Translation elicits a growth rate-dependent, genome-wide, differential protein production in Bacillus subtilis

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Abstract

Complex regulatory programs control cell adaptation to environmental changes by setting condition-specific proteomes. In balanced growth, bacterial protein abundances depend on the dilution rate, transcript abundances and transcript-specific translation efficiencies. We revisited the current theory claiming the invariance of bacterial translation efficiency. By integrating genome-wide transcriptome datasets and datasets from a library of synthetic gfp-reporter fusions, we demonstrated that translation efficiencies in Bacillus subtilis decreased up to fourfold from slow to fast growth. The translation initiation regions elicited a growth rate-dependent, differential production of proteins without regulators, hence revealing a unique, hard-coded, growth rate-dependent mode of regulation. We combined model-based data analyses of transcript and protein abundances genome-wide and revealed that this global regulation is extensively used in B. subtilis. We eventually developed a knowledge-based, three-step translation initiation model, experimentally challenged the model predictions and proposed that a growth rate-dependent drop in free ribosome abundance accounted for the differential protein production.

Keywords Bacillus subtilis; global regulation; growth rate; protein production; translation efficiency

Subject Categories Genome-Scale & Integrative Biology; Quantitative Biology & Dynamical Systems; Protein Biosynthesis & Quality Control

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Introduction

The physiological state of the cell results from a complex interplay between environmental stimuli and the molecular mechanisms generating the major cellular functions (Crick, 1970; Bollenbach et al, 2009; Scott et al, 2010). As a result, each environmental condition coincides with a specific growth rate and a growth rate-dependent macromolecular composition (Schaechter et al, 1958; Bremer & Dennis, 2008). The abundance of the molecular machines (DNA and RNA polymerases, ribosome) strongly increases with increasing growth rate (Bremer & Dennis, 2008; Klumpp & Hwa, 2008; Klumpp et al, 2009). The pool of free polymerase (i.e. RNAPr70 available to initiate transcription) as well as the overall transcription efficiency increases, which in turn leads to a significant increase in total RNA and total rRNA abundances (Schaechter et al, 1958; Maaløe & Kjeldgaard, 1966; Marr, 1991; Bremer & Dennis, 2008; Klumpp & Hwa, 2008). In addition, the global growth rate-dependent variation in the transcription machinery abundance strongly and specifically influences the expression of each gene on the basis of their promoter sequence (Klumpp & Hwa, 2008; Gerosa et al, 2013). This effect is referred to as “global regulation”, and the promoter activity can be described by a Michaelis–Menten-type rate law as a function of the growth rate (Gerosa et al, 2013).

Global regulation operates at the level of translation by identically altering the production of each protein due the growth-related dilution (Liang et al, 2000; Bremer & Dennis, 2008; Klumpp et al, 2009). In contrast to transcription, the global growth rate-dependent variation in the translation machinery does not seem to trigger an additional global regulation specific to the genetic sequence of the translation initiation region (TIR). Indeed, the transcript-specific translation efficiency, defined as the number of proteins produced per mRNA per hour, was estimated to be invariant (Bremer & Dennis, 2008). As a consequence, the translation efficiency ($\lambda_i$) of each transcript ($m_i$) can be described by a constant (i.e. leading to the protein abundance: $P_i = \frac{\mu}{\mu_i} \lambda_i$ with $\mu$ being the rate of growth in h$^{-1}$ (Klumpp et al, 2009)).

The advent of high-resolution technologies and the consecutive generation of quantitative, genome-wide transcriptomic and proteomic datasets (Nicolas et al, 2012; Muntel et al, 2014; Goelzer et al, 2015) enable the estimation of the transcript-specific translation efficiencies genome-wide. The comparison of these proteome
and transcriptome datasets immediately suggests that the current model of the invariant transcript-specific translation efficiency is not satisfactory at the genome-scale level. The aim of this work was therefore to investigate the transcript-specific translation efficiency across growth conditions and to revisit the global regulation operating at the translation level. To this purpose, we combined genome-wide arrays, proteomics, qPCR and fluorescent reporter fusions to quantify transcript and protein abundances and deduce the transcript-specific translation efficiencies across growth conditions. We first demonstrated that translation efficiency does not remain constant but drops when growth rate increases. We furthermore revealed that the gene-specific translation initiation region (TIR) can drive a differential production of single proteins in the absence of any dedicated, specific regulators. We showed that the transcript-specific translation efficiency can be described by a Michaelis-Menten-type rate law as a function of the free ribosome abundance. We proposed that the drop in translation efficiency can result from a drop in abundance of the free ribosomes with increasing growth rates and estimated the drop in free ribosome abundance as well as the parameters of the model for over a thousand of transcripts. We further explored the sensitivity of the translational global regulation with respect to the addition of translation inhibitors. To precisely investigate the growth-rate dependency of the translation efficiency, we eventually developed a knowledge-based mathematical model of protein production and explored the possible interdependence of free ribosome, total ribosome and mRNA abundances.

Results

Growth-rate dependence of bacterial transcript abundances

We determined the intracellular abundance (per mass) of total RNA species in B. subtilis with increasing growth rate ($\mu$). We extracted total RNA from cells grown at various growth rates (from $\mu = 0.25$ to 1.70 h$^{-1}$), measured the amount of total RNA and the total amount of ribosomal RNA species and inferred the corresponding abundances (see Appendix section 1.3). Total RNA abundance increased as fast as the growth rate increased (Figs 1A and EV1A). The proportion of rRNA in total RNA remained constant at ~85% (Fig EV1B–D). We next developed a dedicated experimental approach to quantify the proportion of total mRNA in total RNA for each sample using genome-wide expression microarrays and a set of control “spike-in” transcripts (Materials and Methods, Fig 1B, Appendix section 1.4). A scaling factor was inferred from the intensity values of the retrotranscribed, in vitro-synthesized transcripts for each microarray (Fig 1C). The result was that the proportion of total mRNA in total RNA remained constant across samples, which directly implies that total mRNA abundance in B. subtilis does increase proportionally to the growth rate (Fig 1D).

By comparing the transcriptomes acquired in slow vs. fast growth conditions (Fig EV1E and F), we sorted the transcripts into two groups (Fig 1E): transcripts whose abundances increased faster than the total mRNA abundance (enriched species) and the remaining ones (depleted species). The first group represented ~39% of the total mRNA abundance at slow growth and reached up to 81% at fast growth. In particular, transcripts coding for ribosomal proteins that belong to the first group increased 2.5-fold from slow to fast growth (ribosomal mRNAs). Several unregulated (constitutive) genes fell into either of the two groups (Fig EV1G). Taken together, these results reveal a global, growth rate-dependent reorganization of the transcriptome (Fig 1E) and are consistent with the global regulation of the transcription machinery (Bremer & Dennis, 2008; Klumpp & Hwa, 2008; Gerosa et al, 2013).

Growth-rate dependence of the bacterial translation efficiency

In this work, we aimed at experimentally determining the transcript-specific translation efficiency across growth conditions. The transcript-specific translation efficiency ($\lambda_i$) is defined as the number of proteins produced per mRNA per hour, that is $\lambda_i = \frac{\mu_P}{m_i}$ (Bremer & Dennis, 2008). In order to accurately estimate the translation efficiency, we determined the abundance of a the stable variant of the green fluorescent protein (GFPmut3 (Botella et al, 2010)) at various growth rates in B. subtilis strains carrying GFPmut3 under the control of the promoter and translation initiation region (TIR) of the constitutive fbaA gene at the fbaA genomic locus ($\lambda_{fbaA}^{TIR_{fbaA}}$gfpmRNA, Table 1). GFP abundance from the $\lambda_{fbaA}^{TIR_{fbaA}}$gfpmRNA-reporter strain exhibited a fivefold decrease from slow to fast growth (Fig 2A). In order to systematically correct for possible differential stability between the $\lambda_{fbaA}^{TIR_{fbaA}}$gfpmRNA and the fbaA mRNA (Fig 2B) across growth conditions, we assessed the growth rate-dependent variation in $\lambda_{fbaA}^{TIR_{fbaA}}$gfpmRNA abundance by quantitative PCR (qPCR, Appendix section 1.5). The $\lambda_{fbaA}^{TIR_{fbaA}}$gfpmRNA transcript was slightly less stable than the fbaA transcript, in a growth rate-dependent manner (Fig 2C). Combining total mRNA quantification and qPCR-corrected transcriptome data, we then deduced that the translation efficiency of the $\lambda_{fbaA}^{TIR_{fbaA}}$gfpmRNA construct decreased fourfold from slow to fast growth (Fig 2D). We concluded that the resulting protein abundance decreased with increasing growth rate (from 0.4 to 1.7 h$^{-1}$), due equally to growth-related dilution (~fourfold) and to a decrease in the growth rate-dependent translation efficiency (~fourfold).

Translation initiation regions trigger different translation efficiency's growth-rate dependencies, and consequently differential growth rate-dependent protein production

Since the translation efficiency of a given transcript also depends on the genetic sequence of its translation initiation region (TIR (Vellanoweth & Rabinowitz, 1992)), we wondered whether the same constitutive protein expressed under the control of different TIRs may exhibit a variety of growth-rate dependencies. We therefore constructed a series of gfpmRNA-reporter strains combining one of the two promoters, $P_{fbaA}$ and $P_{hs}$ (a synthetic, isopropyl β-D-1-thio-galactopyranoside (IPTG)-inducible promoter) with one of eight additional synthetic translation initiation regions (TIRs, Table 1). The synthetic TIRs were derived from the natural TIR of fbaA (TIR$_{fbaA}$) by introducing point mutations into the RBS, the accommodation region of the ribosome, and/or changing both the sequence and the size of the accommodation region and modifying the start codon (Table 1). The synthetic TIRs were then inserted downstream of either the promoter and 5’UTR of fbaA ($\lambda_{fbaA}^{TIR}$) or the promoter and 5’UTR of the artificial $P_{hs}$ ($\lambda_{hs}^{TIR}$). For each synthetic strain, mRNAs were extracted from cells grown at
various growth rates and qPCRs were performed to correct for the stability differences between the various synthetic transcripts (Appendix section 1.5; Fig EV2A–D). We next quantified in media supporting growth rates ranging from 0.25 to 1.70 h⁻¹ the GFP abundances of 6 strains, which exhibited significantly dissimilar gene expression profiles with respect to the growth rate (fbaA/TIR fbaA gfp, fbaA/TIR short gfp, fbaA/TIR0 gfp, fbaA/TIR modif1 gfp, fbaA/TIR modif2 gfp and hs/TIR0 gfp; Figs 3A and EV2E and F, Appendix Table S1, Dataset EV2) and computed the ratios of the TIR-specific translation efficiencies. Hence, we performed minimization of mean-square-error-based estimation of the ratios of the translation efficiencies for every pair of strains (12 technical replicates * 2

**Figure 1.** Total RNA and mRNA abundances increase proportionally to the growth rate.

A Total RNA abundance in *B. subtilis* (blue dots) at different growth rates (0.25 h⁻¹, 0.40 h⁻¹, 0.60 h⁻¹, 1.2 h⁻¹ and 1.7 h⁻¹; this work). The 95% confidence intervals are shown with horizontal and vertical bars. Previously quantified total RNA abundance in *B. subtilis* (green dots) from Dauner et al (2001). Data are fit with a first-order polynomial.

B Scheme of the experimental design developed to quantify the relative abundance of total mRNA and of each transcript across growth conditions. \( r_1 + t_1 \) and \( r_2 + t_2 \) stand for total ribosomal RNA amounts, \( m_1 \) and \( m_2 \) stand for total transfer RNA amounts, and \( m_1 \) and \( m_2 \) stand for total messenger RNA amounts in growth conditions 1 and 2, respectively. S corresponds to the amount of the ten different in vitro-synthesized (spike-in) transcripts. \( c_1 \) stands for the amount of cDNA reverse transcribed from \( m_1 \) and \( S_i \). Note that the amount of \( S_i \) added to the total RNA extract of either growth condition is identical, which straightforwardly allows calculating the scaling factor between growth conditions (see Appendix section 1.4).

C Mean-normalized scaling factor.

D First-order polynomial fit of normalized total mRNA abundance (as inferred from the scaling factor and total RNA abundance). The total mRNA abundance measured at slow growth (\( \mu = 0.4 \) h⁻¹) was then assigned a value of 1.0 and total mRNA abundances across the other conditions were reported normalized to this value (in arbitrary units, AU; note that AU is a quantity). The 95% confidence interval is shown with shaded areas.

E The population of transcripts was sorted into two groups using a slow (\( \mu = 0.4 \) h⁻¹) and a fast growth condition (\( \mu = 1.7 \) h⁻¹). The first group contained transcripts for which the abundance increased faster than the growth rate (green), and the second group included all other transcripts (orange). The enriched sub-group of transcripts at fast growth contained the ribosomal mRNAs (dark green) and several non-ribosomal mRNAs (light green). The corresponding datasets can be found in Dataset EV1.
biological replicates, i.e. 24 replicates) grown under identical conditions (Appendix sections 3.1 and 3.2). The ratio of translation efficiencies between strains $\text{modif} \Delta \text{TIR}_0 \text{gfp}$ and $\text{short} \Delta \text{TIR}_0 \text{gfp}$ approximately doubled from 0.3 to 1.5 h$^{-1}$ (Fig 3B), indicating that the number of proteins produced per mRNA and per hour for these two synthetic constructs differently varies with growth rate as a result of only single point mutations in the TIR. We also observed a slight (1.3-fold) increase in the ratio of translation efficiencies between strains $\text{modif} \Delta \text{TIR}_0 \text{gfp}$ and $\text{modif} \Delta \text{TIR}_0 \text{gfp}$ from 0.30 to 0.38 (Fig 3B), which is the result of only a few point mutations 

 Experiment-based estimation of the translation efficiency parameters of the library of synthetic reporter fusions

Mathematical models of protein production that can handle a transcript-specific, growth rate-dependent translation efficiency (Appendix section 2.5) have previously been proposed (Kremling, 2007; Tadmor & Tlusty, 2008)). What we above defined as the translation efficiency is represented in these models by a Michaelis–Menten rate law, that is $\lambda_i = \frac{K_i}{R_{\text{free}}} \times [\text{gFP}]$, with two transcript-specific $K_i$ and $K_2$ constants, and where $R_{\text{free}}$ (free ribosome) abundance corresponds to the fraction of ribosomes ready to initiate translation (Kremling, 2007; Bremer & Dennis, 2008; Klumpp et al, 2009). According to a Michaelis–Menten-type translation initiation model, a drop in translation efficiency implies that $R_{\text{free}}$ abundance decreases with increasing growth rate. We therefore made use of the model and solved a constrained optimization problem using the GFP expression profiles from a representative subset of our synthetic strains (Table 1) to precisely infer the model parameters and the $R_{\text{free}}$ relative abundance with increasing growth rate (Appendix section 3.3). The inferred behaviour of $R_{\text{free}}$ abundance exhibited at least a fourfold decrease from slow to fast growth (Fig 4A). A sharp drop occurred between growth rates from 0.25 to 1.2 h$^{-1}$, and $R_{\text{free}}$ abundance reached a plateau thereafter. We also obtained a set of $\{K_1, K_2\}$ pairs for each construct that enabled us to accurately fit the corresponding GFP abundances (Fig 4B). Interestingly, the $K_2$ values ranged over 25-fold ($\text{modif} \Delta \text{TIR}_0 \text{gfp}$ vs. $\text{short} \Delta \text{TIR}_0 \text{gfp}$), which is the result of only a few point mutations

### Table 1. Strains and translation initiation regions (TIRs) used in this study.

| Strains* | Explicit naming | Genetic sequence upstream of the reporter gene (if relevant) |
|----------|-----------------|----------------------------------------------------------|
| Name†   | Promoter | Upstream 5′ UTR‡ | Pre-sequence | RBS§ | AR§ | IC‖ |
| OB01    | –       | –               | –           | –     | –   | –   |
| OB02    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC A TGG |
| OB03    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |
| OB04    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |
| OB05    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |
| OB06    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |
| OB07    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |
| OB08    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |
| OB09    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |
| OB10    | $\text{modif} \Delta \text{TIR}_0 \text{gfp}$  | fbaA           | GGA...88bp...ACA | GGT GGA | AGG AGG | TGA TCC AGT TGG |

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*Strains were constructed as indicated in Appendix Table S3.
†The sequence upstream of the pre-sequence depends on the promoter sequence (P$_\text{prom}$ or P$_\text{null}$).
‡RBS, ribosome-binding site.
§AR, Accommodation region. The sequence and length of the accommodation region affect ribosomal positioning onto the initiation codon.
‖IC, Initiation codon.
*Name of the B. subtilis strains which contain the GFP under control of the described TIR.
within the accommodation region (Table 1). As a consequence, the translation efficiencies showed various profiles as a function of the growth rate (Fig 4C). The translation efficiency of \( fbaA \) decreased by twofold from slow to fast growth, a behaviour that is clearly explained by a \( K_{2i} \) value (of 0.3) in the range of the \( R_{\text{free}} \) abundance variation (from 0.7 down to 0.2; Fig 4A and B). Eventually, when \( K_{2i} \) is very high compared to \( R_{\text{free}} \) abundance (e.g. \( fbaA^{\text{TIR}\text{mod1}} \ gfp \)), translation efficiency decreased as much as \( R_{\text{free}} \) abundance with increasing growth rate. Altogether, this analysis confirmed that translation efficiency can be entirely represented in terms of translation initiation complex recruitment to the ribosome (Fig EV3). This analysis led us to partition the transcriptome into three classes of transcripts: \( R_{\text{free}} \)-saturated, \( R_{\text{free}} \)-unsaturated and \( R_{\text{free}} \)-undersaturated (Fig 5A). Two-hundred and twenty-three transcripts (\( R_{\text{free}} \)-saturated) exhibited invariant translation efficiencies (i.e. independent of the variation in the \( R_{\text{free}} \) ribosome abundance: \( K_{2i} \ll R_{\text{free}} \); Fig EV3A; Dataset EV3). Six hundred and ninety-eight transcripts (\( R_{\text{free}} \)-unsaturated) exhibited translation efficiencies that nonlinearly depend on \( R_{\text{free}} \) ribosome abundance (with \( K_{2i} \) values in the range of \( R_{\text{free}} \) values). The two transcript-specific \( K_{1i} \) and \( K_{2i} \) aggregated constants were weakly correlated (\( r_{\text{Pearson}} = 0.60; r_{\text{Pearson}} = 0.56 \); Fig 5A; Dataset EV3). Finally, 81 transcripts (\( R_{\text{free}} \)-undersaturated) exhibited translation efficiencies that dropped with increasing growth rate proportionally to the free ribosome abundance (\( K_{2i} \gg R_{\text{free}} \)) with 95% confidence intervals spanned over several orders of magnitude (Fig EV3B; Dataset EV3).

The structural determinants at the ribosome-binding sites are key determinants for the recruitment of the initiation complex onto the mRNA (Milon et al., 2012). We therefore wondered whether by higher recruitment of the translation initiation complex, a long 5’UTR may be correlated with low \( K_{2i} \) values in \( B. subtilis \),

**Figure 2.** Protein abundance and translation efficiency of \( fbaA^{\text{TIR}\text{mod1}} \) drop at fast growth.

A. Blue dots represent the GFP abundance in different growth conditions (average of 12 technical replicates); the 95% confidence intervals are given as horizontal and vertical bars. Data were fit with a third-order polynomial (dashed line), and the 95% confidence interval of the fit is shown in grey.

B. Normalized \( fbaA \) transcript abundance (i.e. proportion of \( fbaA \) transcript within total mRNA × normalized total mRNA abundance from Fig 10).

C. Relative abundance (measured by qPCR) of the \( fbaA \) mRNA to the endogenous \( fbaA \) mRNA in strain \( fbaA^{\text{TIR}\text{mod1}} \) as a function of the growth rate. Filled circles are the mean of eight replicates in one medium; the 95% confidence intervals are depicted as bars. Data are fit with a third-order polynomial (dashed line), and the 95% confidence interval of the fit is shown in grey.

D. Deduced translation efficiency (\( h^i \)) of the \( fbaA^{\text{TIR}\text{mod1}} \) construct as a function of the growth rate. Note that translation efficiency in h^i is a slight abuse of notation since we divided protein abundance (in FAU OD600^-1) by the normalized transcript abundance (AU, quantity OD600^-1). The 95% confidence interval is shown in grey.

The corresponding datasets can be found in Datasets EV1 and EV2.

**Exploration of the variety of translation efficiency’s growth-rate dependencies in \( B. subtilis \) using proteomic datasets**

We wondered how representative of variation among endogenous \( B. subtilis \) transcripts are the TIR variants we constructed. We therefore explored the variety of translation efficiency’s growth-rate dependencies by combining the above computational approach, and quantitative transcriptome (this work) and proteome datasets obtained from identical growth conditions we recently published in Muntel et al (2014) and in Goelzer et al (2015). We estimated the \( K_{1i} \) and \( K_{2i} \) constants associated with each gene for which the cognate protein has been detected and correctly quantified in at least three growth conditions (i.e. 1,002 proteins; Appendix section 3.4 and Dataset EV3). This analysis led us to partition the transcriptome into three classes of transcripts: \( R_{\text{free}} \)-saturated, \( R_{\text{free}} \)-unsaturated and \( R_{\text{free}} \)-undersaturated (Fig 5A). Two-hundred and twenty-three transcripts (\( R_{\text{free}} \)-saturated) exhibited invariant translation efficiencies (i.e. independent of the variation in the \( R_{\text{free}} \) ribosome abundance: \( K_{2i} \ll R_{\text{free}} \); Fig EV3A; Dataset EV3). Six hundred and ninety-eight transcripts (\( R_{\text{free}} \)-unsaturated) exhibited translation efficiencies that nonlinearly depend on \( R_{\text{free}} \) ribosome abundance (with \( K_{2i} \) values in the range of \( R_{\text{free}} \) values). The two transcript-specific \( K_{1i} \) and \( K_{2i} \) aggregated constants were weakly correlated (\( r_{\text{Pearson}} = 0.60; r_{\text{Pearson}} = 0.56 \); Fig 5A; Dataset EV3). Finally, 81 transcripts (\( R_{\text{free}} \)-undersaturated) exhibited translation efficiencies that dropped with increasing growth rate proportionally to the free ribosome abundance (\( K_{2i} \gg R_{\text{free}} \)) with 95% confidence intervals spanned over several orders of magnitude (Fig EV3B; Dataset EV3).

The structural determinants at the ribosome-binding sites are key determinants for the recruitment of the initiation complex onto the mRNA (Milon et al., 2012). We therefore wondered whether by higher recruitment of the translation initiation complex, a long 5’UTR may be correlated with low \( K_{2i} \) values in \( B. subtilis \),
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Structural sensitivity of the global regulation of translation with respect to perturbations

We next wondered whether structurally disturbing the global regulation of translation would affect the translation efficiencies across growth conditions or whether there exists an unknown feedback regulation (most probably acting on free ribosome) that would rectify protein production with respect to the rate of growth (Klumpp et al, 2009). A straightforward approach to alter the translation process is to use translation inhibitors. When either chloramphenicol or tetracycline was added to the growth medium, Scott et al (2010) observed an increase in the total ribosome abundance in Escherichia coli to compensate for the inhibition of the elongation phase (Fig 6A). We therefore used tetracycline to disturb the translation process. In the presence of sub-inhibitory concentrations of tetracycline (0.5 and 1 mg l\(^{-1}\)), the growth rate was reduced and the ratios between the GFP proteins expressed under the control of \(\text{fbaA}\) translated \(gfp\) and \(\text{fbaA}\) translated \(\text{shortgfp}\) decreased, as compared to the same growth conditions in the absence of antibiotic (Fig 6B). With 1 mg l\(^{-1}\) of tetracycline, the ratio between \(\text{fbaA}\) translated \(gfp\) and \(\text{fbaA}\) translated \(\text{shortgfp}\) decreased by 50% in rich medium (CHG) and by 5% in poor medium (M9P; Fig 6B). By contrast, in the presence of 0.5 mg l\(^{-1}\) of tetracycline, the ratio only decreased by 25% in rich medium and by 5% in poor medium (Fig 6B). Overall, when translation was disturbed in the presence of tetracycline, the ratios between translation efficiencies were significantly altered for all growth rates over 0.4 h\(^{-1}\). Altogether, our results indicated that there is no feedback regulation that strictly rectified protein production with respect to the rate of growth, which consequently suggested that the growth-rate dependency of the global regulation of translation resulted from the variation in a molecular entity related to the total ribosome abundance.

An elementary three-step translation initiation model to explain the different translation efficiency’s growth-rate dependencies

In order to provide a rationale for modelling the translation efficiency in the form of a Michaelis–Menten-like equation, we developed a mechanistic model of protein production that decomposes translation initiation into three main molecular steps (Fig 7A, Table 2): i) ribosome binding to the mRNA; ii) accommodation of the ribosome to the start codon; and iii) initiation of translation elongation (Tomsic et al, 2000; Ramakrishnan, 2002; Milon et al, 2012). Translation initiation is then followed by the translation elongation and termination steps. The underlying molecular assumptions for modelling this process are described in the Appendix (sections 2.1 and 2.2). Due to the succession of reversible and irreversible steps (Fig 7A), the mathematical formalization of this elementary three-step translation initiation process leads to a Michaelis–Menten-type rate law of the translation efficiency (\(\lambda_i\)).

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One-fourth (157) over barely 600 mapped transcription starts are over 80 nucleotides upstream the coding sequence (Irnov et al, 2010). The \(K_S\) values of the 71 long 5’UTR messengers identified by our analysis (over the 157 long 5’UTR messengers known so far) were not significantly over-represented in any of the three classes of transcripts (Fig 5A and Dataset EV3). This analysis suggested that long 5’UTR is not the only molecular determinant for the growth-rate-dependent regulator-independent global regulation of translation.

We eventually (re)inferred the variation in \(R_{\text{free}}\) abundance using this extended dataset (Fig EV3C) and obtained, as expected, a relative drop in \(R_{\text{free}}\) abundance slightly larger than that obtained using the \(gfp\)-reporter strains from the synthetic library (see Appendix section 3.4). As shown in Fig 5B, the normalized translation efficiencies for the 1,002 transcripts therefore exhibited various growth-rate dependencies (from invariance to drop). Although we do not exclude the occurrence of a few growth condition-specific post-transcriptional regulation and/or degradation for a subset of proteins, our results demonstrated that \(B.\ subtilis\) extensively makes use of the newly identified regulator-independent mode of regulation.

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\(\text{Figure 3. Nonlinear translation efficiencies entail differential drops in protein abundances with increasing growth rate.}\)

\(\text{A} \quad \text{Circles represent the GFP abundance (in fluorescence arbitrary units by}\)
\(\text{optical density units: FAU OD}_{560}\) measured during exponential growth for 12 replicates at a given growth rate for constructs \(\text{fbaA}\) translated \(gfp\), \(\text{fbaA}\) translated \(\text{modif1 gfp}\) and \(\text{fbaA}\) translated \(\text{short gfp}\). The GFP levels were fit with a third-degree polynomial (dashed line); 95% confidence intervals are given in the coloured areas.

\(\text{B, C} \quad \text{Black dots represent the ratio of the GFP abundance after correction of}\)
\(\text{the different mRNA stability by qPCR (i.e. ratio of translation efficiencies,}\)
\(\lambda\) between two strains \(\text{fbaA}\) translated \(gfp\) vs. \(\text{fbaA}\) translated \(gfp\) in panel B;
\(\text{fbaA}\) translated \(\text{modif1 gfp}\) vs. \(\text{fbaA}\) translated \(\text{short gfp}\) in panel C) and the 95% confidence intervals are given as vertical bars (see Appendix section 3.1). The dashed curves are computed using the parameters estimated on Fig 4. The corresponding datasets can be found in Dataset EV2.

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\(\text{Figure 4. Growth rate-dependent bacterial translation}\)

\(\text{A, B, C}\)

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with a limiting molecular entity, the so-called free ribosome abundance ([R_{free}], Fig 7B). Following the common consensus used in the study of Bremer & Dennis, 2008; Klumpp et al., 2009; Kremling, 2007; Tadmor & Tlusty, 2008; R_{free} corresponds hereafter to the translation initiation complex and is composed of the 30S subunit of the ribosome, the initiation factors IF1, IF2 associated with GTP, and IF3, and the initiator tRNA, fMet-tRNAMet (30S-mRNA-IFs-GTP-fMet-tRNAMet; Fig 7A). The complete model (Fig EV3D) can be written in the form of a Michaelis–Menten-like equation (Fig 7B) using two integrative, growth rate-independent constants, K_{1i} and K_{2i}, which are composed of the primary TIR-dependent constants. It is worth to note that the two integrative constants shared several primary constants (Fig EV3D). Our model encompassed the simplest molecular scenario of the translation process; we nonetheless explored several other scenarios and alternative ribosomal assembly pathways by developing alternative models (Appendix section 2.4). The alternative models gave rise to translation efficiencies in the form of Michaelis–Menten-like equations except that the two aggregated constants (K_{1i} and K_{2i}) were constituted of different primary, kinetic parameters and/or that free ribosome was constituted of different ribosomal subunits. Altogether, our complete model turned to be a reasonable proxy of the translation process to account for the growth rate-dependent translation efficiencies.

Interdependence of free ribosome, total ribosome and mRNA abundances

The interdependence of free ribosome, total ribosome and mRNA abundances may directly set the free ribosome abundance across growth conditions due to the growth-rate dependency of both the RNA and ribosome production. We therefore theoretically explored how R_{free} abundance can naturally vary with growth rate by extending our knowledge-based model to the entire proteome (Appendix section 2.3). We demonstrated the formal relationship relating total ribosome (R_{total}) and mRNA species (m_i) with R_{free} (Fig 7C). Although R_{total} abundance increases with growth rate (Schaechter et al., 1958; Bremer & Dennis, 2008), an analysis of this relationship showed that the two following solutions can trigger a drop in R_{free} abundance (Fig 7D). The first solution consists of a strong increase in the abundances of all or many individual mRNAs with increasing growth rate (as shown in Fig 1D), which at least counterbalances the increase in total ribosome abundance to eventually lower the amount of available ribosomes (R_{free}). The second solution consists of a global reorganization of mRNA synthesis (as shown in Fig 1E), during which a class of transcripts (i.e. exhibiting low K_{2i} values such as the R_{free}-saturated transcripts) is relatively upregulated, while a class of transcripts exhibiting the converse properties is downregulated with increasing growth rate. A likely

Figure 4. R_{free} abundance drops at fast growth and results in different TIR-specific translation efficiencies.

A Inference of R_{free} abundance. R_{free} abundance was fit with a third-order polynomial (black line); the 95% confidence interval is given by the grey area (see Appendix section 3.3).

B Set of (K_{1i}, K_{2i}) pairs for six synthetic constructs. The three upper plots correspond to synthetic constructs having a translation efficiency as \( \lambda = \frac{K_{1i}/R_{free}}{K_{2i}} \), and the three lower plots correspond to synthetic constructs having a translation efficiency as \( \lambda = \frac{K_{1i}}{K_{2i}} \), with K_{2i} large as compared to [R_{free}]. The ratio \( \frac{K_{1i}}{K_{2i}} \) was scaled to 1 in order to relatively estimate the entire set of K_{1i} and K_{2i} constants (for detailed explanation, see Appendix section 3.3). Red curves represent model-based fits of GFP abundance quantified in more than 120 cultures for each bacterial strain (black dots).

C Relative translation efficiencies of the six strains from panel (B) (**TIR_{short}**) in blue, **TIR_{modif}** in red, **TIR_{modif2}** in green and **TIR_{modif3}** in black. The grey area corresponds to the space of possible translation efficiencies, which is bounded by the translation efficiency when K_{1i} is much larger than R_{free} (**TIR_{modif}** and **TIR_{modif3}**), and when R_{free} is much larger than K_{2i} (theoretical values, dashed black line). The corresponding datasets can be found in Dataset EV2.
biological interpretation is that such a reorganization of mRNA synthesis would enhance titration of free ribosome at fast growth and eventually lowers the amount of available ribosomes ($R_{\text{free}}$).

This prompted us to analyse the possible role of ribosomal mRNAs due to the enriched, large proportion of ribosomal proteins in total proteins at fast growth. Our combined statistical and model-based data analyses of genome-wide transcript and protein abundances have identified the $K^2_i$ values of 32 ribosomal proteins (among about 50 known ribosomal proteins, Dataset EV3). As expected, most of the 32 $K^2_i$ values were close to zero, which indicated that their translation is barely affected by the rate of growth. Actually, the rate of ribosomal protein synthesis is cross-coordinated with the available amount of rRNA by an autogenous feedback regulation (Nomura’s model) in $B. subtilis$ (Grundy & Henkin, 1991; Choonee et al, 2007) similar to that in $E. coli$ (Kaczanowska & Ryden-Aulin, 2007). We then theoretically explored how this feedback regulation interferes with our whole-proteome modelling approach and demonstrated that the enrichment of ribosomal mRNAs with increasing growth rate (Fig 1E) could not alone explain the drop in $R_{\text{free}}$ abundance (Appendix section 2.3). As a consequence, if the growth rate-dependent variation in $R_{\text{free}}$ abundance only resulted from the trade-off between the titrations of free ribosomes with the non-ribosomal and ribosomal mRNAs, several other transcripts contributed to the observed drop in $R_{\text{free}}$ abundance. Altogether, our
analysis suggested that the interdependence of free ribosome, total ribosome and mRNA abundances is the cornerstone of growth condition-specific cell (re)programming.

Discussion

Prokaryotes adapt to environmental changes by adjusting their transcriptome in a complex manner mostly via the use of DNA-binding regulators that respond to environmental signals or metabolic effectors (Lu et al, 2007; Goelzer et al, 2008; Buescher et al, 2012; Nicolas et al, 2012). Yet, the growth rate-dependent variation in abundance of the transcription machinery also strongly influences gene expression (Klumpp & Hwa, 2008; Gerosa et al, 2013). Indeed, total RNA abundance (per mass) is well known to increase with increasing growth rate in both Gram-negative (E. coli and Salmonella typhimurium) and Gram-positive (B. subtilis) bacteria (Schaechter et al, 1958; Dauner et al, 2001; Bremer & Dennis, 2008). We reassessed total RNA and total mRNA using up-to-date, high-resolution technologies in B. subtilis. We confirmed that total RNA abundance increases twofold when the growth rate doubles and showed that total mRNA abundance represents a constant fraction of the total RNA abundance for all tested growth rates (Fig 1C). Altogether, it implies that the total mRNA abundance in B. subtilis increases twofold when the growth rate doubles. These results are consistent with a recent investigation reporting that mRNA
Table 2. Molecular steps of the knowledge-based model of protein production, degradation and dilution.

| Step | Molecular reaction | Parameters |
|------|-------------------|------------|
| ①   | Ribosome binding  | $R_{\text{free}} = 30S+1F1+F2+3\text{tRNA}^{\text{Met}}$ (free ribosome) |
|      | $R_{\text{free}} + mF_i F_i R_{\text{free}} \xrightarrow{k_{\text{on}} + k_{\text{off}}} R_{\text{active}}$ |
|      | $m_F = $ free mRNA coded by the gene \( i \) |
|      | $R_{\text{active}} = 30S+F1+F2+3\text{tRNA}^{\text{Met}}$ (active ribosome) |
|      | $k_{\text{on}} = $ rate of free ribosome binding |
|      | $k_{\text{off}} = $ rate of free ribosome release |
| ②   | Ribosome accommodation | $R_{\text{active}} \xrightarrow{k_{\text{u}}} R_{\text{active}}$ |
|      | $k_{\text{u}} = $ rate of ribosome positioning onto the start codon |
|      | $k_{\text{u}} = $ rate of ribosome release from the start codon |
| ③   | Initiation of translation elongation | $R_{\text{active}} + 50\text{S}^{\text{free}} \xrightarrow{k_i} R_{\text{active}}$ |
|      | $50\text{S}^{\text{free}} = $ free 50S subunit of the ribosome |
|      | $k_i = $ rate of initiation of translation elongation |
| Completion | $R_{\text{active}} \xrightarrow{k_i} P_i$ |
|      | $P_i = $ protein coded by the gene \( i \) |
|      | $k_p = $ protein elongation aggregated parameter (equal to the inverse the population averaged time of protein elongation) |
| Degradation | $P_i \xrightarrow{\gamma_i} \emptyset$ |
|      | $\gamma_i = $ rate of protein degradation |
| Dilution | $P_i \xrightarrow{\mu} \emptyset$ |
|      | $\mu = $ growth rate |

abundance in *E. coli* at least doubles when growth rate increases from 0.11 to 0.49 h\(^{-1}\) (Valgepea et al., 2013).

Translation efficiency based on Michaelis–Menten kinetics: the what, why and how

In Gram-positive and Gram-negative bacteria, the drop in abundance of constitutively expressed proteins is comparable (Bremer & Dennis, 2008; Klumpp et al., 2009; Scott et al., 2010) and this work). In this study, we demonstrated that this drop in *B. subtilis* is not only due to the dilution but also to an unexpected decrease in translation efficiency (Fig 2). We also observed that the translation efficiencies of two proteins can exhibit different growth-rate dependencies (Fig 3B and C). To provide a rationale to the observed translation efficiency’s growth-rate dependencies (Appendix Fig S1) across normal growth conditions or upon antibiotic addition (Figs 3 and 6), we developed and analysed an elementary knowledge-based model of translation in which translation initiation is decomposed into three main steps. Such formalization ensued from straightforward, reasonable biological assumptions and took into account the commonly accepted scenario of ribosome assembly and translation initiation in *vivo*. However, several others scenarios and pathways of ribosome assembly have recently been characterized in *vivo* with *E. coli* cell-free extracts (Tsai et al., 2012). We thoroughly explored these scenarios and the alternative ribosomal assembly pathways by developing alternative models (Appendix section 2.4). The alternative models also gave rise to translation efficiencies in the form of Michaelis–Menten-like equations except that the two aggregated constants ($K_{11}$ and $K_{22}$) were constituted of different primary, kinetic parameters. Altogether, it means that modelling the translation efficiency in the form of a Michaelis–Menten-like equation is relevant to investigate bacterial translation. Interestingly, an early study from the nineties in *E. coli* also tends to support the existence of differential protein productions (Jacques et al., 1992), which in turn supposes a variation in $R_{\text{free}}$ abundance in Gram-negative bacteria. In order to draw firm conclusions, dedicated experiments should now be performed with *E. coli* to confirm Gram-negative bacteria that translation efficiencies can exhibit different growth-rate dependencies, especially by altering the TIR-related $K_{11}$ and $K_{22}$ constants and $R_{\text{free}}$ abundance.

Our whole-proteome modelling approach suggested that the global regulation of translation mediated by the growth rate-dependent variation in $R_{\text{free}}$ abundance may only result from the interdependence of the *free* ribosome, total ribosome and mRNA abundances across the growth conditions. Because of the strong increase in total ribosome abundance with increasing growth rate, the increase in ribosomal mRNA abundance could not trigger alone the observed drop in *free* ribosome abundance. This suggested that the growth rate-dependent reorganization of the expression of each transcript in the cell is specifically hard-coded on the genome to set the proper amount of *free* ribosomes for a given growth condition. As a consequence, there might not exist any specific feedback regulation controlling the abundance of *free* ribosome and therefore the translation efficiency’s growth-rate dependencies of each transcript in the cell. As a corollary, perturbation of the growth rate-dependent variation in the *free* ribosome abundance is expected to alter the gene-specific translation efficiency’s growth-rate dependencies. In this study, we demonstrated that the gene-specific translation efficiency’s growth-rate dependencies were sensitive to the presence in the growth media of sub-inhibitory concentrations of tetracycline. It is tempting to postulate that tetracycline modified the growth rate-dependent drop in the *free* ribosome abundance, which in turn altered the gene-specific translation efficiency’s growth-rate dependencies. We therefore inferred the growth rate-dependent evolution of the *free* ribosome abundance across growth conditions in the presence of 1 mg l\(^{-1}\) of tetracycline, which now exhibited a maximum twofold decrease from slow to fast growth (Fig EV3E). However, we cannot exclude that tetracycline affected translation initiation in a way that altered the two $K_{11}$ and $K_{22}$ growth rate-independent aggregated constants. Nevertheless, in the presence of tetracycline cells were unable to set right the production of proteins with respect to the rate of growth. Altogether, it suggests that there is no strong feedback control on the abundance of *free* ribosome across growth conditions.

The space of translation efficiencies allows *B. subtilis* cells to adjust single protein abundances across growth conditions

Our combined statistical and model-based data analyses of genome-wide transcript and protein abundances uncovered a large range (several orders of magnitude) of $K_{22}$ aggregated constants and revealed that the drop in *free* ribosome abundance altered...
genome-wide the translation efficiency’s growth-rate dependencies. According to the model, the translation efficiency can be constant at any growth rates if the $K_2$ value is very small as compared to that of $R_{free}$. If such is the case, the gene-specific translation efficiencies simplify into the $K_1$ values and the current model of Hwa and colleagues (Scott et al, 2010) perfectly describes the process of protein production. Our results showed that it was indeed the case for about one-fourth of the transcripts (i.e. 223 genes) for which we have characterized the \{$K_1$, $K_2$\} pairs. In addition, several other transcripts (about 100 among the 698 transcripts from Fig 5A) are only barely affected by the drop in free ribosome abundance, so that the resulting dilution-corrected protein abundances may also be perceived as invariant. Remarkably, the 1,002 transcript-related translation efficiencies fully filled in the space predicted by the three-step translation initiation model between $K_1$ and $K_2$ (Fig 5B vs. Fig 4C). Till now, translation initiation regions were believed to be shaped by evolution in order to optimize the trade-off between the level of expression and the resulting gene expression noise. The gene expression noise is indeed important for the highly translated transcripts (Ozbudak et al, 2002; Ferguson et al, 2012). However and in view of our results, TIRs must also be regarded as targets for selective evolution towards optimized cellular networks, in particular by efficient adjustment of the growth-rate dependencies. An open issue is whether evolutionary constraints provided more incentives to set protein abundances across growth conditions (see Appendix section 3.5) or the resulting noise, and whether independently modulating the $K_1$ and $K_2$ variables for a given protein abundance will generate different levels of noise.

Transcriptional and translational growth rate-dependent global regulations optimize cellular fitness without dedicated regulators

The growth rate-dependent regulation (also referred to as global regulation) of bacterial translation provides cells with a remarkable toolbox to tweak protein production. However, translation efficiency is either a constant or a decreasing function of growth rate. By contrast, the global regulation of transcription via the transcription efficiency is an increasing function of growth rate (Klumpp & Hwa, 2008; Gerosa et al, 2013). When combined, the global regulations of transcription and translation may provide cells with a much larger range of protein expression in the absence of dedicated regulators and may allow prokaryotes to fine-tune the abundance of each protein as a function of the growth rate even in the absence of dedicated regulators (Figs 8, EV4 and EV5). We can therefore question why do cells need dedicated regulators? By

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Figure 8. Schematic representation of the interdependence of free ribosome abundance and both mRNA and ribosome abundances. The $K_2$-related global reorganization of mRNA expression leads to a drop in $R_{free}$ with increasing growth rate, which in turn promotes differential protein production. From low to high growth rates, the total mRNA and total ribosomal abundances as well as the protein elongation aggregated parameter $R_{tot}$, Table 2; Bremer & Dennis, 2008) increase, while the free ribosome abundance drops. Titration of $R_{free}$ is weaker at low than at fast growth because transcripts exhibiting strong $K_2$ and low $K_1$ (green TIR) are relatively upregulated and those exhibiting converse properties (orange TIR) downregulated with increasing growth rate. The ribosome density along the mRNA is the combined outcome of the translation efficiency and the elongation rate. The "red" and "green" proteins are differentially produced with increasing growth rate due to the growth rate-dependent translation efficiency. See also a more detailed representation in Fig EV5.
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responding to specific signals, regulators allow bringing discontinuity in protein expression across growth conditions, as opposed to the nonlinear but continuous transcription and translation efficiencies functions. Evolutionary theories suggest that protein expression levels maximize fitness (Dekel & Alon, 2005; Molenaar et al., 2009; Goelzer et al., 2011; Goelzer & Fromion, 2011). In particular, the growth rate-dependent regulations may contribute to cost minimization by providing reasonable solutions to the cost–benefit optimization of the cellular and metabolic processes that must be active under all growth conditions and consequently to fitness increase across growth conditions (Goelzer et al., 2015).

We revealed that the variations in the free ribosome abundance can globally contribute to proper cellular (re)programming and have evidenced a unique, hard-coded, growth rate-dependent mode of translation regulation, which manages genome-wide gene expression in addition to specific post-transcriptional and translational regulations. Our findings on the global regulation of translation, together with previous findings on the global regulation of transcription (Klumpp & Hwa, 2008; Gerosa et al., 2013), will open pioneering opportunities for the differential modulation of complex synthetic circuits. By coupling high-throughput, precise genome editing technologies, we can now envision a rational, in silico design and experimental (re)programming of a high performing cell factory genomically streamlined to optimize growth and production properties at a given growth rate.

Materials and Methods

Biological materials

Escherichia coli TG1 was used for plasmid constructions and transformations using standard techniques (Sambrook et al., 1989). A tryptophan prototrophic B. subtilis 168 (BSB168) strain (Botella et al., 2010) was transformed using standard procedures (Anagnostopoulou & Spizizen, 1961). The reference OB1 strain, containing the P\text{hs} (IPTG-inducible hyperspangk promoter), derived from BSB168 by double crossover insertion in the amyE locus of the pOB1 plasmid. To generate the Cm\text{R} pOB1 plasmid, the pDR111 plasmid (kind gift of David Rudner), which carries the P\text{hs} and lacI gene between two arms of the amyE gene, was digested by SacII/EcoRI and the 3,083-bp fragment was sub-cloned into the SacII/EcoRI sites of pDG1661 (Guérout-Fleury et al., 1996). To generate vectors that contained the gfp\text{mut3} gene downstream of a given translation initiation region (TIR), we amplified by inverse PCR the entire p8ha\text{SysBioI} plasmid (Botella et al., 2010) using primers introducing point mutations in the original TIR but conserving the ligation-independent cloning site (LIC). For LIC, the vector-related PCR products were gel-purified, treated with T4 DNA polymerase and 2.5 mM dATP, and resulting fragments phosphorylated with T4 polynucleotide kinase. Promoter regions (400 bp) of fbaA (P\text{fbaA}) and hyperspangk (P\text{hs}) were generated by PCR from OB1 genomic DNA using the appropriate primers listed in Appendix Table S2. Then, 0.2 pmol of each PCR amplification was incubated with 2.5 mM dTTP and T4 DNA polymerase. A mix of 5 ng of prepared vector-related fragment and 15 ng of promoter-related PCR amplification was used to transform E. coli. The resulting plasmids were extracted from E. coli, used to transform OB1 strain by single crossover either at the fbaA locus or at the hyperspangk locus (within amyE), leading to the strains listed in Table 1.

Growth conditions

LB was used to grow E. coli and B. subtilis. The eight growth media used for B. subtilis strain to reach growth rates were modified from previously described growth media (Harwood & Cutting, 1990; Partridge & Errington, 1993; Sharpe et al., 1998; Kleijn et al., 2010; Chubukov et al., 2013). The composition of the different media is described in Appendix (section 1.1). When required, media were supplemented with antibiotics at the following abundances for B. subtilis/E. coli: 100 μg ml\textsuperscript{–}1 of ampicillin (only for E. coli), 200/5 μg ml\textsuperscript{–}1 of spectinomycin or 20/5 μg ml\textsuperscript{–}1 of chloramphenicol. The related bacterial growth rates were 0.25 h\textsuperscript{–}1 in M9P (M9 pyruvate), 0.40 h\textsuperscript{–}1 in M9SE (M9 succinate/glutamate), 0.60 h\textsuperscript{–}1 in S, 0.75 h\textsuperscript{–}1 in M9G (M9 glucose), 0.80 h\textsuperscript{–}1 in M9M (M9 malate), 0.90 h\textsuperscript{–}1 in TS, 1.20 h\textsuperscript{–}1 in CH and 1.70 h\textsuperscript{–}1 in CHG. To alter ribosome abundance, the M9P, M9SE, S, TS, CH and CHG media were supplemented with 0.5 or 1 mg l\textsuperscript{–}1 of tetracycline.

Quantification of RNA molecular species

RNA extraction was modified from Nicolas et al. (2012) (Appendix section 1.4). Total RNA quantification was performed using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). RNA quality and rRNA quantification were assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies). In order to scale the gene-level intensities from each microarray to and determine the total mRNA fraction out of the total RNA pool, an equal amount of in vitro-synthesized transcripts (One Color RNA Spike-In Kit, Agilent Technologies) was added to 10 μg of each total RNA sample. Synthesis, one-colour hybridization of fluorescently labelled cDNA to Agilent custom microarrays and data processing are described in Appendix (section 1.4). Experimental procedure for real-time quantitative PCR is depicted in Appendix (section 1.5).

Protein abundance determination by Live Cell Array

For live cell array experiments, a single colony of B. subtilis was grown in a well of a 96-well microtitre plate (Cellstar\textsuperscript{®}, Greiner bio-one) with Luria–Bertani (LB) medium until an OD\textsubscript{600} of 0.4–0.5. For precultures in the medium of interest, LB-grown cells were diluted 400-fold into 96-well microtitre plates and incubated overnight under constant shaking at 37°C until OD\textsubscript{600} reached 0.3. The cultures, with a dilution that yielded exponentially growing cultures next morning, were diluted in 100 μl of the same medium to an OD\textsubscript{600} of 0.001 into 96-well microtitre plates and incubated at 37°C with constant shaking in a Synergy\textsuperscript{™} 2 multimode microplate reader (BioTek) for at least 20 hours. OD\textsubscript{600} and fluorescence (excitation: 485/20 nm, emission: 528/20 nm) were measured at an interval of 7 minutes. OD\textsubscript{600} and OD\textsubscript{377} were measured once at the beginning of the experiment in order to correct the optical path length to 1 cm using the following equation: (OD\textsubscript{377}–OD\textsubscript{600})/0.18. Data were extracted and processed as previously described (Aichaoui et al., 2012; Botella et al., 2010; Buescher et al., 2012; see Appendix sections 1.6 and 1.7).

Data availability

Data generated in this work are provided in Dataset EV files. The microarray data have been made publically available in Gene
Expression Omnibus (GEO) database with the accession number GSE78108. Proteomic datasets used in this study are available in Goelzer et al. (2015).

Expanded View for this article is available online.

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Author contributions
OB constructed the library of plasmids and strains, collected data, performed qPCR and transcriptomic studies, mathematical modelling and analysed data; AG was involved in mathematical modelling; MS and UM performed RNA hybridization; MC collected data; SA was involved in study design and discussion of the results; and MJ and VF designed the study, analysed data and wrote the paper.

Conflict of interest
The authors declare that they have no conflict of interest.

References
Aichaoui L, Jules M, Le Chat L, Aymerich S, Fromion V, Goelzer A (2012) BasyLiCA: a tool for automatic processing of a Bacterial Live Cell Array. Bioinformatics 28: 2705 – 2706
Anagnostopoulous C, Spizizen J (1961) Requirements for transformation in Bacillus subtilis. J Bacteriol 81: 741 – 746
Bollenbach T, Quan S, Chait R, Kishony R (2007) Modulation of chemical composition and other parameters of the cell at different exponential growth rates. In: EcoSal — Escherichia coli and Salmonella: Cellular and Molecular Biology, Böck A, Curtiss III R, Kaper JB, Karp PD, Neidhardt FC, Nyström T, Slauch JM, Squires CL, Ussery D (eds), 730 – 738. Washington, DC: ASM Press
Buescher JM, Liebermeister W, Jules M, Uhr M, Muntel J, Botella E, Hessling B, Kleijn RJ, Le Chat L, Leconte F, Mader U, Nicolas P, Piersma S, Sugheimer F, Becker D, Bessieres P, Benkeno E, Denham EL, Derun V, Devine KM et al. (2012) Global network reorganization during dynamic adaptations of Bacillus subtilis metabolism. Science 335: 1099 – 1103
Choonee N, Even S, Zig L, Putzer H (2007) Ribosomal protein L20 controls expression of the Bacillus subtilis infC operon via a transcription attenuation mechanism. Nucleic Acids Res 35: 1578 – 1588
Chubukov V, Uhr M, Le Chat L, Kleijn RJ, Jules M, Link H, Aymerich S, Stelling J, Sauer U (2013) Transcriptional regulation is insufficient to explain substrate-induced flux changes in Bacillus subtilis. Mol Syst Biol 9: 709
Crick F (1970) Central dogma of molecular biology. Nature 227: 561 – 563
Dauner M, Stormi T, Sauer U (2001) Bacillus subtilis metabolism and energetics in carbon-limited and excess-carbon chemostat culture. J Bacteriol 183: 7308 – 7317
Dekel E, Alon U (2005) Optimality and evolutionary tuning of the expression level of a protein. Nature 436: 588 – 592
Ferguson ML, Le Coq D, Jules M, Aymerich S, Radulescu O, Declerck N, Royer CA (2012) Reconciling molecular regulatory mechanisms with noise patterns of bacterial metabolic promoters in induced and repressed states. Proc Natl Acad Sci USA 109: 155 – 160
Gerosa L, Kochanowski K, Heinemann M, Sauer U (2013) Dissecting specific and global transcriptional regulation of bacterial gene expression. Mol Syst Biol 9: 658
Goelzer A, Brikci FB, Martin-Verstraete I, Noirot P, Bessieres P, Aymerich S, Fromion V (2008) Reconstruction and analysis of the genetic and metabolic regulatory networks of the central metabolism of Bacillus subtilis. BMC Syst Biol 2: 20
Goelzer A, Fromion V (2011) Bacterial growth rate reflects a bottleneck in resource allocation. Biochim Biophys Acta 1810: 978 – 988
Goelzer A, Fromion V, Scorletti G (2011) Cell design in bacteria as a convex optimization problem. Automatica 47: 1210 – 1218
Goelzer A, Muntel J, Chubukov V, Jules M, Prestel E, Nolker R, Mariadassou M, Aymerich S, Hecker M, Noirot P, Becher D, Fromion V (2015) Quantitative prediction of genome-wide resource allocation in bacteria. Metab Eng 32: 232 – 243
Grundy Fj, Henkin TM (1991) The rpsD gene, encoding ribosomal protein S4, is autogenously regulated in Bacillus subtilis. J Bacteriol 173: 4595 – 4602
Guerout-Fleury AM, Frandsen N, Streger P (1996) Plasmids for ectopic integration in Bacillus subtilis. Gene 180: 57 – 61
Harwood CR, Cutting SM (1990) Chemically defined growth media and supplements. In: Molecular Biological Methods for Bacillus, Harwood CR, Cutting SM (eds), p 548. Chichester, UK: Wiley
Imov I, Sharma CM, Vogel J, Winkler WC (2010) Identification of regulatory RNAs in Bacillus subtilis. Nucleic Acids Res 38: 6637 – 6651
Jacques N, Guillerrez J, Dreyfus M (1992) Culture conditions differentially affect the translation of individual Escherichia coli mRNAs. J Mol Biol 226: 579 – 608
Kaczanowska M, Ryden-Aulin M (2007) Ribosome biogenesis and the translation process in Escherichia coli. Microbial Mol Biol Rev 71: 477 – 494
Kleijn RJ, Buescher JM, Le CL, Jules M, Aymerich S, Sauer U (2010) Metabolic fluxes during strong carbon catabolite repression by malate in Bacillus subtilis. J Biol Chem 285: 1587 – 1596
Klumpp S, Hwa T (2008) Growth-rate-dependent partitioning of RNA polymerases in bacteria. Proc Natl Acad Sci USA 105: 20245 – 20250
Klumpp S, Zhang Z, Hwa T (2009) Growth rate-dependent global effects on gene expression in bacteria. Cell 139: 1366 – 1375
Kremling A (2007) Comment on mathematical models which describe transcription and calculate the relationship between mRNA and protein expression ratio. Biotechnol Bioeng 96: 815 – 819
Liang ST, Xu YC, Dennis P, Bremer H (2000) mRNA composition and control of bacterial gene expression. J Bacteriol 182: 3037 – 3044
Li P, Vogel C, Wang R, Yao X, Marcotte EM (2007) Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation. Nat Biotechnol 25: 117 – 124
Maaløe O, Kjeldgaard NO (1966) Control of Macromolecular Synthesis: A Study of DNA, RNA, and Protein Synthesis in Bacteria. New York: W. A. Benjamin, Inc.
Marr AG (1991) Growth rate of Escherichia coli. Microbiol Rev 55: 316–333
Milon P, Maracci C, Filonava L, Gualerzi CO, Rodnina MV (2012) Real-time assembly landscape of bacterial 30S translation initiation complex. Nat Struct Mol Biol 19: 609–615
Molenaar D, van Berlo R, de Ridder D, Teusink B (2009) Shifts in growth strategies reflect tradeoffs in cellular economics. Mol Syst Biol 5: 323
Muntel J, Fromion V, Goelzer A, Maabeta S, Mader U, Buttnert K, Hecker M, Becher D (2014) Comprehensive absolute quantification of the cytosolic proteome of Bacillus subtilis by data independent, parallel fragmentation in liquid chromatography/mass spectrometry (LC/MS[E]). Mol Cell Proteomics 13: 1008–1019
Nicolas P, Mader U, Dervyn E, Roche T, Leduc A, Pigeonneau N, Bidenko E, Marchadier E, Hoebeke M, Aymerich S, Botella E, Delumeau O, Doherty G, Denham EL, Fogg MJ, Fromion V, Goelzer A, Hansen A et al (2012) Condition-dependent transcriptome reveals high-level regulatory architecture in Bacillus subtilis. Science 335: 1103–1106
Ozbudak EM, Thattai M, Kurtser I, Grossman AD, van Oudenaarden A (2002) Regulation of noise in the expression of a single gene. Nat Genet 31: 69–73
Partridge SR, Errington J (1993) The importance of morphological events and intercellular interactions in the regulation of prespore-specific gene expression during sporulation in Bacillus subtilis. Mol Microbiol 8: 945–955
Ramakrishnan V (2002) Ribosome structure and the mechanism of translation. Cell 108: 557–572
Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, 2nd edn. New York: Cold Spring Harbor Laboratory
Schaechter M, Maaloe O, Kjeldgaard NO (1958) Dependency on medium and temperature of cell size and chemical composition during balanced growth of Salmonella typhimurium. J Gen Microbiol 19: 592–606
Scott M, Gunderson CW, Mateescu EM, Zhang Z, Hwa T (2010) Interdependence of cell growth and gene expression: origins and consequences. Science 330: 1099–1102
Sharpe ME, Hauser PM, Sharpe RG, Errington J (1998) Bacillus subtilis cell cycle as studied by fluorescence microscopy: constancy of cell length at initiation of DNA replication and evidence for active nucleoid partitioning. J Bacteriol 180: 547–555
Tadmor AD, Tlusty T (2008) A coarse-grained biophysical model of E. coli and its application to perturbation of the rRNA operon copy number. PLoS Comput Biol 4: e1000038
Tomsic J, Vitali LA, Daviter T, Savelserbergh A, Spurio R, Striebeck P, Wintemeyer W, Rodnina MV, Gualerzi CO (2000) Late events of translation initiation in bacteria: a kinetic analysis. EMBO J 19: 2127–2136
Tsai A, Petrov A, Marshall RA, Korlach J, Uemura S, Puglisi JD (2012) Heterogeneous pathways and timing of factor departure during translation initiation. Nature 487: 390–393
Valgepea K, Adamberg K, Seiman A, Vilu R (2013) Escherichia coli achieves faster growth by increasing catalytic and translation rates of proteins. Mol BioSyst 9: 2344–2358
Vellanoweth RL, Rabinowitz JC (1992) The influence of ribosome-binding-site elements on translational efficiency in Bacillus subtilis and Escherichia coli in vivo. Mol Microbiol 6: 1105–1114

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