A decrease in transcription capacity limits growth rate upon translation inhibition

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Abstract

In bacterial cells, inhibition of ribosomes by sublethal concentrations of antibiotics leads to a decrease in growth rate despite an increase in ribosome content. This has been proposed to result from a decrease in the resources available for the production of non-ribosomal proteins that become limiting for cellular metabolism, mediated by changes in ppGpp concentration. Here, by the comparison of the activity of promoters that are differentially regulated by ppGpp and with different affinities for RNAP, we show that increased ribosome inhibition instead results in a decrease in total RNAP activity, independently of the changes in ppGpp. This is due to the decrease in ribosome processivity which affects more strongly the translation of longer genes, such as those coding for the β and β' subunits of RNAP, amongst the longest genes in the *E. coli* genome, while the expression of the very short ribosomal proteins is not limited to the same extent. Furthermore, the decrease in the total amount of free RNAP favors the expression of genes with promoters with a high RNAP affinity, such as ribosomal promoters, over the expression of other, growth-limiting, proteins. The decrease in transcription capacity could thus lead to increased survival whenever ribosome activity is compromised.

Bacteria often encounter sub-lethal levels of antibiotics produced by other microorganisms in their environment that can lead to a decrease in growth rate, allowing a strain to survive long enough until the inhibitor is no longer present or, in some cases, until the bacteria becomes resistant to the antibiotic via the selection of pre-existing mutations or an increase in mutation rates [1–4]. However, the mechanistic details of these response pathways often remain to be described. The cellular response to the limitation of translation activity is thought to be related to the pathway involved in the stringent response, the regulatory mechanism that decreases

49 ribosome production in response to a decrease in amino acid availability. This is mediated by
50 the change in concentration of the secondary messenger molecule, (p)ppGpp, that is produced
51 by the RelA enzyme when the pool of amino acids decreases and ribosomes are not loaded with
52 charged tRNAs[5,6]. ppGpp can directly inhibit ribosome assembly[7,8] and the activity of RNA
53 polymerase (RNAP) at ribosomal promoters, while increasing the activity of the promoters of
54 genes for amino acid biosynthesis[9]**.** The transcription of ribosomal operons can use a large
55 fraction of the free RNA polymerase pool in the cell because of the high affinity of the ribosomal
56 promoters for the enzyme and a high frequency of transcription initiation. Therefore, the
57 regulation of ribosomal promoter activity can be a means by which the pool of free RNA
58 polymerase can be repartitioned between ribosomal operon transcription and non-ribosomal
59 mRNA synthesis (Fig. 1A). This has been referred to as the passive control of transcription
60 regulation [10–14].
61 The ppGpp dependent feedback loop also plays a role in the regulation of ribosome content as
62 a function of growth rate[15–17]. Growth rate dependent regulation of gene expression determines
63 the allocation of cellular resources between the production of ribosomes and that of other
64 proteins and results in a linear increase in ribosome content with increasing growth rate[15,18–20]
65 . In richer growth media, when the amount of amino acids is higher, ppGpp levels are lower,
66 favoring ribosome production and a higher fraction of active ribosomes (Figs. 1 and S1A). In
67 poorer media it is the inverse, accumulation of ppGpp slows down the production of new
68 ribosomes and a smaller fraction of the ribosome pool is in an active form[19] (Fig. S1B). The
69 activity of ribosomal promoters is also regulated as a function of the growth rate by changes in
70 the activity of the Fis and H-NS proteins and by changes in the levels of negative supercoiling of
71 the DNA [21–24], which can explain the increase in rRNA with increasing growth rate in the mutant
72 strains devoid of ppGpp [25–27].

73 When ribosome activity is inhibited by sublethal concentrations of antibiotics, amino acids are
74 used more slowly and their concentration increases, thus decreasing the intracellular ppGpp
75 pool. The cellular response, as predicted by the ppGpp feedback loop, is to produce a higher
76 amount of ribosomes and an increased translation rate, however, despite this increase, the
77 cell's growth rate is reduced[18,19]. This has been proposed to result from a decrease in the
78 resources available for the production of non-ribosomal proteins that become limiting for
79 cellular metabolism [18,28]. More recent results point to a decrease in the fraction of active
80 ribosomes to explain the decrease in the total protein production rate[1] .
81 To measure the effect that ppGpp can play on gene expression in translation-limiting conditions,
82 and its possible effect on the repartition of RNAP, we have compared the activity of promoters
83 that are differentially regulated by ppGpp and that have different affinities for RNAP (Figs 1A
84 and S1). In parallel, we analyzed data from the literature on the direct and indirect effects of
85 changing ppGpp concentration on gene expression and compared them to the genes that
86 change expression upon translation inhibition[29,30]. The results from this analysis show that the
87 direct effect of ppGpp at the transcriptional level can only partially explain the change in protein
88 expression levels under translation limitation and that instead there is a decrease in RNA
89 polymerase production that increases the competition between high and low affinity
90 promoters, favoring the transcription of ribosomal operons. We propose that it is this decrease
91 in the total transcriptional capacity that results in a decrease the cell's growth rate even when
92 the translational capacity is increased.
93
94 **Results**
95 ***Decrease in total RNAP activity with increasing translation inhibition independently of***
96 ***changes in ppGpp.*** In order to measure the effect of increasing ribosome inhibition on RNAP

activity via changes in ppGpp we chose three promoters with different RNAP affinities and that are differently regulated by ppGpp. The first is a shortened version of the well characterized ribosomal *rrnB* operon promoter *rrnB*P1, here called P1, that terminates at -69 from the transcription start site[23]. This promoter has a GC-rich discriminator region at the transcription initiation site that makes the open complex sensitive to ppGpp inhibition (Table S1). The second is a constitutive promoter, P5, that has consensus -10 and -35 sequences and no discriminator region. The third is PLtet, also a strong constitutive promoter with no discriminator region but with a lower affinity for RNA polymerase due to a non-consensus -10 sequence[31]. Each of these promoters was placed upstream of the *gfpmut2* gene and this cassette was inserted in the chromosome together with a kanamycin resistance gene expressed divergently from the chosen promoter (Fig. S2). Growth of these strains in a 96-well plate allowed us to measure the changes in growth rate, the GFP production rate (Gpr), and the resulting GFP concentration as a function of chloramphenicol concentration (Figs. 1B-D and S2). We compared four different growth media, M9 with glucose, M9 with glycerol and these two media supplemented with casamino acids (CAA). This results in four different growth rates. In addition, it has been shown that cells growing in a growth medium containing amino acids have lower levels of ppGpp[16], allowing us to compare the effects due to changing concentrations of this key metabolite without the use of mutant strains that can result in secondary effects on cell metabolism[32].

In the absence of translation inhibition, the change in promoter activity measured as a function of growth rate is consistent with previous measurements on constitutive promoters and *rrnB*P1 promoters[11,11] (Fig. 1B-D dashed lines). Previous work had shown that as the concentration of chloramphenicol is increased, the ribosome content increases and the concentration of a reporter protein expressed from a constitutive promoter decreases[18]. Therefore, the expected

121 result here is that the GFP concentration from a ribosomal promoter (P1) should increase while
122 the concentration from a constitutive promoter (P5 or PLtet) should decrease, with the lower
123 affinity constitutive promoter (PLtet) decreasing at a faster rate if increasing amounts of RNAP
124 are being used for transcription of ribosomal operons. Unexpectedly, however, the pattern of
125 the change in Gpr and GFP concentration for the P1 and P5 promoters is very similar. The
126 pattern of the change of expression for the three promoter constructs depends strongly on the
127 growth medium and whether or not it contains casamino acids. In the absence of CAA, in the
128 cells starting with a higher amount of ppGpp, the increase in Gpr at low Cm is significantly
129 greater than in their presence (blue lines compared to the red lines in Fig. 1). This shows that a
130 decrease in ppGpp increases not only ribosomal promoter activity but also the translation rate
131 of GFP, independently of promoter sequence, as expected from recent studies on the effect of
132 ppGpp on ribosome assembly and activity[7,8]. The similarity in the pattern of the change in GFP
133 expression from the *rrnB*P1 and P5 promoters indicates in fact that upon translation limitation
134 the main effect of ppGpp on gene expression is via the translation rate rather than the
135 transcription rate (Fig. 1 and Fig. S16). Nevertheless, for each promoter the change in Gpr results
136 in a distinct pattern in the change of GFP concentration as a function of the presence of CAA in
137 the growth medium. In the two growth media with casamino acids [GFP] increases at the lower
138 Cm concentrations for P1 and P5, to then decrease again, while for PLtet it decreases with
139 increasing Cm.
140
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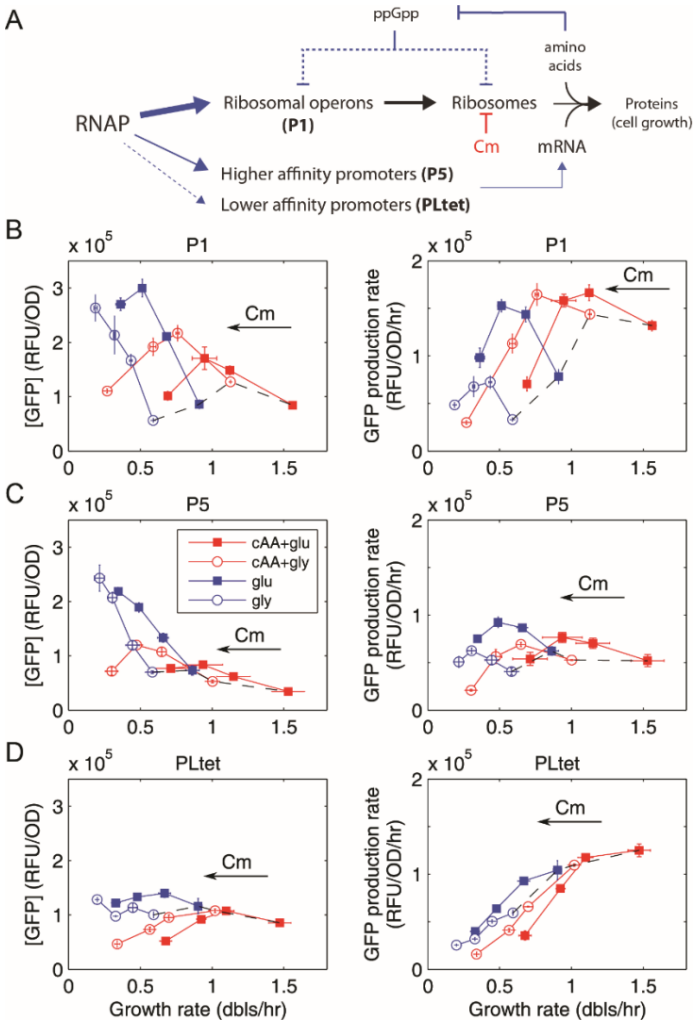

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Figure 1. Effect of translation limitation on GFP expression from three different promoters. A. The negative feedback loop mediated by ppGpp maintains ribosome production and activity proportional to the amount of available amino acids[5,6]. During the stringent response, upon a decrease in nutrients, an increase in ppGpp reduces the activity of ribosomal promoters and more RNAP is available for the transcription of non-ribosomal genes. In the presence of translation inhibitors, on the other hand, the pool of available amino acids increases, decreasing the concentration of ppGpp (Fig. S1). The three promoters used are: P1, a shortened version of the rRNA *rrnB*P1 promoter, P5, a constitutive promoter with a high affinity for RNA polymerase and PLtet, a constitutive promoter with a lower affinity for RNAP. These promoters can be used as reporters of the changes in ppGpp, by comparing P1 and P5, and of the changes in the activity of RNA polymerase, by comparing PLtet and P5. **B-D** Change in GFP concentration and GFP production rate in the three strains as a function of growth rate at increasing Cm concentrations in growth media with and without casamino acids (red and blue lines respectively). In the growth media with casamino acids the cells have a lower concentration of ppGpp [16]. The dashed black lines connect the points obtained in the absence of Cm. The growth rate decreases with increasing chloramphenicol concentration (black arrow). The four points correspond to 0, 2, 4 and 8 μM final chloramphenicol concentration. The error bars represent the SEM from 3 independent experiments. The error bars smaller than the size of the symbols are not shown.

143 One of the predictions from previous studies is that the induction of the ribosomal operons
144 promoters by decreasing ppGpp will result in a decrease in the amount of RNAP that is available
145 to transcribe mRNA[10–14]. In order to obtain an estimate of the magnitude of the effect of ppGpp
146 on promoter activity we measured the ratios of Gpr. Since the translation rate of GFP is shared
147 similarly by the three promoter constructs, this operation will leave the ratio of the
148 transcription-specific effect (Fig. 2). The comparison of two promoters with differing affinities
149 for RNAP can be used to estimate the change in the amount of RNAP that is available for
150 transcription[33]. In the absence of Cm, the ratio of PLtet to P5 Gpr increases with increasing
151 growth rate, in agreement with previous estimates of the change in free RNAP as a function of
152 doubling time[34]. The ratio of P1 to P5 instead increases rapidly between M9-glu and M9-CAA-
153 gly, consistent with a lower level of ppGpp in the cells growing in the presence of CAA increasing
154 the probability of transcription initiation specifically from P1. The PLtet/P5 and P1/P5 ratios are
155 greater than 1 because while PLtet and P1 have a lower affinity for RNAP than P5, when RNAP
156 binds at the PLtet or at the P1 promoter it initiates transcription with a higher frequency than
157 at P5 [31].
158 When Cm is increased in the cells with a higher amount of ppGpp (Fig. 2, blue lines), the fold
159 induction of P1 is greater than the one of P5, consistent with a decrease in ppGpp levels. As the
160 Cm concentration is increased further, the difference between the two promoters decreases
161 again to the initial level, suggesting that the ppGpp levels could increase again with the increase
162 in the cellular ribosome content (Fig. S4). On the other hand, in the growth media with casamino
163 acids, and thus lower levels of ppGpp, the P1 to P5 ratio decreases, indicating that the change
164 in Gpr from P1 is smaller than that of P5 as a function of increasing Cm. Here the decrease in
165 ppGpp is not large enough to increase the transcription initiation rate from P1 to the same
166 extent as in the poorer growth media, therefore the repartition of RNAP between ribosomal

167 and non-ribosomal promoters is expected to have a smaller influence on transcription
168 regulation. However, this still does not explain why the P1/P5 ratio should decrease with
169 increasing translation inhibition. One possibility is that there is an increase in ppGpp, however
170 a similar result is also observed for the PLtet to P5 ratio, independently of the growth medium.
171 Instead, this is consistent with a decrease in the amount of free RNA polymerase in the cell with
172 increasing Cm, independently of ppGpp regulation of ribosomal promoters.
173 The values of the RNAP affinities for these promoters and the relative changes in Gpr can be
174 used to estimate the change in the amount of free RNAP taking place in the cells. Fig. 2C and
175 Fig. S3 illustrate this point. The RNAP binding affinity for each promoter was estimated based
176 on a statistical-mechanical selection model developed by Berg and von Hippel[35] (see details in
177 the SI). The binding curves for RNAP to each promoter are shown. The vertical bars indicate the
178 ratio of promoter activity at a given RNAP concentration. The values of the Cm concentration
179 for each of these ratios is shown on the top. The decrease in free RNAP with increasing
180 chloramphenicol will have a stronger effect on PLtet first, then on P1 and finally on P5. Similarly,
181 using the P1 to P5 Gpr ratio it is possible to estimate the change in ppGpp as a function of growth
182 rate and as a function of Cm (Fig. S4). In summary, the decrease in free RNAP, as measured by
183 the decrease in the PLtet to P5 and P1 to P5 ratios, appears to be independent of a ppGpp-
184 mediated repartition between ribosomal and non-ribosomal promoters, since it can also be
185 observed in the growth media where ppGpp levels are very low.

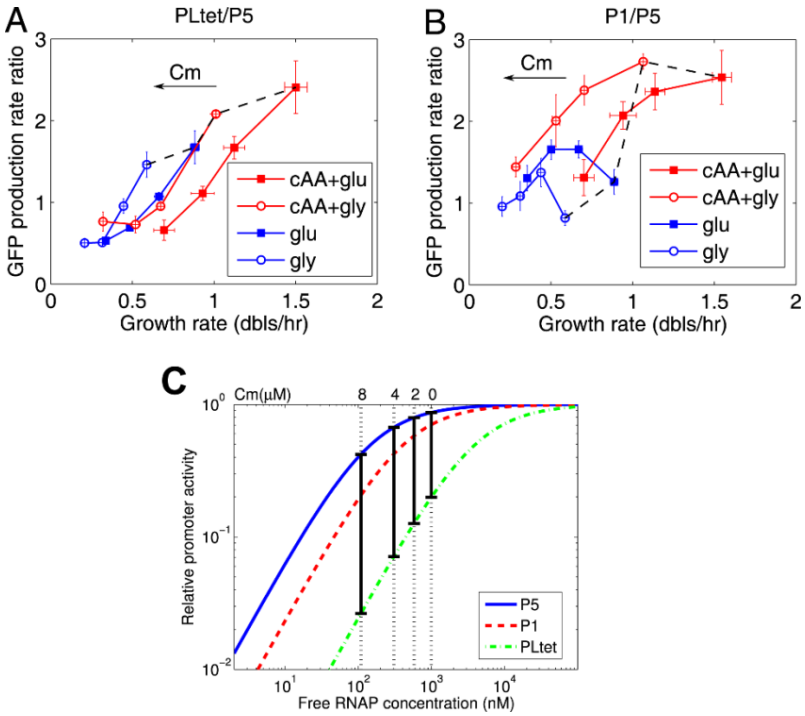

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Figure 2. Ratios of GFP production rate for the different promoters reveal promoter sequence-specific effects of translation limitation and ppGpp concentration. A. Ratio of PLtet to P5. The activity of the lower affinity constitutive promoter, PLtet, decreases more than that of P5 upon increasing Cm. **B.** Ratio of P1 to P5. Changes in ppGpp concentration in the growth media without cAA (blue lines) have a specific effect on the P1 promoter's activity compared to the constitutive promoter P5. The dashed lines connect the points obtained in the absence of Cm. **C.** The change in the ratios of promoter binding as a function of chloramphenicol concentration can be used to estimate the decrease in the amount of free RNAP. The relative activity of the promoters as a function of free RNAP concentration (c_f) was estimated from the respective RNAP binding constants, $K_i \in \{K_1, K_5, K_{Ltet}\}$ using $c_f/(K_i + c_f)$. K_5 is known, K_1 and K_{Ltet} can be derived by the formula $K_5 \exp(\Delta E)$, where ΔE is the difference between the binding energies of RNAP with P1 (or PLtet) and P5 (see Supporting Information). The black vertical bars indicate the concentrations of RNAP that give the measured GFP production rate ratios shown in (A) for different Cm concentrations, shown above the plot. The ratio of PLtet and P5 activities for example is given by: $Gpr(PLtet)/Gpr(P5) = a(K_5 + c_f)/(K_{Ltet} + c_f)$, where a is a scaling factor. K_5 and a can be fixed by fitting the data in absence of Cm from our experiments and the literature. Data from the cells growing in cAA-glu was used so that ppGpp-dependent regulation of P1 is small and can be ignored. Fig. S3 in Supplementary Materials shows the estimation of RNAP concentration for the four growth media used. See details of the analysis in the Supporting Information.

187

188 ***The decrease in ribosome processivity by chloramphenicol decreases the expression of longer***

189 ***genes more than shorter ones.*** A possible cause for the decrease in the pool of free RNAP

190 independently of the changes in ppGpp is a decrease in the amount of RNAP per cell. The RNA

191 polymerase holoenzyme is composed of 5 subunits: β, β', ω, 2 subunits of α, and a σ factor. β,
192 β' are among the longest genes in *E. coli* with a length of 4029 bp and 4224 bp respectively (the
193 average gene length in *E. coli* is about 900 bp). We hypothesized that translation of such long
194 genes would have a higher probability to end prematurely in the presence of ribosome
195 inhibitors such as chloramphenicol.

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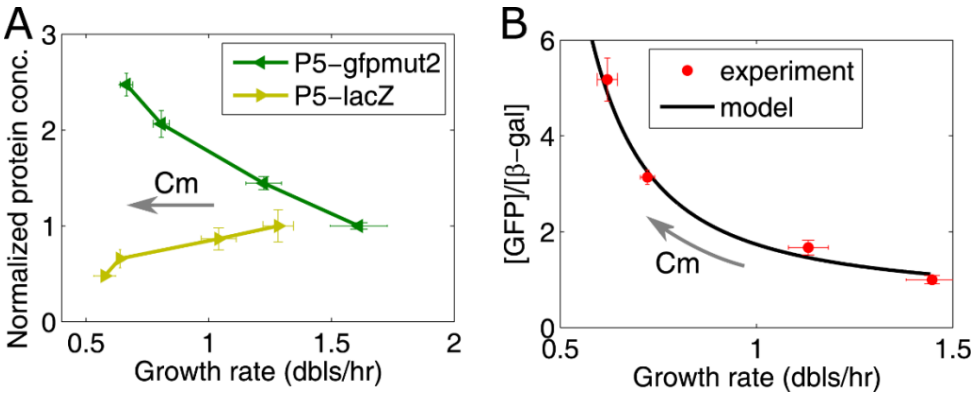

197

198 **Figure 3. Gene length can influence gene expression under translation limitation. A.** Change in GFP
199 (238 aa) and β-galactosidase (1024 aa) expressed from the P5 promoter as a function of increasing
200 chloramphenicol concentrations. The bacteria were grown in M9 glu+cAA with chloramphenicol (Cm) at
201 concentrations of 0, 2 μM , 4 μM , or 6 μM in flasks. The concentrations were normalized by dividing
202 the data by the point without Cm. **B.** Fit of the model to the ratio of GFP to β-galactosidase
203 concentrations from the data in (A). The growth rate at each chloramphenicol concentration is the
204 average over the two strains. The error bars correspond to the SEM from three independent
205 experiments.
206

207 The processivity of translation has been shown to decrease exponentially with increasing gene
208 length[36]. If, in addition, ribosome processivity were decreased by an inhibitor, then the
209 probability to finish the translation of a long gene would be lower compared to a shorter gene,
210 decreasing the rate of expression of the longer gene to a greater extent. To test this hypothesis,
211 we compared the changes in gene expression from the same constitutive promoter, P5, of two
212 different genes, *gfpmut2*, 714 bp long, and *lacZ*, 3072 bp long and coding for the enzyme β-

213 galactosidase, in the presence of increasing chloramphenicol concentrations (Fig. 3A). The
214 results show that while the concentration of GFP increases as a function of Cm concentration,
215 β-galactosidase concentration decreases (Fig. 3A). Fig. 3B shows the change in the ratio of the
216 shorter to the longer protein as a function of Cm concentration. The black line shows the fit
217 obtained to a model of ribosome processivity. Briefly, it is possible to estimate the probability
218 that a ribosome will be inhibited by the antibiotic before it reaches the end of the mRNA, P_{hit},
219 as described by Dai et al[19]. Their equation can be extended to describe the dependence of P_{hit}
220 on protein length, i.e.

221 $$P_{hit} = 1 - \exp(-k_{on}[Cm]L/v) \quad (1)$$

222

223 where k_{on} denotes the binding constant of Cm with ribosome ($k_{on} = 0.034(\mu M \cdot min)^{-1}$ [37]),
224 L indicates protein length, and v is the translation elongation rate dependent on the
225 RNA/protein mass ratio (see the Materials and Methods; ref.[19]). Using equation 1, at 8 μM Cm
226 (the highest concentration used here) P_{hit} is 23% for LacZ (1024 aa), while for GFP it is 6% (238
227 aa). These results therefore indicate that a gene's length, in addition to its promoter's affinity
228 for RNAP, can influence how its expression levels change in the presence of sub-lethal
229 concentrations of ribosome inhibitors.

230

231 We have extended this analysis and this model to measure the difference in the expression of
232 early and late genes within the same operon upon decreased translation processivity and we
233 have found that indeed late operon genes are less expressed than early ones due to mRNA
234 degradation induced by the presence of a stalled ribosome (SI Fig. S8). However, at these low
235 Cm concentrations transcription-translation coupling is not significantly perturbed (SI Fig. S8
236 and S9). Finally, we have analyzed data from previously published transcriptomics and

237 proteomics studies[29,32] and we have found that the effect of promoter affinity, gene length and
238 operon position can help explain the global changes in gene expression in the presence of
239 sublethal levels of chloramphenicol (SI Fig.s S12,S13,S15).
240
241 **Discussion**
242 Understanding how bacteria modulate their growth rate and resource allocation in response to
243 inhibition of growth has paramount importance in biotechnological and health applications[38–
244 41]. The evidence provided here points to a possible cellular adaptation mechanism leading to a
245 reduction of transcription capacity when ribosome activity is compromised. This decreases the
246 cost of transcription of untranslated mRNAs[42] and allows for more resources to be available for
247 the synthesis of increased amounts of ribosomes to respond to the presence of translation
248 inhibitors. Here we found that as the sublethal concentration of ribosome inhibitor is increased
249 the growth rate decreases linearly with the decrease in the amount of available RNA
250 polymerase, pointing to a growth-limiting role for this enzyme in these conditions (Fig. 2). These
251 results are coherent with the observation in a previous study on the quantitation of over 1000
252 proteins in the presence of increasing concentrations of Cm[29] that the β and β' subunits of RNAP
253 remain a constant fraction of the proteome with increasing Cm and decreasing growth rate. This
254 is what would be expected if RNAP were to play an important role in determining the total rate
255 of protein production and the cell's growth rate.
256
257 The comparison of two different constitutive promoters with differing RNAP affinity, PLtet and
258 P5, shows a striking difference in the change in Gpr and the resulting GFP concentration with
259 increasing ribosome inhibition (Fig. 1). The decrease in the amount of free RNAP with increasing
260 sublethal concentrations of chloramphenicol estimated by measuring the ratio of gene

261 expression from these two promoters (PLtet/P5) is about 10-fold (Fig. 2 and Fig. S3) and is
262 independent of the change in ppGpp in the different growth media (Fig. 2). This can be used to
263 predict the change in transcription rate for the P5 promoter, for example, and therefore the
264 GFP translation rate that results in the measured Gpr (Fig. S16). The translation of short proteins
265 such as GFP is barely affected by the presence of sublethal concentrations of Cm (8 % probability
266 of drop off at the highest concentration used here) and can therefore be used to estimate the
267 changes in total translation activity. The translation rate increases and mirrors the ratio of P1 to
268 P5 activity reflecting the changes in ppGpp (Fig. S4). Several factors can contribute to this
269 increase in translation rate: the shorter than average length of ribosomal genes (Fig. S12),
270 whose translation is not affected as strongly by translation inhibition compared to longer genes;
271 the high affinity of RNAP for ribosomal promoters, insuring transcription despite a decrease in
272 the amount of free RNAP (Fig. S11); the decrease in ppGpp in the growth media lacking
273 casamino acids (Fig. S4), resulting in an increase in both ribosomal promoter activity (Fig. 2)[5,43]
274 and in the fraction of active ribosomes due to the effect of ppGpp on ribosome assembly[7,8,19],
275 and the increase in translation elongation rate[19]. The higher increase in translation rate in the
276 growth media without cAA is consistent with a larger fraction of inactive ribosomes stored in
277 the cells growing at these slower growth rates that can be quickly reactivated by a decrease in
278 ppGpp concentration[19,44–46]. Furthermore, gene length could also have an effect on translation
279 via increased probability of ribosome recycling on shorter mRNAs, increasing the fraction of
280 ribosomes translating ribosomal genes[47].
281
282 The similarity in the pattern of the change in GFP expression from the rrnBP1 and P5 promoters
283 despite their different regulation by ppGpp indicates that upon translation limitation the main
284 effect of ppGpp on gene expression from high affinity promoters is via the translation rate

285 rather than the transcription rate (Fig. 1 and Fig. S16). The effect of decreasing ppGpp on
286 transcription from the P1 promoter is just strong enough to compensate for the decreased
287 availability of RNAP (Fig. S16). In the richer growth media, where ppGpp levels are low and the
288 ribosomes are working at near maximum capacity, a specific induction of the ribosomal
289 promoter is practically absent and the increase in translation rate is lower, consistent with a
290 smaller change in ppGpp levels. The decrease in transcription capacity however is maintained.
291 In these growth conditions, in rich media when ppGpp levels are low, a decrease in transcription
292 rate when translation is compromised might play a crucial role for the cell's survival, as the
293 capacity of the cell to increase its translation capacity to respond to the presence of the inhibitor
294 is limited by the small fraction of inactive ribosomes[19,48].
295 The ppGpp independent decrease in transcription capacity can be explained by the fact that the
296 RNA polymerase core contains two of the longest proteins in *E. coli*, the β and β' subunits (Fig.
297 S12), and by the increased probability that a ribosome will stall before reaching the end of a
298 long mRNA than a short mRNA, particularly in the presence of inhibitors such as
299 chloramphenicol, as shown by the difference between the expression of the *lacZ* and *gfp* genes
300 (Fig. 3). These results can shed light on a recent study by Dai et al[19] where it was proposed that
301 in the presence of sublethal concentrations of Cm, despite an increase in the translation
302 elongation rate due to a higher concentration of ternary complexes, the reduction in the total
303 protein production rate results from a decrease in the active ribosome fraction, or the fraction
304 of ribosomes that can reach the end of a mRNA in the presence of the inhibitor[19]. Here we
305 identify RNAP as one of the genes that is likely to be most affected by the decrease in ribosome
306 processivity, while shorter genes are affected to a lesser extent.
307 The extreme length of the β and β' subunits is conserved throughout bacteria[49]. In the case of
308 *Helicobacteraceae* and *Wolbachia* the two genes are even fused together[49,50]. The gene length

309 of β and β' subunits can vary in different strains since they are composed of independent
310 structural modules separated by spacers of differing length[49]. In *E. coli* the spacer sequences,
311 that account for more than 25% of the total sequence, can be deleted without causing a
312 significant decrease in activity. In archea and chloroplasts some of the conserved protein
313 modules are found in separate genes and in *E. coli* they can be split from each other to produce
314 an active enzyme[51]. The reason why the RNAP and ribosomal proteins find themselves at
315 opposite ends of the spectrum of gene lengths in bacteria is likely linked to the assembly
316 process, structural flexibility and stability of the final multi-protein complex[52,53]; however, these
317 results suggest that it could also play an important role for the cell's survival in the presence of
318 antibiotics that decrease translation processivity.
319
320 It is possible to obtain an estimate the change in ppGpp from the ratio of the Gpr of the P1 and
321 P5 promoters since they both have a similar affinity for RNA polymerase while P1 (*rrnBP1)* is
322 also negatively regulated by ppGpp (Fig. S4). ppGpp decreases at the lower Cm concentrations
323 and then remains constant or increases again as Cm is increased, depending on the growth
324 medium. An increase in ppGpp coupled to an activation of the RpoS regulon has been implicated
325 in a general hormetic stress response to sublethal levels of antibiotics[1] and in the emergence of
326 tolerance and persister cells[3], a subset of the population that resists antibiotic inhibition due to
327 decreased growth[54,55]. Here we measured gene expression when the cells are still in exponential
328 phase, before the amounts of amino acids become limiting and before the adaptation to early
329 stationary phase. However, the repartition of the RNAP core among the sigma factors
330 associated with stress response[12,56] could also contribute to a further decrease the amount of
331 free sigma 70 RNAP as the cells enter stationary phase or in the presence of other antibiotics
332 that are not specifically targeting translation[1]. The results presented here provide a new cellular

333 mechanism by which bacterial cells can decrease their growth rate in response to antibiotic
334 stress[1,57,58] and can help explain the observation from a previous report that the induction of
335 persistence and β-lactam tolerance by chloramphenicol is also independent of the presence of
336 the RelA enzyme[59]. In conclusion, the results presented here provide evidence that in the
337 presence of sublethal concentrations of ribosome inhibitors, it is not translation that becomes
338 limiting for the cell's growth rate, or the ppGpp dependent repartition of RNAP between
339 ribosomal and non-ribosomal promoters, but it is the decrease in total transcription capacity.
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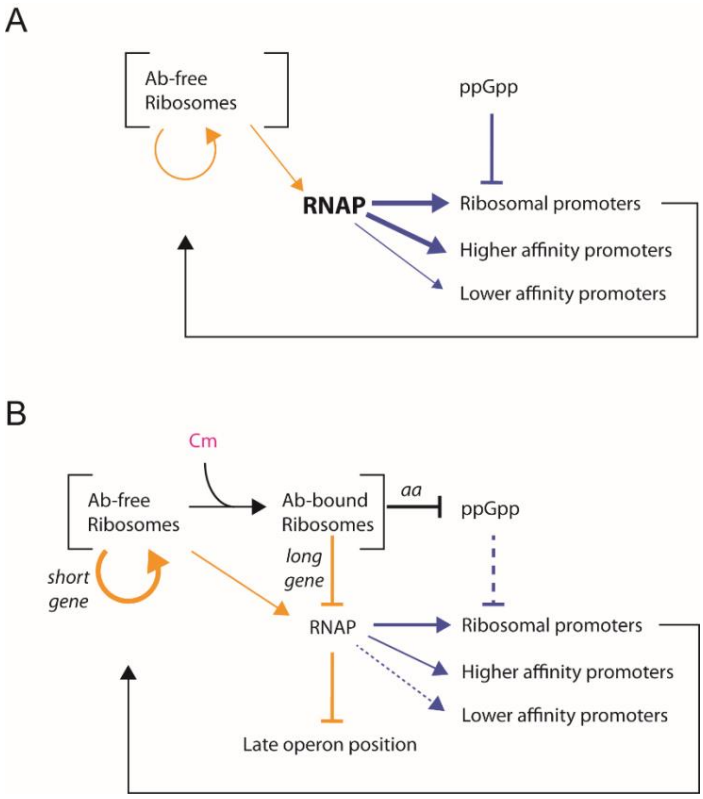

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Figure 4. Summary. Orange arrows show translation effects and blue arrows show transcription effects. **A.** In the absence of translation limitation ribosomes translate both ribosomal and non-ribosomal genes with similar rates. The amount of RNAP available is regulated in part by the changes in ppGpp and the ensuing transcription rate of ribosomal promoters. **B.** The decrease in ribosome processivity by inhibitors such as chloramphenicol results in translation of longer genes being prematurely terminated more frequently than translation of shorter genes. RNAP subunits β and β' are among the longest genes in *E. coli*, while ribosomal proteins are among the shortest. The decrease in free RNAP can be measured by a decreasing ratio of high affinity to low affinity promoter genes expression rate. The decrease in ribosome processivity increases the probability of mRNA degradation, thus penalizing the expression of genes at the end of operons. In nutrient poor media, inhibition of ribosome activity by chloramphenicol increases the pool of amino acids and decreases the levels of ppGpp, increasing both ribosome production and ribosome activity. Ab: antibiotic.

344 **Materials and Methods**

345 **Strains, promoters and reporters**

346 GFP and β-galactosidase were used as the reporter proteins to measure the rate of gene
347 expression from a specific promoter. The GFP gene used is *gfpmut2* coding for a fast-folding
348 GFP[60]. The β-galactosidase gene is a 5'-end-modified *lacZ* from the pCMVbeta plasmid[61].
349 Comparison with the wild type *lacZ* gene shows that the additional 23 amino acids do not
350 change the results obtained with this version of the reporter gene (data not shown). The
351 promoters include two constitutive promoters (P5, obtained from T5 phage and PLtet, i.e. P_{LtetO-}
352 $_{1}$[31] and a shortened version of a rRNA promoter (*rrnB*P1 without the upstream Fis and H-NS
353 sites) (Table S1). The constructs of P1-*gfpmut2*, P5-*gfpmut2*, PLtet-*gfpmut2* and P5-*lacZ* with a
354 divergent kanamycin resistance gene were inserted in the chromosome of the BW25113
355 *Escherichia coli* strain. P1-*gfpmut2* and P5-*gfpmut2* (for Fig. 1) were inserted at position 258235
356 between the convergent *crl* and *phoE* genes, PLtet-*gfpmut2* was at position 356850 between
357 *cynR* and *codA*, and P5-*lacZ* and P5-*gfpmut2* (for Fig. 3) were at position 1395689 between *uspE*
358 and *ynaJ*. Genome position did not have an effect on the change in reporter gene expression as
359 a function of Cm. The double fluorescent protein system (RFP-GFP constructs) has been
360 described previously[62]. The ribosome binding sites (RBS) i.e. Shine-Dalgarno sequences, used in
361 above constructs are all similar to the consensus UAAGGAGGU[63]. The RBS for GFP (*gfpmut2*)
362 and β-gal (*lacZ*) is GAAGGAGAU, for RFP (*mCherry*) it is AGAGGAGAA.

363

364 **Bacterial growth and fluorescence measurements.**

365 Bacterial growth was carried out in M9 minimal growth medium supplemented with 0.5%
366 glycerol (gly), 0.5% glucose (glu), 0.5% glycerol+0.2% casamino acids (cAA+gly) and 0.5%
367 glucose+0.2% casamino acids (cAA+glu). The pre-culture was obtained from the inoculation of
368 one bacterial colony in LB growth medium. After overnight growth, the seed culture was washed

369 once with PBS and diluted 200 times with the corresponding growth medium containing a
370 specific concentration of chloramphenicol (Cm). This culture was diluted again 200 times once
371 it reached exponential phase. The cultures were grown in flasks, shaking at 37°C and 170 rpm,
372 and optical density and fluorescence were measured with a plate reader (Tecan, Infinite 200Pro)
373 every 30 - 50 min. Alternatively, the cultures were grown in a 96 well plate, with 150 µL of
374 bacterial culture per well covered by 70µL mineral oil. The culture plate was kept at 37°C in the
375 plate reader, shaking and measuring fluorescence and OD_{600} every 5 minutes. The auto-
376 fluorescence measured from the wild type strain Bw25113 was subtracted from the
377 fluorescence of the fluorescent strains at the same OD (dependent on the medium). The
378 experimental procedure of the β-galactosidase assay followed the protocol of Zhang et al.[64]
379 except that the bacterial strains were cultivated in flasks instead of 48-well plates[18,65,66]. The
380 measurement of RFP-GFP constructs followed the protocol described previously[62].
381
382 **Analysis of GFP reporter expression data.**
383 Experimental data obtained from the plate reader were analyzed with Matlab to obtain growth
384 rate, protein concentration and protein expression rate. The pipeline is shown in Fig. S2. The
385 window in the growth curve corresponding to the exponential growth phase was defined as a
386 linear range between an upper and a lower threshold in the growth curve plot of $\log(OD_{600})$
387 versus time (the thresholds determined manually or from an automated method[67] gave similar
388 results). Growth rate was derived from the slope of $\log(OD_{600})$ versus time in exponential phase
389 (Fig. S2B and C). GFP concentration was derived as the slope of the plot of GFP versus OD_{600} in
390 the exponential growth phase (Fig. S2B). β-galactosidase concentration in Miller Units was
391 obtained by the following formula (Fig. S2C)

392 $$\beta - \text{gal activity} \triangleq 1000 \cdot \mathrm{A} \cdot \frac{1}{0.01} \cdot 20 \cdot \frac{1}{\mathrm{OD}_{600}} = 2 \times 10^6 \times \frac{\mathrm{A}}{\mathrm{OD}_{600}} \quad (2)$$

393 where A comes from the fit of OD_{600} as a function of time with the formula $A(1-e^{-\gamma t})/\gamma$

394 and γ is a decay factor from taking into account that the reaction product o-nitrophenol is

395 volatile[64]. The rate of protein expression is defined as the product of protein concentration and

396 growth rate (μ).

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398

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