) or 19 \hfill (R9)

This general model can be tuned based on the numbers of each \( \sigma \) factor present in the conditions considered, and the consensus sequences to each \( \sigma \) factor (Table S5).

In addition, following the findings in Section 2.8, it should be feasible to introduce factors to account for the timing of the changes in the transition period.

### 3. Discussion

From information on the promoters' sequence and dynamics, we proposed and validated a sequence-dependent, kinetic model of genes controlled by promoters that are responsive to both \( \sigma^{70} \) and \( \sigma^{38} \). This model of \( \sigma^{70+38} \) genes, which accurately predicts how their dynamics change for exponential and stationary growth, is an expansion of a past model of promoters with preference for \( \sigma^{70} \) alone [26], but has two competing reactions for transcription by RNAP when bound to \( \sigma^{70} \) and when bound to \( \sigma^{38} \). In addition, these reactions’ rate constants are sequence-dependent, in line with the hypothesis that the consensus sequence of promoters with preference for a given \( \sigma \) factor should provide the strongest affinity to that \( \sigma \) factor. The model also accounts for the period of the growth phase transition of the cell, which is linked to \( \sigma^{38} \) levels (from lowest to highest). Finally, we proposed (yet to validate) a general model, applicable to promoters with any given \( \sigma \) factor(s) preference.
Relevantly, we identified a simple correlation between the promoter sequence of $\sigma^{70+38}$ genes and their response strength to the growth phase transition, making it predictable. So far, this predictability has not been reported for any cohort of natural promoters of *E. coli*, even in stable growth conditions. Similar relationships have only been reported for synthetic libraries of promoter variants under stable growth [5,6,30,42].

Since it remains challenging to predict if a sequence can act as a promoter and, if so, with which strength and under what regulatory mechanisms [5], this predictability of the dynamics from the sequence for the natural cohort of $\sigma^{70+38}$ genes is of interest for three main reasons. First, these promoters and their variants (with varying $p$-distances from the consensus sequences of $\sigma^{70}$ and $\sigma^{38}$ genes) could become key components of a toolbox for engineering circuits whose response kinetics is predictable from the promoter sequences. Further, such circuits should be functional in both exponential and stationary growth phases, with tunable responsiveness to the phase transition. Finally, their model could be a starting point for broader models of sequence-predictable adaptability of natural promoters to various stresses.

It may prove difficult to find similar relationships between the sequences of other natural promoters and their responsiveness to other $\sigma$ factors (or global regulators). E.g., we failed to find a relationship between the sequences of promoters responsive to $\sigma^{38}$ alone and their response strength to $\sigma^{38}$ (even for genes without TF inputs (Fig 5b)). Also, it remains to be proved whether promoters responsive to $\sigma^{70}$, $\sigma^{54}$, $\sigma^{32}$, $\sigma^{28}$, $\sigma^{24}$, or dual combinations, have similar behaviors when those $\sigma$ factors change.
It also remains to be studied whether the sequence-dynamics relationship in $\sigma^{70+38}$ genes is causal or coincidental. While the large numbers of promoters studied here support the causality, a definitive proof likely requires producing synthetic promoters similar to the natural ones but differing in p-distances to the consensus sequences of promoters with preference for $\sigma^{38}$ and for $\sigma^{70}$, respectively.

Also, in the future, it should be investigated if the sequence-dependent responsiveness of $\sigma^{70+38}$ genes to $\sigma^{38}$ levels contributes to why their promoters (from positions -41 to -1) are highly conserved (Fig S1 in S2 Appendix) and/or why the TFs that they code for commonly serve as input TFs to essential genes [43] (2.5 times more than average, Fisher test $p$-value < 0.05).

Finally, over-representation tests of the ontology [44,45] of $\sigma^{70+38}$ genes suggest that they are commonly involved in respiration (Fig S4 in S2 Appendix, Table S4 in S3 Appendix). In agreement, this process is highly affected when changing from exponential to stationary growth [23,46], since aerobic respiration is reduced, while fermentation and anaerobic respiration are enhanced [18]. Moreover, we observed that $\sigma^{70+38}$ genes are amongst the most responsive to the change from growth phase out of the genes associated with these functions (Fig S4 in S2 Appendix). This suggests that they may control the processes that need most alterations during the adaptation. Therefore, externally regulating these genes may thus give significant control over these processes. This is particularly appealing since this control could be exerted by tuning their promoter sequences (i.e., their p-distances) making the effects of their integration on a genetic circuit’s response to the growth phase shift largely predictable as well. This strategy may thus contribute to the engineering of synthetic circuits with tailored responses to growth phase transitions.
4. Materials and Methods

4.1. Bacterial strains and media

E. coli strains and plasmids are listed in Tables S1 and S2 in S3 Appendix. In short, we used YFP strains from the genetic stock center (CGSC) of Yale University, U.S.A [32] and, as support, a low-copy plasmid fusion library of fluorescent reporter strains using GFP to track promoter activity [47]. In both, one measures the levels of fluorescent proteins under the control of the promoters of our genes of interest which are a good proxy for the native protein levels [35][39]. For simplicity, we refer to these fluorescence levels as ‘protein levels’.

We used a RL1314 strain (rpoC::GFP) generously provided by Robert Landick, University of Wisconsin-Madison [48]), to measure RNA Polymerase levels. Their rpoC gene codes for β’ sub-unit endogenously tagged with GFP. Since rpoC codes for the β’ subunit, a limiting factor in the assembly of RNAP holoenzyme [49,50], its numbers serve as a good proxy for RNAP numbers. For simplicity, [RNAP] refers to the sum of RNAP core and holoenzymes in a cell. Further, we used a MGmCherry (rpoS::mCherry) strain to measure RpoS levels (kind gift from James Locke [19]). Their rpoS gene codes for σ38 endogenously tagged with mCherry. Finally, we used wild-type K12 MG1655 strain for control.

We used M9 medium (1xM9 Salts, 2 mM MgSO4, 0.1 mM CaCl2; 5xM9 Salts 34 g/L Na2HPO4, 15 g/L KH2PO4, 2.5 g/L NaCl, 5 g/L NH4Cl) supplemented with 0.2% Casamino acids and 0.4% glucose, and Luria-Bertani (LB) medium with 10 g peptone,
10 g NaCl, and 5 g yeast extract in 1000 ml distilled water. We used the antibiotics kanamycin and chloramphenicol from Sigma Aldrich, U.S.A.

4.2. Growth rates and growth phases

Growth rates were measured by spectrophotometry (BioTek Synergy HTX Multi-Mode Microplate Reader). From a glycerol stock (-80 °C), cells were streaked on LB agar plates (2%) and incubated at 37 °C, overnight. Next, a single colony was picked, inoculated in LB medium with antibiotics (Section 4.1), and incubated at 30°C overnight with shaking. Overnight cultures were further diluted into fresh medium to an optical density of 600 nm (O.D.600) of 0.01 and incubated for growth by shaking at 250 rpm at 37°C. OD600 was recorded every 20 min, for 800 min. Cells were extracted at 150 min and at 700 min after inoculation into fresh medium to represent cells in exponential and stationary growth phases, respectively (Fig 1A).

4.3. Microscopy and Image analysis

To collect microscopy data, cells were placed between a coverslip and agarose gel pad (2%) and visualized by a confocal laser-scanning system, using a 100× objective. Green fluorescence images were captured using a 488 nm laser and a 514/30 nm emission filter. Phase-contrast images were simultaneously acquired for purposes of segmentation and to assess health, morphology, and physiology. Images were analyzed using “CellAging” software [51]. After automatically segmenting cells and manually correcting errors, we applied 2D Gaussian filters to remove measurement noise, and extracted each cell’s total fluorescent intensity.

4.4. RNA-seq
4.4.1. Sample preparation

Cells were grown until reaching either the exponential or stationary growth phases, respectively. At each of these moments, they were treated with a double volume of RNA protect bacteria reagent (Qiagen, Germany) for 5 min, at room temperature, to prevent RNA degradation. Cells were then pelleted and frozen immediately at -80 °C. After unfreezing the cells, total RNA was extracted using RNeasy mini-kit (Qiagen) according to the instructions. RNA was treated twice with DNase (Turbo DNA-free kit, Ambion, Life Technologies, U.S.A.) and quantified using Qubit 2.0 Fluorometer RNA assay (Invitrogen, Carlsbad, CA, USA). Quality of total RNA was determined by gel electrophoresis, using 1% agarose gel stained with SYBR safe (Invitrogen, U.S.A.). RNA was detected using UV with a Chemidoc XRS imager (BioRad, U.S.A.).

4.4.2. Sequencing

Sequencing was performed by GENEWIZ, Inc. (Leipzig, Germany). The RNA integrity number (RIN) of the samples was obtained with Agilent 4200 TapeStation (Agilent Technologies, Palo Alto, CA, USA). Ribosomal RNA depletion was performed using Ribo-Zero Gold Kit (Bacterial probe) (Illumina, San Diego, CA, USA). RNA-seq libraries were constructed using NEBNext Ultra RNA Library Prep Kit (NEB, Ipswich, MA, USA). Sequencing libraries were multiplexed and clustered on 1 lane of a flow-cell. Samples were sequenced using single-index, 2x150bp paired-end (PE) configuration on an Illumina HiSeq instrument. Image analysis and base calling were conducted with HiSeq Control Software (HCS). Raw sequence data (.bcl files) were converted into fastq files and de-multiplexed using Illumina bcl2fastq v.2.20. One mismatch was allowed for index sequence identification.
4.4.3. Data analysis

RNA-seq data analysis pipeline: i) RNA sequencing reads were trimmed to remove adapter sequences and nucleotides with poor quality with Trimmomatic [52] v.0.39. ii) Trimmed reads were then mapped to the reference genome (E. coli MG1655, NC_000913.3) using STAR v.2.5.2b aligner [53]. iii) featureCounts from Rsubread R package (v.1.34.7) was used to calculate unique gene hit counts [54]. Genes with less than 5 counts in more than 3 samples, and genes whose mean counts are less than 10, were removed. iv) Next, the unique gene hit counts were used for subsequent differential expression analysis, using DESeq2 R package (v.1.24.0) [55] to compare gene expression between samples and calculate p-values and log2 of fold changes (LFC) using Wald tests (function nbinomWaldTest). P-values were adjusted for multiple hypotheses testing (Benjamini–Hochberg, BH procedure [56]) and genes with adjusted p-values (False discovery rate (FDR)) smaller than 0.05 were classified as differentially expressed (D.E.).

Due to filtering (iii), only 783 (777 D.E.) out of the 931 σ^70 genes, 87 (all D.E.) out of the 93 σ^38 genes, 57 (56 D.E.) out of the 64 σ^70+38 genes, and 3257 (2737 D.E.) out of 3607 remaining genes were assessed by RNA-seq. Other genes did not produce enough reads.

4.5. Flow cytometry

We used a ACEA NovoCyte Flow Cytometer equipped with yellow (561 nm) and blue lasers (488 nm), controlled by Novo Express (V1.50). Cells were diluted 1:10000 into 1 ml of PBS vortexed for 10 sec. For each gene and condition, we performed 3 biological replicates, acquiring 50,000 events in each. For GFP and YFP, we used the
blue laser for excitation and the FITC-H channel (520/20 nm filter) for emission. For mCherry, we used a yellow laser for excitation and PE-Texas Red (615/20 nm filter) for emission. We collected events at a flow rate of 14 µl/minute, a core diameter of 7.7 µM, and adjusted PMT voltage for each parameter. We have set the FSC-H detection threshold to 5000.

We collected single-cell fluorescence distributions. To remove outliers due to debris, cell doublets, and other undesired events, we performed gating by setting the maximum values of SSC-H to $5 \times 10^4$ and of FSC-H to $10^5$. These limits allowed observing the whole distributions, without cutting their natural tails. The results did not differ widely from using unsupervised gating as in [57,58], if setting $\alpha$ to 0.95.

Finally, we discarded “far out” events using Tukey’s fences [59]. This had little effect on the results. Furthermore, background fluorescence was not subtracted since doing so did not affect the results (Section S1.2 in S1 Appendix and Fig S2 in S2 Appendix).

4.6. Spectrophotometry

Time-lapse protein fluorescence of MG$mCherry$ cells was measured by a BioTek Synergy HTX Multi-Mode Microplate Reader. Overnight cultured cells were diluted 1:1000 times into fresh M9 medium, aliquoted into 24 well dark bottom microplates, and kept at 37°C with shaking of 250 rpm. Fluorescence intensities were recorded for 14 hours, every 20 min, using excitation (590/20 nm) and emission (645/20 nm) filters. Temporal profiles of fluorescence intensity were extracted using Gen5 software, based on 6 biological repeats.

4.7. Statistical analyses of the data
Data points from microscopy, flow cytometry, etc. are based on at least 3 biological replicates each. Meanwhile, to assess if genes measured by flow cytometry were a good representative of the set of all σ^{70-38} genes, we used two-sample Kolmogorov-Smirnov tests (KS tests) to compare their sequences (D_{σ^{38}} and D_{σ^{70}}) and dynamics (LFC_{RNA}, μ, and CV^{2}). Further, gaussian fittings were made by the Distribution Fitter app (MATLAB). To evaluate its fitting, we calculated the coefficient of determination (R^2) between the PDF of the distributions and the PDF of the fitting. Finally, linear correlations were estimated by least-squares fitting (FITLM of MATLAB) and considered significant if the p-value of an F-test on the fitted regression line was smaller than 0.05. Other curves were fitted using MATLAB’s curve fitting toolbox (apart from the fitting of Hill functions, which was done using the Hill function [60]).

As for gene ontology representations, we perform overrepresentation tests using PANTHER Classification [61] for finding significant overrepresentations by Fisher’s exact tests. For p-values < 0.05, the null hypothesis that there are no associations between the gene cohort and the corresponding GO of the biological process is rejected. This p-value is corrected by calculating if the overall False Discovery Rate (FDR) is < 0.05. FDR is calculated by the Benjamini-Hochberg procedure [56].

4.8. Features of the promoter sequences

4.8.1. σ factor preference

Table S3 in S3 Appendix informs on the σ factor preference of the genes. From Regulon DB v10.5 (August 14, 2019), we obtained lists of all transcription units (TUs), promoters (including σ factor preferences), and genes of *E. coli* [62]. Recently, we compared our lists with information on July 1st, 2021 and found no changes that would
affect the conclusions. TUs only differed by ~1%, promoters by ~0.5%, and genes by ~1%.

From the 3548 TUs (gene(s) transcribed from a single promoter), we extracted 2179 with known promoters, containing 2713 genes in total. To minimize interference to the classification of σ factor preferences arising from transcription by multiple promoters, we narrowed our list to 1824 genes transcribed by only one promoter and, of those, to the 1328 genes with known σ factor preference. From those, 1242 have a preference for only one σ factor, including 931 with a preference for σ70, and 93 with a preference for σ38. Conversely, 76 genes have promoters with a preference for two σ factors. Out of these, 64 are transcribed by only one promoter with a preference for σ70 and σ38.

4.8.2. Promoter sequence logos

Promoter sequence logos were created using WebLogo [63]. In each position (from -41 to -1), we counted how many times a nucleotide is present in all promoters considered. Then, we stacked all nucleotides (A, C, T, G) on top of each other, and sorted from the least found one in the bottom to the most present one on the top. For each position, we quantified its ‘bit’, as the difference between the maximum information possible (entropy given the 4 nucleotides) and the information considering the variability of the nucleotides (sum of the entropy for each of the 4 nucleotides) in that position (observed entropy): Bit = \( \log_2 4 - \sum_{n=1}^{4} f_n \times \log_2 (f_n) \). From this, the height of each letter is set to be proportional to its frequency of occurrence. Finally, the height of each position was normalized, so as to equal its corresponding amount of information (with the more conserved positions having more bits) [64].

4.8.3. Promoter sequence p-distance
The p-distance $D$ of a promoter [65] is the fraction of its nucleotides between positions -41 to -1 (assuming that transcription start site starts at position +1) that differ from the consensus (most common) nucleotide in that position of a cohort of genes (here, genes with preference for a given $\sigma$ factor). We extracted the consensus sequences related to each $\sigma$ from RegulonDB [62]. To measure the $D$ of promoters with preference for both $\sigma^{38}$ and $\sigma^{70}$, we calculated the p-distances $D_{\sigma^{38}}$ and $D_{\sigma^{70}}$, and then an overall p-distance, $\Delta$, defined as: $\Delta = D_{\sigma^{38}} - D_{\sigma^{70}}$.

5. Supporting Information

S1 Appendix. Extended Methods and Materials. (Word)

S2 Appendix. Supporting Figure. (Word)

S3 Appendix. Supporting Tables. (Word)

S4 Table. List of genes classified as $\sigma^{70}$, and $\sigma^{38}$ or as both $\sigma^{70}$ and $\sigma^{38}$ dependent (Excel)

S5 Table. Consensus sequences of promoters with preference for one $\sigma$ factor. (Excel)

S6 Table. Fold changes in RNA levels of genes with a promoter with preference for $\sigma^{70}$ and $\sigma^{38}$. Measurements by RNA-seq in the exponential and stationary growth phases. (Excel)

S7 Table. Statistics of single-cell distributions of fluorescence of cells measured by flow cytometry. (Excel)
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