Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

How do bacteria tune translation efficiency?

Gene-Wei Li

Bacterial proteins are translated with precisely determined rates to meet cellular demand. In contrast, efforts to express recombinant proteins in bacteria are often met with large unpredictability in their levels of translation. The disconnect between translation of natural and synthetic mRNA stems from the lack of understanding of the strategy used by bacteria to tune translation efficiency (TE). The development of array-based oligonucleotide synthesis and ribosome profiling provides new approaches to address this issue. Although the major determinant for TE is still unknown, these high-throughput studies point out a statistically significant but mild contribution from the mRNA secondary structure around the start codon. Here I summarize those findings and provide a theoretical framework for measuring TE.

Addresses
Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, United States

Corresponding author: Li, Gene-Wei (gwli@mit.edu)

Current Opinion in Microbiology 2015, **24**:66–71

This review comes from a themed issue on **Cell regulation**

Edited by **Carol Gross** and **Angelika Gründling**

For a complete overview see the Issue and the Editorial

Available online 28th January 2015

http://dx.doi.org/10.1016/j.mib.2015.01.001

Introduction

Soon after Jacob and Monod proposed the existence of polycistronic mRNA [1], it was noticed that different proteins originating from the same mRNA are translated at very different rates [2]. This observation was made for the RNA genome of a bacteriophage that was translated upon entrance to the host bacterium. The difference in translation rates was deemed necessary to synthesize a large excess of bacteriophage coat proteins relative to RNA polymerases for viral particle production. Although the initial studies suggested the use of mRNA secondary structure to modulate translation efficiency (TE) [3], the later discovery of the Shine–Dalgarno (SD) sequence pointed to the potential for tuning TE by changing the affinity of mRNA to the anti-Shine–Dalgarno sequence on the 16S rRNA [4,5]. Detailed studies on individual genes and operons then revealed a plethora of means to modulate TE [6–9]. It remains unclear whether there exists a general principle for setting the TE for the 4000+ genes in *Escherichia coli*.

Recently, the promise of synthetic biology — the design of biological devices from genetic and protein components — increased the demand for better understanding and control of TE. To address this issue, several research groups created large-scale libraries of synthetic mRNAs to probe the sequence features that influence TE [13••,10••,11••,12••]. Meanwhile, with the development of ribosome profiling (deep sequencing of ribosome protected mRNA fragments) it became possible to monitor the TE of endogenous genes at genome-scale [14]. Here, I will summarize the conclusions from these recent studies and discuss the missing pieces of the puzzle.

Defining TE

In this review, 'TE' is referred to as the rate of protein production per mRNA [14–16]. In other contexts, the same phrase has been defined as the rate of translation elongation, which affects the efficiency with which ribosomes are used [13••,17,18]. Both definitions are widely used in the literature, and this can lead to profound confusion when the exact definition is not specified. For example, factors that influence the efficiency of elongation should not be confused with the determinant of protein production per mRNA, or TE as defined here [19–21]. In cells, these two processes are sometimes connected because they both concern the cellular pool of ribosomes [22–24], but they are not the same. The possible connection between elongation and production per mRNA (or lack thereof) has been reviewed in several recent studies [21,25–27]. Here I focus on understanding the meaning and utility of defining TE as the rate of protein production per mRNA.

It is clear that protein abundance is not equal to TE. Protein abundance is a product of mRNA level, TE, and protein lifetime (Box 1). Changes to the mRNA sequence can often affect some or all of these factors, making it difficult to attribute the resulting difference in protein level to changes in TE alone. Moreover, TE itself can also directly influence mRNA levels. If an mRNA is more stable when TE is high, the amount of proteins produced scales nonlinearly with TE (Box 1). It is therefore important to normalize protein abundance by differences in protein lifetime and, in particular, mRNA levels. As described later, the combination of ribosome profiling and RNA-seq enables accurate determination of protein production rate per mRNA.

Using synthetic DNA libraries to interrogate determinant of TE

Examining the effect of mutations on protein production is a common approach to dissect the determinants of TE.

Box 1 Definition of translation efficiency.

Various definitions of translation efficiency have been a source of confusion. To make a clear definition of the term used in this review, consider the four basic kinetic parameters in the central dogma (Figure 1). mRNAs are produced at a rate k_1, and proteins are produced from mRNA with a first-order rate constant k_2. λ_1 and λ_2 are the first-order decay rate constants for mRNA and protein, respectively. The master equations for the scheme in Figure 1 are

$$\frac{d}{dt}M = k_1 - \lambda_1 M \quad (1)$$

$$\frac{d}{dt}P = k_2 M - \lambda_2 P \quad (2)$$

where M and P are the concentration of mRNA and protein, respectively. In this review, translation efficiency is defined as the rate of protein production per mRNA, which is equal to k_2.

$$TE \equiv k_2 \quad (3)$$

Operationally, most studies report changes in P or P/M as a measure for changes in TE. At steady state, the master equations yield

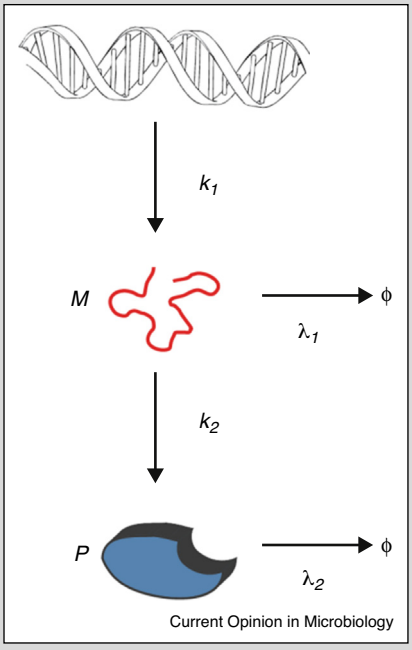
$$M = \frac{k_1}{\lambda_1} \quad (4)$$

$$P = \frac{k_2}{\lambda_2} M = \frac{k_2}{\lambda_2}\frac{k_1}{\lambda_1} \quad (5)$$

Therefore, both P and P/M are sensitive to changes in the degradation rate. In contrast, ribosome profiling in combination with RNA-seq reports k_2, a direct measure for TE (Box 2).

Consider a simple case in which mRNA degradation depends on the level of translation, which is common in bacteria [37]. If the mRNA decay rate is inversely proportional to TE ($\lambda_1 = A/k_2$), the amount of protein produced then scales quadratically with TE ($P = ((k_1 k_2^2)/(A\lambda_2)) \propto k_2^2$), whereas $P/M = k_2/\lambda_2$ still scales linearly with TE ($P/M = \propto k_2$). This example illustrates the importance of normalizing protein levels by mRNA levels when reporting TE. In ribosome profiling, the ratio between ribosome density and mRNA level is not affected by changes in mRNA decay.

Figure 1



Defining translation efficiency and other kinetic constants in gene expression. The mRNA (M) is transcribed from DNA at a rate k_1 and degraded (ϕ) with a rate constant λ_1. The corresponding protein (P) is translated from mRNA with a rate constant k_2 and degraded (ϕ) with a rate constant λ_2. Translation efficiency is defined as k_2 in this review. See Box 1.

An important limitation is that it is not feasible to explore every possible combination of mutations, as a 200-aa protein can have 10^{120} possible synonymous coding variants multiplied by additional variations for the untranslated regions of mRNA. Therefore, even with high-throughput oligonucleotide synthesis technology, a library of mutations can only cover a very small subset of the sequence space. Therefore, instead of aiming for unbiased coverage, synthetic libraries are often designed to test specific hypotheses. For example, a library of ~100 mutations in the 5′ un-translated region (UTR) was constructed to systematically examine the effects of sequences surrounding the ribosome binding site using RFP fluorescence as a readout [10••]. Note that this approach assumes that the potential impact of 5′ UTR on mRNA stability is negligible. The results of this study suggested that TE was influenced by multiple factors including the SD sequence, the thermostability of RNA secondary structure, and other features of the ribosome binding site.

In parallel to the 5′ UTR study, several groups constructed fluorescent reporter libraries of similar size (~10^2) to test the effect of different synonymous mutations within the open reading frame (ORF) [11••,13••]. These studies found that the usage of rare codons has little or no effect on protein abundance. Instead, the lack of mRNA secondary structure at the start site has the most significant, albeit weak, correlation with protein abundance [11••,13••]. As was true of the previous study, the parameter measured was the final amount of protein produced. Thus, it was unclear whether the effects of RNA folding were on mRNA decay or TE. In fact, a later study reanalyzed the data and found that when protein abundance is normalized by mRNA levels, the correlation with RNA folding vanishes even though TE remains variable over two orders of magnitude [28]. How the observed TE is modulated is still unknown.

Bacterial mRNAs tend to have a lower amount of secondary structure around the translational start site, both in

Box 2 Ribosome density as a measure of translation efficiency.

To understand the relationship between ribosome density and rate of protein synthesis, consider the following simplified model for translation of an mRNA (Figure 2). The codon positions for the ORF are labeled from 1 to N. Let q_i denote the probability that the ith codon is occupied by a ribosome ('occupancy'), and r_i denote the ribosome translocation rate constant from i to $i+1$. The overall rate of translocating from i to $i+1$ is r_i multiplied by the occupancy at i. Assuming that ribosomes do not dissociate until the end of the ORF, which is valid for most genes [35••], the net rate of change in q_i is the difference between the translocation rates from $i-1$ and from i.

$$\frac{d}{dt}q_i = r_{i-1}q_{i-1} - r_i q_i \tag{6}$$

For the master equation above I made an approximation that the occupancy is much less than unity ($q_i \ll 1$), so that the translocation rate from $i-1$ to i is independent of q_i. There are several ways to justify this approximation. One approach is based on the length of rRNA (4.6 kb) and the ratio between rRNA and mRNA content in a cell (~20 nt of rRNA per 1 nt of mRNA) during steady state growth [38,39]. These give 4 ribosomes per 1 kb of mRNA. Because each ribosome occupies ~30 nt of mRNA, the maximal occupancy is $4 \times 30/1000 = 0.12$. Because only ~70% of ribosomes are actively translating, the actual occupancy is close to 0.08. Therefore we can assume $q_i \ll 1$ and that there is no traffic jam.

At the end of the ORF, the rate of ribosome leaving the mRNA is equal to the rate of protein synthesis from this molecule of transcript (k_2, Figure 2). On the basis of conservation of flux, the rate of ribosomes initiating translation on this transcript must also be k_2. Thus, the master equations for the boundaries are

$$\frac{d}{dt}q_1 = k_2 - r_1 q_1 \tag{7}$$

$$\frac{d}{dt}q_N = r_{N-1}q_{N-1} - k_2 \tag{8}$$

At steady state, the solutions give

$$r_1 q_1 = \cdots = r_{i-1}q_{i-1} = r_i q_i = \cdots = k_2 \tag{9}$$

In other words,

$$q_i = \frac{k_2}{r_i} \tag{10}$$

Averaging the occupancy over the ORF gives

$$\langle q \rangle = k_2 \left\langle \frac{1}{r} \right\rangle = k_2 \langle \tau \rangle \tag{11}$$

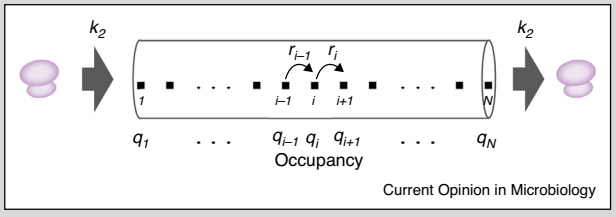
where $\langle \tau \rangle$ is the average translocation time per codon. We can now rewrite k_2 in terms of the observables in RNA-seq (M) and in ribosome profiling ($\langle q \rangle M$):

$$k_2 = \frac{1}{\langle \tau \rangle}\frac{\langle q \rangle M}{M} = \frac{1}{\langle \tau \rangle}\frac{\text{ribsome density}}{\text{RNA level}} \tag{12}$$

Therefore, the ribosome density per mRNA is proportional to TE, provided that $\langle \tau \rangle$, or the average translocation time, is the same for each transcript.

The average translocation time could differ among transcripts with different codon usage if each codon is decoded at different rates. However, studies using ribosome profiling have observed a similar elongation rate (well below twofold difference) for every codon during steady state growth [27,40–44], which is likely due to balanced codon usage and tRNA abundance. Furthermore, local variations due to internal SD sequences and, to a lesser extent, different codons are often averaged out and the residual can be corrected for when considering the average translocation time. The balance between codon usage and charged tRNA level can be perturbed under nutrient limitation or overexpression of heterologous genes [25–27,45]. In these scenarios, it is important to correct for the difference in $\langle \tau \rangle$ when using ribosome density to infer TE. During steady state growth, the average translocation time can be considered constant across messages.

Figure 2



Calculation of ribosome density. Consider a molecule of mRNA of length N (cylinder). The transition rate constant from position i to $i+1$ is r_i. The ribosome occupancy at position i is q_i. Assuming that every ribosome that initiates on the mRNA finishes translation, the steady state condition requires that the rate of producing a full-length protein from this mRNA (k_2) is the same as the rate of initiation. See Box 2.

the 5′ UTR and the initial ORF region [29–31]. What is the role of this structure-free region? To address this issue, Goodman *et al.* constructed a much larger scale library (~10^4) combining variants in both the 5′ UTR and the first 11 codons from endogenous *E. coli* genes [12••]. As previously reported, codon substitutions that increase mRNA folding stability have a negative impact on protein abundance. Interestingly, rare codons in the N-terminal region tend to decrease the amount of secondary structure and thereby increase TE, explaining why rare codons are enriched in the beginning of ORFs. Despite this progress, the predicted strength of ribosome binding site and mRNA folding are still not sufficient to explain the variations in TE, pointing to the need both for improved RNA structural prediction and for a better mechanistic understanding of translation [32–34].

Using ribosome profiling to measure TE for endogenous genes

Ribosome profiling, developed at about the same time as the synthetic oligo approach described in the previous section, has emerged as a methodology that enables the direct monitoring of translation [14]. Based on deep sequencing of ribosome protected mRNA fragments, ribosome profiling reports the number of ribosomes translating each gene. Provided that most ribosomes complete translation to yield full-length polypeptides and the elongation time averaged across the entire transcript is the same, then the density of ribosomes (number of ribosome per unit length of a gene) is proportional to the rate of protein synthesis [35••]. Indeed, studies show that these two assumptions are generally correct (Box 2). Moreover, ribosome density is highly correlated with the individually quantified copy numbers of stable proteins in *E. coli* [35••]. Additionally, ribosome density is proportional to the stoichiometry of members of multi-protein complexes ('proportional synthesis'), indicating that each subunit is synthesized proportionally. These lines of evidence confirm that ribosome density can be used to report the rates of protein synthesis [35••].

One can then obtain TE by dividing rate of synthesis of each protein (ribosome density) by the corresponding mRNA levels as measured by RNA-seq [14] (Box 2). This metric is independent of mRNA and protein lifetimes. Application of ribosome profiling to *E. coli* revealed that the TE for endogenous genes can vary by >100 fold even among ORFs on the same polycistronic transcript [36]. In fact, modulation of TE is broadly used by bacteria to enable differential production of proteins in the same operon. For example, ribosomal proteins are often translated at much higher levels than other proteins, such as those involved in DNA repair and membrane translocation, that share the same transcript. Among functional modules, one component is often translated at a higher level according to its hierarchical role. The level of fine-tuning is best illustrated by the observation that operons that encode multi-protein complexes often modulate TE to match the stoichiometry of the different proteins in the complex [35••].

Understanding how bacteria tune their own TE promises to provide crucial information for designing synthetic constructs. Surprisingly, the strength of the SD sequence has no predictive power for the variation in TE for endogenous genes, that is, genes with a weak SD sequence do not, in general, have lower TE than those with strong SDs [35••]. TE is weakly anti-correlated with predicted thermal stability of RNA folding around the start codon. Importantly, neither the predicted accessibility nor strength of the ribosome binding site can explain the level of fine control of translation that bacteria have evolved [35••]. These results suggest that bacteria do not use the SD sequence to tune TE. Interestingly, the same conclusion, which seems paradoxical at the current time, was first drawn when Shine and Dalgarno described the SD sequence in 1974 [4]. They noted that the gene with the strongest SD site (protein A) on the MS2 bacteriophage RNA is in fact most weakly translated [4]. 40 years after their discovery, the major determinant of TE is still largely unknown.

Conclusion

The ability to control TE is fundamental to the operon strategy in bacteria. Organizing functionally related genes into the same operon allows a simple for transcription so as to regulate the overall level of the functional group. Meanwhile, tuning TE is essential to differential protein production within the group. This strategy is analogous to a single-handle faucet design in which one direction controls the overall flow rate and the other controls the ratio between hot and cold water. An alternative strategy that uses two independent handles for hot and cold water makes it difficult to reach the desired output in pressure and temperature. From a practical perspective, the bacterial operon strategy can be applied to synthetic biology only when it becomes possible to control TE artificially.

From the recent high-throughput studies reviewed here, it is apparent that we currently cannot predict TE in a quantitative way. A major bottleneck could lie in the biophysical algorithms that compute the most physiologically relevant RNA secondary structure. Alternatively, there might be uncharacterized molecular mechanisms that broadly influence TE. The combination of ribosome profiling and large-scale synthetic DNA libraries will likely shed light on the major mechanism for the translational control of protein synthesis.

Acknowledgements

I am grateful to Carol Gross, Jonathan Weissman, and David Burkhardt for their critical reading and discussion of this review. This work is supported by the NIH Pathway to Independence Award (GM105913).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jacob F, Monod J: **Genetic regulatory mechanisms in the synthesis of proteins**. *J Mol Biol* 1961, **3**:318-356.

2. Ohtaka Y, Spiegelman S: **Translational control of protein synthesis in a cell-free system directed by a polycistronic viral RNA**. *Science* 1963, **142**:493-497.

3. Lodish HF: **Bacteriophage f2 RNA: control of translation and gene order**. *Nature* 1968, **220**:345-350.

4. Shine J, Dalgarno L: **The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites**. *Proc Natl Acad Sci U S A* 1974, **71**:1342-1346.

5. Steitz JA, Jakes K: **How ribosomes select initiator regions in mRNA: base pair formation between the 3′ terminus of 16S rRNA and the mRNA during initiation of protein synthesis in *Escherichia coli***. *Proc Natl Acad Sci U S A* 1975, **72**:4734-4738.

6. Nomura M, Gourse R, Baughman G: **Regulation of the synthesis of ribosomes and ribosomal components**. *Annu Rev Biochem* 1984, **53**:75-117.

7. Baranov PV, Gesteland RF, Atkins JF: **Recoding: translational bifurcations in gene expression**. *Gene* 2002, **286**:187-201.

8. Ito K, Chiba S, Pogliano K: **Divergent stalling sequences sense and control cellular physiology**. *Biochem Biophys Res Commun* 2010, **393**:1-5.

9. Winkler WC, Breaker RR: **Regulation of bacterial gene expression by riboswitches**. *Annu Rev Microbiol* 2005, **59**: 487-517.

10. •• Salis HM, Mirsky EA, Voigt CA: **Automated design of synthetic ribosome binding sites to control protein expression**. *Nat Biotechnol* 2009, **27**:946-950.
Using an engineering approach to study the effect of 5′ UTR on protein production, the authors constructed a thermodynamics model for predicting translation efficiency.

11. •• Allert M, Cox JC, Hellinga HW: **Multifactorial determinants of protein expression in prokaryotic open reading frames**. *J Mol Biol* 2010, **402**:905-918.
The authors created a synthetic library of 285 genes and determined the expression levels *in vitro* in *E. coli* extracts. High AU content and low secondary structure in the ORF 5′ region of the mRNA have the most significant effect on expression.

12. •• Goodman DB, Church GM, Kosuri S: **Causes and effects of N-terminal codon bias in bacterial genes**. *Science* 2013, **342**:475-479.
The authors synthesized a library of >14,000 reporters and used FlowSeq to measure the protein levels. Synonymous mutations in the N-terminal region were found to be associated with decreased mRNA secondary structure and higher protein levels.

13. •• Kudla G, Murray AW, Tollervey D, Plotkin JB: **Coding-sequence determinants of gene expression in *Escherichia coli***. *Science* 2009, **324**:255-258.
The authors engineered a synthetic library of 154 genes that code for GFP and found that codon usage is not correlated with the level of fluorescence. The stability of mRNA folding around the ribosome binding site has the most significant correlation with fluorescence levels.

14. Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS: **Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling**. *Science* 2009, **324**: 218-223.

15. de Smit MH, van Duin J: **Secondary structure of the ribosome binding site determines translational efficiency: a quantitative analysis**. *Proc Natl Acad Sci U S A* 1990, **87**:7668-7672.

16. Hsu WT, Weiss SB: **Selective translation of T4 template RNA by ribosomes from T4-infected *Escherichia coli***. *Proc Natl Acad Sci U S A* 1969, **64**:345-351.

17. Ikemura T: **Codon usage and tRNA content in unicellular and multicellular organisms**. *Mol Biol Evol* 1985, **2**:13-34.

18. Pechmann S, Frydman J: **Evolutionary conservation of codon optimality reveals hidden signatures of cotranslational folding**. *Nat Struct Mol Biol* 2013, **20**:237-243.

19. Xu Y, Ma P, Shah P *et al.*: **Non-optimal codon usage is a mechanism to achieve circadian clock conditionality**. *Nature* 2013, **495**:116-120.

20. Gingold H, Pilpel Y: **Determinants of translation efficiency and accuracy**. *Mol Syst Biol* 2011, **7**:481.

21. Plotkin JB, Kudla G: **Synonymous but not the same: the causes and consequences of codon bias**. *Nat Rev Genet* 2011, **12**: 32-42.

22. Dong H, Nilsson L, Kurland CG: **Co-variation of tRNA abundance and codon usage in *Escherichia coli* at different growth rates**. *J Mol Biol* 1996, **260**:649-663.

23. Shah P, Gilchrist MA: **Explaining complex codon usage patterns with selection for translational efficiency, mutation bias, and genetic drift**. *Proc Natl Acad Sci U S A* 2011, **108**:10231-10236.

24. Andersson SG, Kurland CG: **Codon preferences in free-living microorganisms**. *Microbiol Rev* 1990, **54**:198-210.

25. Klumpp S, Dong J, Hwa T: **On ribosome load, codon bias and protein abundance**. *PLoS One* 2012, **7**:e48542.

26. Shah P, Ding Y, Niemczyk M *et al.*: **Rate-limiting steps in yeast protein translation**. *Cell* 2013, **153**:1589-1601.

27. Subramaniam AR, Zid BM, O'Shea EK: **An integrated approach reveals regulatory controls on bacterial translation elongation**. *Cell* 2014, **159**:1200-1211.

28. Tuller T, Waldman YY, Kupiec M, Ruppin E: **Translation efficiency is determined by both codon bias and folding energy**. *Proc Natl Acad Sci U S A* 2010, **107**:3645-3650.

29. Eyre-Walker A, Bulmer M: **Reduced synonymous substitution rate at the start of enterobacterial genes**. *Nucleic Acids Res* 1993, **21**:4599-4603.

30. Scharff LB, Childs L, Walther D, Bock R: **Local absence of secondary structure permits translation of mRNAs that lack ribosome-binding sites**. *PLoS Genet* 2011, **7**:e1002155.

31. Bentele K, Saffert P, Rauscher R *et al.*: **Efficient translation initiation dictates codon usage at gene start**. *Mol Syst Biol* 2013, **9**:675.

32. Kosuri S, Goodman DB, Cambray G *et al.*: **Composability of regulatory sequences controlling transcription and translation in *Escherichia coli***. *Proc Natl Acad Sci U S A* 2013, **110**:14024-14029.

33. Qi L, Haurwitz RE, Shao W *et al.*: **RNA processing enables predictable programming of gene expression**. *Nat Biotechnol* 2012, **30**:1002-1006.

34. Mutalik VK, Guimaraes JC, Cambray G *et al.*: **Precise and reliable gene expression via standard transcription and translation initiation elements**. *Nat Methods* 2013, **10**: 354-360.

35. •• Li GW, Burkhardt D, Gross C, Weissman JS: **Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources**. *Cell* 2014, **157**:624-635.
Using ribosome profiling and RNAseq, the authors directly measured the TE of endogenous genes in *E. coli*. Modulation of TE was found to be broadly used by bacteria to enable differential production of proteins in the same operon.

36. Oh E, Becker AH, Sandikci A *et al.*: **Selective ribosome profiling reveals the cotranslational chaperone action of trigger factor in vivo**. *Cell* 2011, **147**:1295-1308.

37. Deana A, Belasco JG: **Lost in translation: the influence of ribosomes on bacterial mRNA decay**. *Genes Dev* 2005, **19**:2526-2533.

38. Dennis PP, Bremer H: **Macromolecular composition during steady-state growth of *Escherichia coli* B-r**. *J Bacteriol* 1974, **119**:270-281.

39. Neidhardt FC: ***Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology**. Washington, DC: American Society for Microbiology; 1987, .

40. Ingolia NT, Lareau LF, Weissman JS: **Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of Mammalian proteomes**. *Cell* 2011, **147**: 789-802.

41. Li GW, Oh E, Weissman JS: **The anti-Shine–Dalgarno sequence drives translational pausing and codon choice in bacteria**. *Nature* 2012, **484**:538-541.

42. Stadler M, Fire A: **Wobble base-pairing slows in vivo translation elongation in metazoans**. *RNA* 2011, **17**:2063-2073.

43. Zinshteyn B, Gilbert WV: **Loss of a conserved tRNA anticodon modification perturbs cellular signaling**. *PLoS Genet* 2013, **9**:e1003675.

44. Charneski CA, Hurst LD: **Positively charged residues are the major determinants of ribosomal velocity**. *PLoS Biol* 2013, **11**:e1001508.

45. Subramaniam AR, Pan T, Cluzel P: **Environmental perturbations lift the degeneracy of the genetic code to regulate protein levels in bacteria**. *Proc Natl Acad Sci U S A* 2013, **110**:2419-2424.