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**Genome-comparative computational approach for
investigating prokaryotic ORF expression
potentialities, in relation with potentially high
transcription**

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Genome-comparative computational approach for investigating prokaryotic ORF expression potentialities, in relation with potentially high transcription

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Abstract

To synthesize a protein of interest, biotechnological techniques associate a strong promoter and a synthetic 5' Untranslated Region (UTR) optimized to enhance gene expression. Besides, in prokaryotic genomes, transcription and translation are temporally and spatially related events. Therefore, it is worth investigating whether potentially high transcription is associated or not with high translation potentiality. Secondly, since bacteria share common characteristics related to transcription (binding sites); a cross-genome comparison of 5' UTRs' features is of importance for insights into genome organization.

We implemented BACTRANS², a software platform dedicated to the identification of genes harbouring putative strong promoters in prokaryotic genomes. Through a complementary software suite, we analysed such genes identified in 32 bacterial genomes with respect to various characteristics related to translation: nature of start codon, location of Shine-Dalgarno sequence upstream of the start codon, AT-richness in the regions flanking translation signals, interference of secondary structure with translation signals, possible influence of mRNA 5' terminal secondary structure on mRNA stability. In particular, regarding AT-richness and interference with secondary structure, we show that putative strong promoters are characterized by a typical bias common to the known functional promoters of *Escherichia coli* genome. Generally speaking, all five criteria related to translation indicate that putative strong promoters are not favoured over known functional promoters. On the other hand, the large-scale genome-comparison implemented highlights the similarity of the 5' UTR characteristics between genes harbouring putative strong promoters.

Platform BACTRANS² is available at <http://www.sciences.univ-nantes.fr/lina/bioserv/BacTrans2/>. The present work required implementing many more softwares devoted to the analysis of mRNA translatability in relation with potentially transcription. The source code is freely distributed to academic users upon request to the authors. The annotation files relative to the 32 genomes studied are available at http://www.sciences.univ-nantes.fr/lina/bioserv/BacTrans2/putative_strong_promoters.

1 Introduction

In bacterial genomes, transcription and translation are temporally and spatially related events. Transcription, or mRNA synthesis, is promoted by two short sequences at -10 and -35 positions or thereabouts upstream of the Transcription Start Site (TSS). Binding to RNA polymerase, these sequences initiate transcription. Translation, which is the process of synthesizing a protein from an mRNA transcript, is regulated by the sequence and structure of the 5' untranslated region (UTR). Within the 5'UTR, the Shine-Dalgarno sequence binds to the ribosome. Transcription has been widely studied in *E. coli* K-12 (Hawley and McClure, 1983; Harley and Reynolds, 1987; Collado-Vides *et al.*, 1991; Lissner and Margalit, 1993; Fenton *et al.*, 2000; Gruber and Gross, 2003; Pager and Helmann, 2003; Herring *et al.*, 2005; to cite but a few works). In the meanwhile, various methods devoted to bacterial promoter prediction have been proposed (Huerta and Collado-Vides, 2003; Eskin *et al.*, 2003; Bulyk *et al.*, 2004; Shultzaberger *et al.*, 2007; to restrain to a few examples). Furthermore, since the RNA polymerase is conserved through evolution in bacteria, research was extended to other bacteria (Gross *et al.*, 1992; Gralla and Collado-Vides, 1996; Li *et al.*, 2002; Martinez-Antonio and Collado-Vides (2003); Huerta *et al.*, 2006; for some illustrations).

In vivo, transcriptional regulations are known to compensate for promoter weakness (Gross *et al.*, 1998; Browning and Busby, 2004). Thus, identifying putative strong promoters is valuable to biotechnological applications for enhancing the expression of genes of interest. Besides, evaluating mRNA translatability associated with potentially high transcription is of importance to advances in biotechnologies as well as fundamental knowledge. Our study deals with the $\sigma 70$ promoter, including a non-canonical variant consisting of three binding sites. In addition to the usual -10 and -35 boxes, the Upstream Promoter element (UP element) is located approximately 4 bp upstream of the -35 region, conferring additional strength to the promoter. The UP element was experimentally identified in *E. coli* (Ross *et al.*, 1993; Estrem *et al.*, 1998; Estrem *et al.*, 1999), *Bacillus subtilis* (Fredrick *et al.*, 1995), *Vibrio natriegens* (Aiyar *et al.*, 2002) and *Geobacillus stearothermophilus* (Savchenko *et al.*, 1998). For our purpose, we analyse five classes of features related to translation potentiality. We compare the trends between 32 prokaryotic genomes. Then we compare these trends with the trends observed for genes harbouring known functional promoters in *Escherichia coli* genome. In the sequel, genes harbouring Putative Strong Promoters are denoted PSP genes and genes harbouring Functional Promoters are referred to as FP genes.

2 Systems and Methods

2.1 Annotation of genomes with potentially strong $\sigma 70$ promoters

Thirty-two annotated genome files were taken from the publicly available database GenBank. We selected the two well-studied models *Escherichia coli* and *Bacillus subtilis*, as well as an extremophilic model, *Thermotoga maritima*. Our selection provides a good representation of the bacteria spectrum (*Actinobacteria*, *Chlamydia*, *Firmicutes*, *Proteobacteria*, *Spirochaetales*). We restrained our investigation to genes coding for mRNAs. The canonical $\sigma 70$ promoter structure is described in 5' to 3' direction as [-35 box] <spacer> [-10 box]. In Genbank files, the only location annotation available is the start codon location. Hence, for each gene, the start codon (SC) is considered a right anchor and each region upstream of SC is scanned to retrieve in priority the structured motif [UP element] <spacer4> [-35 box] <spacer3> [-10 box] <spacer2> [SD] <spacer1>, where SD denotes the Shine-Dalgarno sequence. The full motif identification is performed in the 3' to 5' direction, successively considering each possible occurrence of the current box as a right anchor. In the absence of any UP element, the structured motif [-35 box] <spacer3> [-10 box] <spacer2> [SD] <spacer1> is looked for. Three types of constraints must be set: box contents (consensuses) are specified, a minimal similarity threshold relative to each such box is fixed, the spacers are described through the minimal and maximal bp distances allowed for them. For

each box of the structured motif, the minimal similarity threshold is expressed as the maximal number of mismatches allowed. To identify the best candidate in the regulatory region, an exact combinatorial approach is implemented rather than a probabilistic approach: reducing the number of mismatches with regard to each box consensus is the optimization criterion used instead of maximizing information content (Schneider *et al.*, 1986). The justification for this is that our aim is identifying potentially strong promoters, not predicting functional promoters. Thus, in essence, there are no strong promoters available over the 32 genomes studied to learn models from, contrary to previous works ((Huerta *et al.*, 2003), where 288 Position-Specific Scoring Matrices were learned from *E. coli*; (Shultzaberger *et al.*, 2007), which considered 6 models for functional promoters in *E. coli*).

In the work reported here, the structured motif is tuned as $[NNAAAWWTWTTTTNAAAANN] \langle 0-15 \rangle [UUGAC] \langle 15-20 \rangle [TATAAT] \langle 10-200 \rangle [GGAGG] \langle 2-10 \rangle$. The maximal mismatch errors allowed are respectively: 0 (SD), 2 (-10 box), 3 (-35 box) and 4 (UP element). The increase of the specificity requirement in the 5' to 3' direction is modeled after observations relative to functional $\sigma 70$ promoters. Moreover, we intentionally focus on the subset of putative strong $\sigma 70$ promoters already potentially favoured by the presence of an optimal SD sequence. The presence of the SD sequence has been ascertained for a large number of bacteria (Osada *et al.*, 1999) and it was established that the extent to which a SD sequence is conserved relates to its translation efficiency (Ma *et al.*, 2002). On the other hand, our choice of a shorter consensus for the -35 box rather than the canonical -35 box itself (UUGACA) is motivated by sixth nucleotide having the lowest conservation level. Our choice simply amounts to taking into account the canonical -35 box allowing 4 mismatches at most. Besides, at most 4 mismatches may seem a low constraint for such a 22 bp-long box as the UP element. But indeed, the content description is already rather flexible with 5 wild-card N nucleotides and 3 W nucleotides (T or A). Finally, when given a genome file, BACTRANS² outputs 0 or 1 putative strong promoter per gene coding for an mRNA.

2.2 Sorting the strong promoters

For each gene encoding a protein, a sequence of interest upstream of the start codon is investigated for potentially strong promoter candidates. This sequence of interest encompasses the largest possible configuration resulting from the user's specification (283 nucleotides in our case). To identify the strong promoter with the highest potentiality, a scoring function is defined, based on bp distances and mismatch error numbers. In the sequel, $d_{35.10}$ and $d_{UP.35}$ respectively denote the bp distances between the -10 and -35 boxes, and between the -35 box and the UP element; e_{10} , e_{35} and e_{UP} respectively describe the maximal numbers of mismatches allowed with respect to the -10 box, the -35 box and the UP element. In the present study, the score is computed as follows: $score = 0.60 \times e_{10} + 0.40 \times e_{35} + e_{UP} + t_1 + t_2$ where $t_1 = 0$ if $d_{35.10}$ belongs to interval [17, 19] else $t_1 = 5 \times d_{35.10}$, and $t_2 = 0$ if $d_{UP.35}$ ranges in interval [3, 5] else $t_2 = 3 \times d_{UP.35}$. When no UP element can be found, the score becomes: $score = penalty + 0.60 \times e_{10} + 0.40 \times e_{35} + t_1$. The lower the score, the more likely the identified putative promoter is a strong one. The penalty value is set in order to systematically favour a candidate with an UP element within the regulatory region.

2.3 Features describing transcription and translation potentialities

For each gene harbouring a putative strong promoter, the following characteristics relative to transcription are provided: score, putative binding site contents, corresponding numbers of mismatches, bp distances between all boxes of the structured motif aforementioned. The transcription start site (TSS) is identified as the 9th nucleotide downstream of the extremity of the -10 box.

On the one hand, several experimental works demonstrated that the use of AUG start codon could increase gene expression level in *E. coli* and *B. subtilis* genomes (Roy *et al.*, 1988; Vellanoweth and

Rabinowitz, 1992). On the other hand, the secondary structure of mRNA 5' extremity is known to play an important role in post-transcriptional gene regulation. First, such a structure is likely to perturb translation initiation when interfering with the start codon or the Shine-Dalgarno sequence. Here, interference means that a translation signal is located in a stem (partially or entirely). Besides, AT-richness is known to prevent the formation of steady secondary structures and it was shown for *E. coli* that the AT-richness of the region following the initiation codon plays a significant enhancing role in gene expression (Sato *et al.*, 2001; Qing *et al.*, 2003; Voges *et al.*, 2004). Finally, hairpins at mRNA 5' extremity have been shown to play various roles regarding mRNA stabilization: (i) the possibility to form a hairpin very close to the 5' end is known to stabilize the mRNA molecule in *Escherichia coli* (Emory *et al.*, 1992; Bricker and Belasco, 1999; Carrier and Keasling, 1999; Cebe and Geiser, 2006); (ii) stem-loop structures are also likely to protect mRNA against the attacks by the main decay-initiating ribonuclease, RNase E (Bechhofer, 1993; Unniraman *et al.*, 2002); (iii) the presence of double-stranded RNA is also required for mRNA degradation by RNase III enzyme. Such stabilization regulations are used by prokaryotes for expression control purpose (Belasco and Brawerman, 1993; Cohen and McDowall, 1997; Carrier and Keasling, 1997).

In this work, we describe genes harbouring putative strong promoters through five classes of features related to translation: (i) start codon nature, (ii) location of the Shine-Dalgarno sequence upstream of the start codon, (iii) AT-richness of the regions flanking the Shine-Dalgarno sequence and the start codon, (iv) interference between translation signals and mRNA 5' terminal secondary structure, (v) possible presence of a stabilizing secondary structure at mRNA 5' extremity. Regarding AT-richness context of translation signals, we examine 5 bp-long left and right flanking regions.

2.4 Processing the mRNA 5' terminal secondary structure

Transcription and translation are quasi simultaneous processes in prokaryotic genomes. Hence the ribosome flattens the mRNA secondary structure when it proceeds along this molecule. Therefore we need only consider a sub-region at mRNA 5' extremity for folding. In the sequel, this sub-region is called the mRNA 5' terminal sub-region; it obligatorily contains both transcription signals. A threshold l_θ is specified for discriminating between short and long 5'UTR regions. In the case of a long 5'UTR region, the 5' terminal sub-region considered is restrained to the 5'UTR region and is automatically sized to l_d nucleotides downstream, including the start codon. When the 5'UTR region's length l is less than or equal to l_θ , we consider up to n downstream nucleotides, including the start codon, with $l + n$ equal to $l_\theta + l_d$. In this study, l_θ and l_d are respectively tuned to 70 and 10. For each gene with a putative strong promoter identified, the mRNA 5' terminal sub-region is folded using the program RNAsubopt of the Vienna RNA Package (Hofacker *et al.*, 1994; Wuchty *et al.*, 1999). The resulting folding depends on the temperature optimal for the life of the prokaryote considered. This temperature is tuned to 37°C for all species considered except for hyperthermophilic prokaryotes (*Thermoanaerobacter tengcongensis* (75°C), *Aquifex aeolicus* (96°C) and *Thermotoga maritima* (80°C)). For each gene harbouring a potentially strong promoter, we select the folding predicted with the lowest free energy, among all suboptimal secondary structures calculated within 1 kcal/mol above the minimum free energy.

Table 1 describes seven types of stem-loop or helix structures whose average frequencies were computed. The first four types were searched for within close distance from TSS, namely in the range [+1, +4]. No interior loop is allowed in these four types. Depending on the stem length, the loop length and the number of bulges, some hairpins might favour or not mRNA stability ($T1$ to $T4$). But hairpins are also known to play a direct role regarding mRNA degradation. We looked for type $T5$, characterized as a hairpin containing at least half of the RNase E cleavage site. The reader is referred to Table 2 for a description of the various sites RNase E is able to cleave. Moreover, in this case, we were careful to check that no full RNase E cleavage site was also present in single-stranded RNA located between the former hairpin identified and a second hairpin. Consistently, we also looked for type $T6$, consisting of two hair-

	distance from TSS	interior loop allowed	stem length	loop length	number of bulges	number of nucleotides in bulge	specificity	presumed influence	reference
T1	[+1, +4]	no	≥ 14	≤ 4	≤ 1	≤ 2	—	+	Carrier and Keasling, 1999
T2	" "	yes	—	≥ 7	—	—	—	-	" "
T3	" "	yes	—	—	≥ 2	—	—	-	" "
T4	" "	yes	≥ 4	[4, 6]	—	—	—	+	Arnold <i>et al.</i> , 1998
T5	[+1, +20]	no	≥ 6	≤ 4	—	≤ 2	RNase E cleavage site trapped in stem	+	Naureckiene and Uhlin, 1996
T6	" "	no	" "	" "	" "	" "	two hairpins flanking an RNase cleavage site	-	Régnier and Hajnsdorf, 1991
T7	[+1, +20]	×	—	≥ 20	≤ 3	≤ 2	helix	-	Cheladurai <i>et al.</i> , 1991

Table 1: Seven various types of stem-loop and helix structures and their presumed influence on the stability of mRNA. —: indifferent; ×: inadequate

cleavage site	reference
G/AUUU	Lundberg <i>et al.</i> , 1990
U/AUUU	" "
A/AUUU	Ghora and Apirion, 1978
A/AUAA	" "
U/AUUU	Tomcsanyi and Apirion, 1985
A/AUUA	Muddet <i>et al.</i> , 1988
AAU/UU	Faubladier <i>et al.</i> , 1990
AA/GCU	Régnier and Hajnsdorf, 1991
AG/UUU	" "
G/AUCG	Mackie, 1991
GU/AUU	Nilsson and Uhlin, 1991
AAU/UU	Gross, 1991
GU/UUU	" "
G...U or .AUU. or A...A	Ehretsmann <i>et al.</i> , 1992

Table 2: Various cleavage sites for the RNase E of *Escherichia coli*. The slash character indicates the cleavage location.

pins separated by single-stranded RNA harbouring an RNase E cleavage site. Type *T5* is presumed to prevent mRNA decaying through interference with RNase E cleavage site. On the opposite, in type *T6* conformation, the cleavage site is accessible to RNase E. Moreover, this unfavourable conformation is stabilized by the two flanking hairpins. For both types *T5* and *T6*, we arbitrarily examined the possibility of such a conformation to start in the range [+1, +20] (with respect to TSS). Finally, the presence of a hairpin may be unfavourable to mRNA stability since RNase III enzymes require a double-stranded cleavage site harboured in an hairpin. A specific cleavage site consensus is known for *E. coli*: it is described as **WNAGWGCNNCWUNNN/NAWGNNCWCUNW** in 5' to 3' direction, with its complement **WNUCWCGNNGWAN/NNUWCNNGWG ANW** on the other strand (Krinke and Wulff, 1990). The six boldface characters correspond to conserved nucleotides. The slashes in both halves point out the cleavage site. However, it was also shown *in vitro* that provided an helix had a sufficient length (over 20), it could be subject to degradation by RNase III (Cheladurai *et al.*, 1991). In the present study, *T7* corresponds to this latter case.

2.5 ORF potential expression: functional promoters of *E. coli* versus potentially strong promoters

Known functional $\sigma 70$ promoters of *E. coli* genome are available in repositories such as RegulonDB (Salgado *et al.*, 2006) and PromEC (Hershberg, *et al.*, 2001). RegulonDB is the reference database for *E. coli* curated knowledge; 601 $\sigma 70$ promoters are listed in 5.6 RegulonDB release (january 2007, <http://regulondb.ccg.unam.mx/data/Promoter Set.txt>). PromEC is entirely dedicated to functional $\sigma 70$ *E. coli* promoters (<http://margalit.huji.ac.il/>); it includes 471 entries, among which some are common to RegulonDB. *E. coli* genome contains 4173 genes coding for mRNAs (out of 4288 genes). The trends for the five classes of translation features described in the previous subsection are compared between ORFs associated with functional promoters of *E. coli* genome and ORFs associated with putative strong promoters identified in the 32 genomes studied, *E. coli* genome comprised. In particular, we compare the distributions for the location of Shine Dalgarno sequence upstream of the start codon. But RegulonDB and PromEC only provide experimentally identified TSSs. Therefore, for each functional promoter, we have to infer the Shine-Dalgarno sequence location; we identify the subsequence located between 7 and 15 bp upstream of the start codon, and most similar to the optimal Shine-Dalgarno sequence.

2.6 Implementation

We first implemented a software devoted to the identification of putative strong $\sigma 70$ promoters in the hyperthermophilic model genome of *Thermotoga maritima*. Subsequently, the generic platform BACTRANS² was developed: it allows the exhaustive search of any structured motif consisting of 3 to 4 boxes in any bacterial genome specified. The web-interface assists the user in specifying the description of the structured motif (bp distances between boxes and minimal similarity thresholds); an expert user may modify the tuning of the scoring function. In addition, BACTRANS² provides a browser to navigate through, visualize and classify the best mRNA 5' terminal secondary structures predicted for each PSP gene. Besides, this platform presents intra- and inter-genomic statistics through various plots. The client-server system implemented facilitates efficient handling of sessions. Each session is relative to both a given genome and a specification. In particular, BACTRANS² identification core software has already been used intensively to study the statistical significance of putative strong promoters, in the 32 bacterial genomes studied. Finally, a software suite was developed to thoroughly investigate mRNA translatability in relation with high transcription potentiality. The components of this software suite were adapted to process *E. coli* FP genes. FP genes were obtained merging data from RegulonDB and PromEC repositories, under redundancy control. The software suite was written in Java and the scripts automating the analysis over the 32 genomes selected were written in Unix shell. Figure 1 depicts the various software components used in the present study.

3 Results

3.1 High transcription potentiality

For each genome, a first annotation file was produced, which contains the comprehensive list of potentially strong promoters (see Figure 2 (a)). These putative promoters are sorted in decreasing order, with respect to their scores. Promoters harbouring an UP element are listed at the top of the list. In addition, for visual facilities, we annotated the genes containing putative strong promoters with tags corresponding to all boxes aforementioned (see Figure 2 (b)). In the context of another study devoted to hyperthermophilic bacterium *Thermotoga maritima*, the experimental verification of 7 putative strong promoters also uncovered by BACTRANS² was performed (Sakanyan *et al.*, 2003; Sinoquet *et al.*, 2007): the 7 promoters were proven functional and 6 were actually confirmed to be strong promoters.

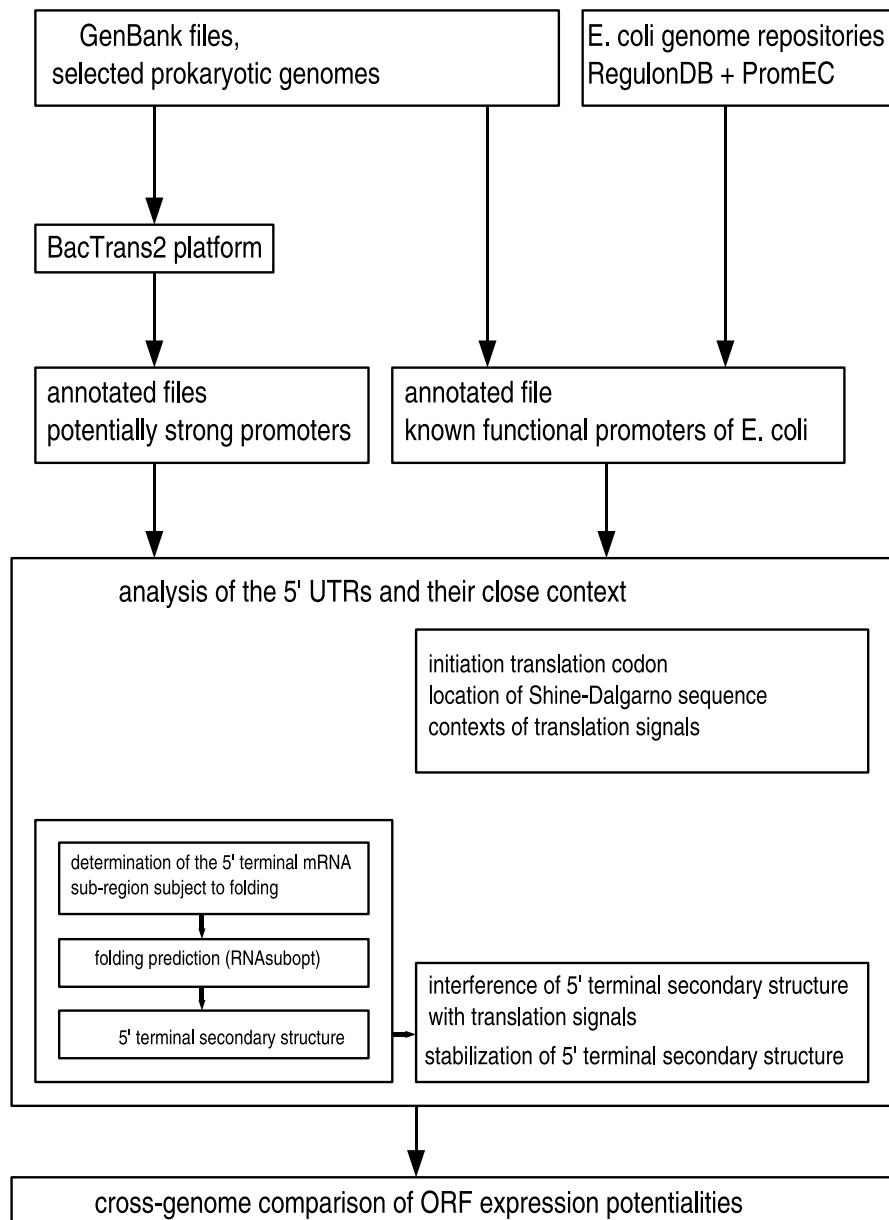


Figure 1: Description of the various software components and data flows implemented for the study of translation potentiality associated with putative strong transcription or known functional transcription.

The 6 smallest genomes are those of either obligate intracellular pathogens, symbionts or animal commensal parasites (*Borrelia burgdorferi* (0.91 Mbp), *Chlamydomphila pneumoniae* (1.22 Mbp), *Mycoplasma genitalium* (0.58 Mbp), *Mycoplasma pneumoniae* (0.81 Mbp), *Rickettsia prowazekii* (1.11 Mbp) and *Treponema pallidum nichols* (1.13 Mbp)). In a prior study, we established that the presence of the putative strong $\sigma 70$ promoter aforescribed is statistically significant for 22 species out of the 26 ones with genomes over 1.5 Mbp (Sinoquet *et al.*, 2007). We performed a statistical analysis based on the comparison of the frequency of PSP genes in each prokaryotic genome and the average frequency calculated over 100 randomly generated genomes. These latter genomes were only constrained to have the same total number of genes coding for mRNAs, and the same proportions of nucleotides A, T, C and G as the bacterial genome. We find interesting to keep *Haemophilus influenzae*, *Helicobacter pylori*, *Vibrio cholerae*, *Xanthomonas campestris* and the 6 species with the smallest genomes in the present genome-comparative analysis. Supplementary Appendix 1 compares the frequencies of putative strong promoters obtained over the 32 genomes, enlightening the proportion of putative strong promoters harbouring an UP element.

3.2 Translation potentiality

3.2.1 Nature of start codon

In *Escherichia coli* genome, the three pre-eminent start codons AUG, GUG and UUG are known to be less favourable to gene expression when considered from AUG to UUG. On average, we check that the respective percentages for AUG, GUG and UUG are 80.36%, 12.18% and 7.19%, over the 32 genomes (for more details, see Supplementary Data, Appendix 2).

3.2.2 Location of Shine-Dalgarno sequence upstream of the start codon

Due to the constraints specified for the present application, Shine-Dalgarno sequence's 5' extremity ranges between 7 and 15 bp upstream of the start codon. For an overwhelming majority of genomes, a single location is clearly pre-eminent. Regarding the 8 *Firmicutes* which are not *Mollicutes*, we observe that the 8 most frequent locations vary in the range [11, 13], 12 being the most represented location. Quite another trend is observed with respect to *Proteobacteria*: for 11 *Proteobacteria* out of the 13 studied, the most frequent locations observed vary between 9 and 10. The other phyla studied show maxima intermediary between those of *Firmicutes* and *Proteobacteria*, namely between 10 and 12. All corresponding plots are displayed in Supplementary Appendix 3.

3.2.3 AT-richness in the regions flanking translation signals

For each genome studied, four AT-richness percentages are computed: average AT-richness in the regions flanking SD, average AT-richness in the regions flanking SC, average AT-richness in the 5'UTRs, average AT-richness calculated over the whole genome (WG) (see Figure 3). The three former averages are computed over the PSP genes. We observe two striking features. First, all four values are highly correlated when considered pairwise (the correlation coefficients range in interval [0.95, 0.99]). This result is all the less trivial as AT-richnesses in the regions flanking SC and SD are calculated considering *only* 10 nucleotides. Secondly, the order for AT-richnesses is always the same, whatever the genome: SD > SC > 5'UTR > WG. *Mycobacterium leprae* and *Mycoplasma genitalium* slightly depart from this trend as showing an inversion between 5'UTR and WG AT-richness ranks. *Streptomyces coelicolor*, *Chlamydomphila pneumoniae* and *Helicobacter pylori* present an inversion between SC context AT-richness and 5'UTR AT-richness. An exception regarding the ranks of SD and SC context AT-richnesses is also observed for *Rickettsia prowazekii*; in the case of *Escherichia coli*, SD and SC context AT-richnesses are very close (56.31% and 56.98%, respectively). The average difference between SD and SC contexts is

4%, with 11% as a maximum (*Helicobacter pylori*). The difference between SD context and 5'UTR is 8% on average (maximum 13% for *Mycoplasma pneumoniae*). The difference between SD context and the whole genome is 10% on average (maximum 17% for *Mycoplasma pneumoniae*).

3.2.4 Interference of secondary structure with translation signals

We recall that the mRNA 5' terminal sub-region considered for its secondary structure includes both Shine-Dalgarno sequence and start codon. The rule described for calculating the length of this sub-region depends on 5'UTR length. According to this rule, the sub-region length is at least equal to $l_\theta + l_d$ (80). An overview of the trend relative to 5'UTR lengths is provided in Supplementary Appendix 4.

Figure 4 (a) describes interference with SD through classes SD_0 to SD_5 . The PSP genes having i SD nucleotides involved in base pairing together define class SD_i . SC_i classes are defined similarly. A common trend is observed for all 32 genomes, which is reflected by the following averages: class SD_0 : 16.70%, class SD_1 : 7.06%, class SD_2 : 14.96%, class SD_3 : 25.51%, class SD_4 : 24.48%, class SD_5 : 11.25%. Between classes 1 and 5, the curve is bell-shaped and the most frequent classes are SD_3 and SD_4 . Thus, about 60% of the genes concerned seem unfavoured with interference involving more than half of the SD nucleotides. However, we note that the most unfavourable case, class SD_5 , is twice less frequent than class SD_3 or class SD_4 . Moreover, interestingly, the most favourable class SD_0 is apart and ranks before classes SD_1 and SD_2 . Neither *Firmicutes* nor *Proteobacteria* with large genomes show a deviation from this trend. Figure 4 (b) depicts interference with start codon through the description of classes SC_0 through SC_3 . Classes SC_0 to SC_3 respectively total 25.76%, 16.50%, 31.54% and 26.15% on average. Again, we observe the outstanding exception of the most favourable class, SC_0 , which departs from the bell-shaped distribution. Furthermore, the proportion of genes without SC interference is greater than that of genes without SD interference (25.76% versus 16.70%, on average). Finally, the trends respectively observed for large *Firmicutes* and *Proteobacteria* genomes are in accordance with that observed over the 32 genomes.

Bad accessibility to SD could be compensated by a conformation locally favourable to SC accessibility and *vice versa*. Though, quite unexpectedly, the joint distribution of #SD and #SC, the numbers of SC and SD nucleotides interfering with the secondary structure, shows a strong bias: (i) half of the 24 possible pairs (#SD, #SC) are *never* represented in any genome; (ii) moreover, pairs with both high (respectively low) #SD and #SC values are pre-eminent. For each possible pair (#SD, #SC), Table 3 recapitulates the average frequencies computed over the 32 genomes: it is clearly shaped as a quasi-diagonal matrix. Supplementary Appendix 5 compares the distributions for the 24 possible pairs (#SD, #SC) over all 32 genomes. Except for small genomes such as *Mollicutes* and *Rickettsia prowazekii*, the trend observed for each genome does not deviate from the average trend. We recapitulate the following rules observed: (i) complete SD interference (#SD=5) implies full start codon interference (#SC=3); (ii) the defavourable #SC value of 3 is never encountered if #SD value is less than or equal to 2; (iii) the favourable #SD value of 1 is only associated with #SC values 0 and 1; (iv) if SD does not interfere with the secondary structure, then neither does SC. Both previous cases (iii) and (iv) total 23.76% on average.

3.2.5 Possible influence of mRNA 5' terminal secondary structure on mRNA stability

Figure 5 depicts the frequencies of the various types exhibited in the 32 genomes. Though the orders of magnitude may differ between genomes, the order $T_3 > T_4 > T_2 > T_5 > T_7$ is constantly observed for frequencies, with minor deviations for 3 small genomes. Favourable type T_1 is rarely encountered: all frequencies are null or close to null. Amongst T_2 and T_3 presumed unfavourable types, less restrictive type T_3 is encountered with the average frequency of 39.16% (minimum and maximum respectively 26.66% and 51.35%) while type T_2 is present for 13.83% of PSP genes on average (minimum and maximum respectively 0% and 33.33%). On average, the presumed favourable type T_4 is identified in

		#SD					
		0	1	2	3	4	5
#SC	0	16.7	4.66	4.4	0	0	0
	1	0	2.4	3.76	10.35	0	0
	2	0	0	6.8	8.84	15.9	0
	3	0	0	0	6.32	8.58	11.25

Table 3: Interference of mRNA 5' terminal secondary structure with translation signals for genes harbouring putative strong $\sigma 70$ promoters, average percentages over 32 prokaryotic genomes. #SD denotes the number of nucleotides in the Shine-Dalgarno sequence which are involved in a stem. #SC is defined similarly with respect to the start codon.

27.54% of PSP genes (minimum and maximum respectively 6.66% and 50%). Favourable type T5 is absent from the two *Mollicutes* genomes as well as *Rickettsia prowazekii*'s; the average frequency is 4.92% (maximum 12.62%). Unfavourable type T6 is shown to be constantly absent from all genomes studied whereas unfavourable type T7 is rarely encountered (average 0.89%, maximum 2.94%). Finally, on average, unfavourable types, *UT*, (*T2*, *T3*, *T6*, *T7*) are slightly more frequent than favourable types, *FT*, (*T1*, *T4*, *T5*). The average ratio $\frac{UT}{FT}$ computed over the 32 genomes is 1.57; it reflects acutely the tendency for each genome (standard deviation 0.23).

3.3 Comparing trends between putative strong promoters and functional promoters of *Escherichia coli* genome

The characteristics of the 255 genes harbouring potentially strong $\sigma 70$ promoters were compared with the characteristics of the 583 genes harbouring functional $\sigma 70$ promoters from RegulonDB and PromEC. Moreover, when possible, we also compared some characteristics with those of the 4173 genes coding for mRNAs (out of a total of 4288 genes). Hereafter, in addition to the PSP and FP notations, respectively referring to genes harbouring putative strong promoters and genes harbouring functional promoters, such genes encoding proteins will be called mRNA genes.

3.3.1 Nature of start codon

Figure 6 describes the distributions relative to mRNA genes, FP genes and PSP genes, in *E. coli* genome. The mRNA genes divide between classes AUG, GUG, UUG and Other as follows : 81.81%, 14.95%, 3.20% and 0.02%. For FP genes, the distribution is 90.05%, 8.74%, 0.85% and 0.34% whereas it is 85.88%, 10.59%, 3.14% and 0.39% for PSP genes. First, we observe that the orders of magnitude for the AUG and GUG percentages are quite conserved through the three gene sets. Moreover, the AUG and GUG percentages relative to PSP genes are intermediary between the percentages of the other two gene sets. Over all three gene sets, AUG is the most represented for FP genes. Consistently, GUG is the least represented for the latter genes. Interestingly, FP genes are associated in the majority with the two most performing start codons AUG and UUG.

3.3.2 Location of Shine-Dalgarno sequence upstream of start codon

Locations quite evenly distribute in interval [10, 13] for both *E. coli* FP genes and *E. coli* PSP genes. In this interval, FP percentages range between 13, 90% and 17, 83%, whereas PSP percentages range in [12, 15%, 18.82%]. The location slightly most frequently encountered for FP genes is 13 (17.83%).

For PSP genes, the maximal frequency, 25.09%, is observed at location 9. Supplementary Appendix 3 outlines the similarity of the two curves relative to *E. coli*.

3.3.3 AT-richness in the regions flanking translation signals

Table 4 compares the AT-richness averages respectively calculated over the regions flanking SD, the regions flanking SC, the 5'UTRs and whole genome (WG). The order observed when studying the PSP genes of the 32 genomes, $SD > SC > 5'UTR > WG$ (Whole Genome), is conserved for FP genes. On average, the bias between SD context and 5'UTR amounts to 9%. For *E. coli* PSP genes, this previous order is conserved, with SD and SC context AT-richnesses quasi similar, and 5'UTR AT-richness only 2% above WG percentage. In Figure 7, the AT-percentage distributions relative to 5'UTRs, SC contexts and SD contexts are compared for FP, PSP (and mRNA) genes. We observe that the histograms relative to SC and SD may be superimposed, whatever the gene set (the shape is roughly a gaussian one). Furthermore, the previous histograms possess a remarkable common characteristic regarding FP and PSP genes: on the left section of the histogram (with respect to the maximum (60%-69%)), FP gene percentages are smaller than PSP gene percentages; on the right section, the opposite is observed. In contrast, the histogram relative to 5'UTRs has a gaussian shape for FP genes only; moreover, AT-richness values observed for PSP genes range in a large interval (from 10% to 90%).

AT-richness	SD context	SC context	5'UTR	WG(4)
(1) FP genes	65.11	60.17	56.24	
(2) PSP genes	56.31	56.98	50.81	49.21
(3) mRNA genes	-	59.24	-	

Table 4. Comparison of average AT-percentages between three gene sets of *E. coli* genome. (1) genes harbouring functional $\sigma 70$ promoters; (2) genes harbouring putative strong $\sigma 70$ promoters; (3) genes encoding proteins; (4) Whole Genome.

3.3.4 Interference of secondary structure with translation signals

We remind that the 5' terminal region considered for folding includes at least the 5'UTR region. FP and PSP distributions of the 5'UTR lengths in *E. coli* are compared in Figure 8. Regarding interference, we observe comparable trends for FP genes and PSP genes (Figure 9). Focusing on the presumed three most favourable classes ($\#SD < 3$ and $\#SC < 2$), $\#SD \leq 1$ and $\#SC = 0$, $\#SD = \#SC = 0$), we observe the respective percentages 33.71%, 24.30% and 16.47% for PSP genes. They are comparable to the percentages 38.73%, 24.16% and 13.20% relative to FP genes (see Table 5).

interference class	$\#SD \leq 2$	$\#SD \leq 1$	$\#SD = 0$
	$\#SC \leq 1$	$\#SC = 0$	$\#SC = 0$
(1) PSP genes	31.92	23.76	16.70
(2) <i>E. coli</i> PSP genes	33.71	20.78	16.47
(3) <i>E. coli</i> FP genes	38.73	17.65	13.20

Table 5. Comparison of the average frequencies of the most favourable interference classes between three gene sets. (1) genes harbouring putative strong $\sigma 70$ promoters (average over 32 genomes), (2) genes harbouring putative strong $\sigma 70$ promoters (*E. coli*), (3) genes harbouring functional $\sigma 70$ promoters (*E. coli*). Interference means that the translation signal considered partially overlaps a stem or is totally contained in a stem. $\#SD$ denotes the number of nucleotides in the Shine-Dalgarno sequence which are involved in base-pairing. $\#SC$ is defined similarly with respect to start codon.

3.3.5 Possible influence of 5' terminal secondary structure on mRNA stability

The frequencies were computed for conformation types $T1$ through $T7$. *E. coli* genome exhibits the order $T3 > T4 > T2 > T5 > T7$, already observed for the PSP genes of other genomes. Moreover, this order holds for FP genes. Among the 255 PSP genes, 9.80% and 41.96% respectively harbour the presumed unfavourable $T2$ and $T3$ conformations. Presumed favourable $T4$ type is identified in 32.94% of the PSP genes. The orders of magnitude are comparable between FP and PSP genes: regarding the FP genes, the frequencies observed for $T2$, $T3$ and $T4$ conformations are 10.12%, 33.61% and 22.98%, respectively. Favourable type $T1$ and unfavourable type $T6$ are absent in both gene sets. Favourable type $T5$ is not much more encountered in PSP genes than in FP genes (6.27% versus 3.25%). The same remark holds for unfavourable type $T7$ (PSP genes: 1.17%, FP genes: 2.91%).

4 Discussion

The striking conclusion of the previous section is that for any criterion examined, the difference is minor between *E. coli* PSP genes and *E. coli* FP genes. The import of this conclusion will be amplified by the next comparison between *E. coli* FP genes and the PSP genes of the other 31 bacterial genomes considered.

4.1 PSP genes are preferentially associated with AUG start codons but without any favouring bias over FP genes

Taking as a reference the average AUG percentage over all genes coding for mRNAs in *E. coli* genome, that is 82%, we show that *E. coli* $\sigma70$ promoters, however functional or potentially strong, are associated with higher AUG percentages. Nonetheless, in *E. coli* genome, PSP genes are less frequently associated with AUG codons than FP genes (86% versus 90%). When considered over 32 genomes, the AUG percentages relative to PSP genes are confirmed to be lower than *E. coli* FP percentage (5 cases excepted). On the other hand, a statistical study focusing on highly expressed genes of *B. subtilis* failed to characterize them by an AUG bias (Rocha *et al.*, 1999). Thus, without denying the enhancing role of AUG in translation, this latter study points out that a high translation level is not obligatorily associated with the AUG codon. Finally, in light of the preceding remark and since no strong bias is shown with respect to FP genes, we are not able to conclude whether the PSP genes are actually less favoured than FP genes.

4.2 The typical distribution for SD locations is common to PSP genes and genes encoding proteins

Regarding the location of Shine-Dalgarno sequence upstream of start codon, it is remarkable that the typical distribution (two maxima) exhibited for genes analysed in 141 prokaryotic genomes (Chang *et al.*, 2006) has been uncovered for the PSP genes, in the 32 genomes studied. Chang and co-workers dealt with all genes coding for mRNAs. They showed that SD sequences are mainly observed at 9th position upstream of the initiation codon, and specifically at 11th position for *Bacillales*, an order of Gram-positive bacteria, placed within the *Firmicutes*. Regarding PSP genes, we observe that the most frequent SD locations vary between 9 and 10 in *Proteobacteria*; in contrast, they range from 11 to 13 in *Firmicutes*, 12 being the most represented value. The slight shift between the two distributions compared is easily explained: we searched for *exact* SD sequences in interval [7, 15] upstream of the start codon, whereas Chang and co-workers considered a set of 6 SD sequence variations. Shine-Dalgarno sequence being located in a narrow region upstream of the start codon, it is likely that FP genes (whether known or not) and PSP genes rely on the same Shine-Dalgarno sequence. Anyway, it was perhaps not expected that 32 would be sufficient enough for the two maxima to be outlined.

4.3 A favourable AT-richness bias in the vicinity of SD and SC characterizes both PSP and FP genes

For an insight into potential protection against secondary structure formation, AT-richness percentages were computed in the vicinity of translation signals. On average, the order $SD > SC > 5'UTR > WG$ (Whole Genome) was constantly observed for the PSP genes of any genome studied, as well as for *E. coli* FP genes. SD and SC context AT-richnesses differ by 4% and 5% respectively for PSP genes (average over the 32 genomes) and FP genes. The difference between SD context AT-richness and 5'UTR AT-richness is more acute (8% and 9% for PSP and FP genes, respectively). As a result, on average, SD AT-richness amounts to 65% for *E. coli* FP genes and reaches up to 75%, for PSP genes in *Firmicutes*. To recapitulate, not only is 5'UTR characterized with a favourable AT-bias; inside or in the close vicinity of the 5'UTR, start codon and Shine-Dalgarno sequence could potentially benefit from a higher protection. Moreover, on average, there is evidence that Shine-Dalgarno sequence is likely to be better protected than start codon.

4.4 For PSP and FP genes, interference with secondary structure is either high or low for both SD and SC signals

A complement to the previous analysis of potential preservation from secondary structure formation, the straight investigation of translation signal interference with predicted 5' terminal mRNA secondary structure is crucial to our conclusions. Though, it must be reminded that inescapable limits are inherent to our large-scale automated approach: adequacy of the rule used to identify what should be the 5' terminal sub-region subject to folding, *in silico* prediction necessarily ignoring *in vivo* environment, choice between several suboptimal predictions. Notwithstanding these drawbacks, we underline the strong convergence of the observations over the 32 prokaryotic genomes: no balanced case with high SD interference and low SC interference is observed; neither is the symmetric case. Thus, the present work establishes that potentially high transcription is characterized with the same bias as functional promoters, regarding translatability: SD and SC contexts AT-enriched over the average, and more strikingly, a typical distribution for the 24 interference classes. It was not worth evaluating through simulation what would be SC or SD interference for an average genome generated at random and having the same nucleotide composition as a given bacterial genome. Indeed, navigating with BACTRANS²'s browser through the 5' terminal secondary structures of various PSP genes convincingly confirms the following statement: due to high local variability, even if SD and SC are in close proximity, it is unlikely that they might be simultaneously present in the same substructure (a loop or a paired region). Thus, in particular, it was not foreseeable that class ($\#SD \leq 2, \#SC \leq 1$), describing low interference for both SD and SC, would total at least a third of the PSP genes in a genome, on average.

Results regarding FP genes are interesting by themselves. First, when all the nucleotides of a translation signal are involved in base-pairing, a translation regulation is likely to exist. Second, if the role of the ribosome was supposed to be strongly hampered when strictly more than 50% of the nucleotides in the translation signal interfere with the secondary structure, then we would observe that only 39% of *E. coli* FP genes would distribute into classes hypothesized favourable to *high* ORF expression ($\#SD \leq 2, \#SC \leq 1$). However, in the remaining 61%, some FP genes might be known to be highly expressed. In this case, the following explanations would be put forward: the actual SD sequence used might not be the one predicted, but a suboptimal sequence in its close vicinity, instead; otherwise, the actual mRNA 5' terminal secondary structure might be determined by post-transcriptional mechanisms. Besides, the hybridization strength is known to vary along stems, in relation with the nature of pairing nucleotides. Then, owing to the possible high local AT-richness of the stem interfering with a given translation signal, it is likely that *in vivo*, some regions predicted paired are actually not. If they were nevertheless paired *in vivo*, a simple local separation of AT-rich regions could occur without need of a further regulation.

This is not novel knowledge regarding FP genes; more interestingly, our study backs up the necessity of regulations for the PSP genes that might be *highly* expressed through their strong promoters, in spite of predicted high interference.

4.5 5' terminal secondary structures presumed unfavourable to mRNA stabilization are slightly pre-eminent in PSP and FP genes

Again, we observe similar trends for PSP genes and FP genes, whatever the favourable or unfavourable influences considered (respectively $T1$, $T4$, $T5$ and $T2$, $T3$, $T6$, $T7$). First, it was not expected that simple stem-loops, instead of embedded secondary structures, would be so frequent at 5' extremity. Secondly, it was neither foreseeable that favourable type $T5$ nor unfavourable type $T7$ would be encountered at so low frequencies. So we show that though the consensus for RNase E cleavage site is rather a flexible motif, its presence in a hairpin is a rare event (type $T5$); it was neither expected that a helix of minimal length 20 nucleotides would be proven to be a rare conformation ($T7$). At least, the absence of the most constrained structures, such as $T1$ hairpins or the two hairpins flanking an RNase E cleavage site in type $T6$, is explainable. The categorization of local secondary structures into favourable and unfavourable types is drawn from various sources accounting for *in vitro* observations performed under heterogeneous experimental conditions, in *E. coli* genome. Besides, the list considered is assuredly not exhaustive. Nevertheless, to our knowledge, the present study offers the first *large-scale* insight into the distribution of potentially stabilizing secondary structures associated with genes harbouring promoters. We retain that $T2$, $T3$ and $T4$ types considered together respectively total 91%, 85% and 67% on average for PSP genes over the 32 genomes studied, *E. coli* PSP genes and *E. coli* FP genes. Finally, in light of observations relative to this restricted list of conformations, we show that unfavourable types are nearly once and a half as frequent as favourable types: 54% versus 33%, 53% versus 39%, 47% versus 26% respectively for the three gene sets aforementioned. Nonetheless, *in vivo*, the role of these various conformations might be minor with respect to the role of RNA binding proteins (translation enhancement through hampering the formation of some secondary structures, mRNA stabilization through masking of RNase E cleavage sites, for example).

5 Conclusion

Together with BACTRANS², the software suite dedicated to translatability analysis allowed the first large-scale insight into translational potentialities coupled with potentially high transcription. Not only does the cross-genome analysis show similar trends for the 5' UTR features studied in 32 prokaryotic genomes; the system implemented brings valuable insight into translatability associated with genes harbouring strong $\sigma70$ promoters and *E. coli* genes harbouring functional $\sigma70$ promoters. Both criteria related to protection against secondary structure formation and translation signal accessibility show typical biases. Besides, regarding potential mRNA stabilization, PSP genes do not seem to be favoured over FP genes. Since no advantage can be shown for PSP genes over FP genes with respect to all criteria examined, we conclude that high translation potentiality is not obligatorily associated with high transcription potentiality. A more thorough analysis failed to identify a class of PSP genes for which all five translation criteria would be simultaneously optimal; moreover, a correlation analysis focusing on genes with a putative strong promoter harbouring an UP element failed to exhibit evidence of potentially high translation (unpublished). In future work, it may be worth extending investigation to PSP genes associated with suboptimal SD sequences like AGGA, GGAG, or GAGG.

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(a)

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(b)

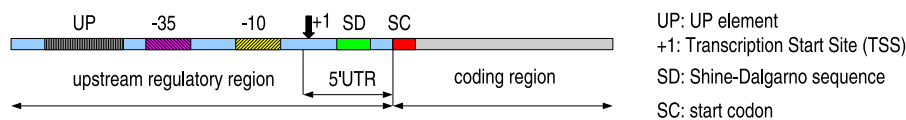
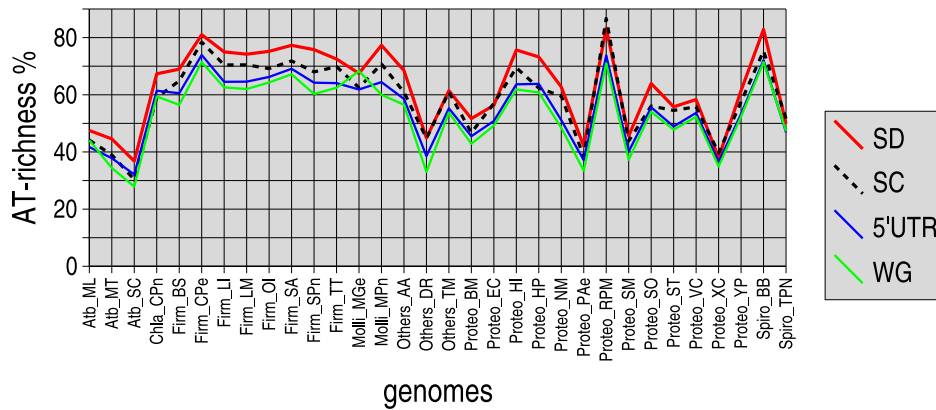


Figure 2: Example of annotations for *Bacillus subtilis*; (a) features describing the genes harbouring putative strong σ_{70} promoters; (b) comparative positioning of transcription binding sites and translation signals on the DNA strand.



Atb_ML:	Mycobacterium leprae tn	Others_TM:	Thermotoga maritima
Atb_MT:	Mycobacterium tuberculosis h37rv	Proteo_BM:	Brucella melitensis 16m
Atb_SC:	Streptomyces coelicolor a3 (2)	Proteo_EC:	Escherichia coli k12
Chla_CPn:	Chlamydomophila pneumoniae ar 39	Proteo_HI:	Haemophilus influenzae rd kw20
Firm_BS:	Bacillus subtilis 168	Proteo_HP:	Helicobacter pylori j99
Firm_CPe:	Clostridium perfringens str13	Proteo_NM:	Neisseria meningitidis mc58
Firm_LI:	Listeria innocua	Proteo_Pae:	Pseudomonas aeruginosa pa01
Firm_LM:	Listeria monocytogenes strain EGD	Proteo_RPM:	Rickettsia prowazekii madrid e
Firm_OI:	Oceanobacillus iheyensis hte831	Proteo_SM:	Sinorhizobium meliloti 1021
Firm_SA:	Staphylococcus aureus mw2	Proteo_SO:	Shewanella oneidensis mrl
Firm_SPN:	Streptococcus pneumoniae r6	Proteo_ST:	Salmonella typhimurium lt2
Firm_TT:	Thermoanaerobacter tengcongensis	Proteo_VC:	Vibrio cholerae n16961
Molli_MGe:	Mycoplasma genitalium G37	Proteo_XC:	Xanthomonas campestris atcc 33913
Molli_MPn:	Mycoplasma pneumoniae M129	Proteo_YP:	Yersinia pestis
Others_AA:	Aquifex aeolicus vF5	Spiro_BB:	Borrelia burgdorferi b31
Others_DR:	Deinococcus radiodurans r1	Spiro_TPN:	Treponema pallidum nichols
Others_TM:	Thermotoga maritima		

Figure 3: AT-richnesses in the regions flanking translation signals, for the genes harbouring putative strong $\sigma 70$ promoters, in 32 prokaryotic genomes. SD and SC respectively refer to Shine-Dalgarno sequence and start codon. For each genome, the average AT-richness is computed over all genes harbouring putative strong promoters, considering the 5 bp-long left and right regions flanking SD and SC. The average AT-richness in 5'UTRs is also computed over all such genes, for each genome. Whole genome AT-richness (WG) is compared with the three previous averages.

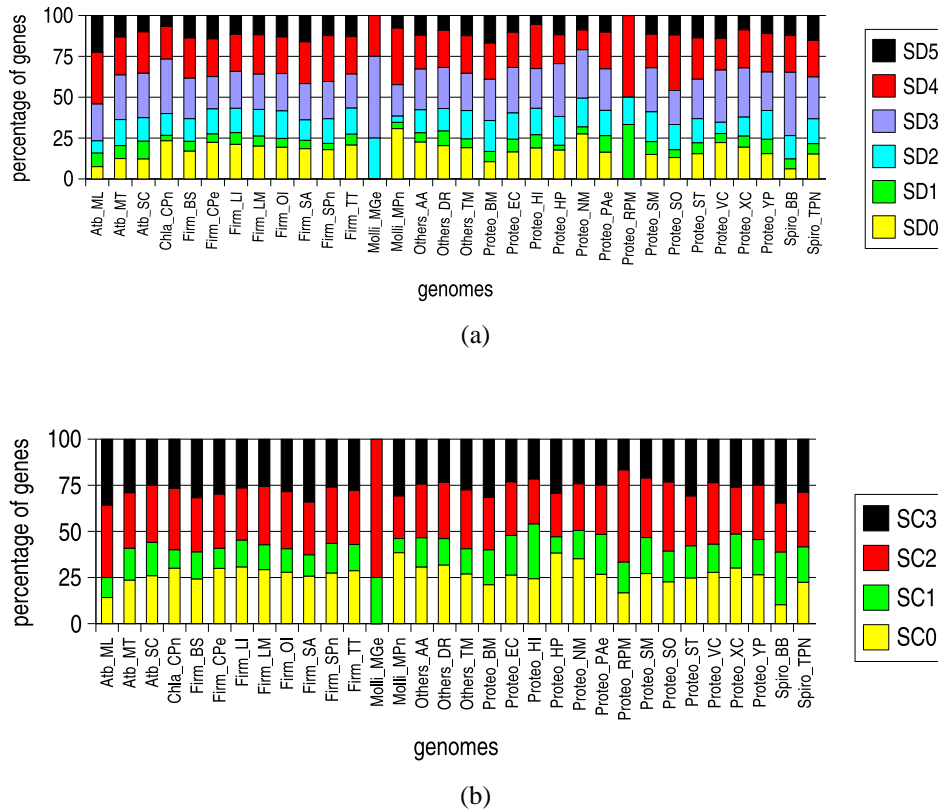


Figure 4: Interference between the translation signals and the mRNA 5' terminal secondary structure for the genes harbouring putative strong $\sigma 70$ promoters, in 32 prokaryotic genomes. Interference means that the translation signal considered partially overlaps a stem or is totally contained in a stem. (a) interference with Shine-Dalgarno sequence: each class through *SD0* to *SD5* corresponds to the number of nucleotides of the Shine-Dalgarno sequence which are involved in base-pairing. (b) interference with start codon: each class through *SC0* to *SC3* corresponds to the number of nucleotides of the start codon which are involved in base-pairing. See Figure 3 for species nomenclature.

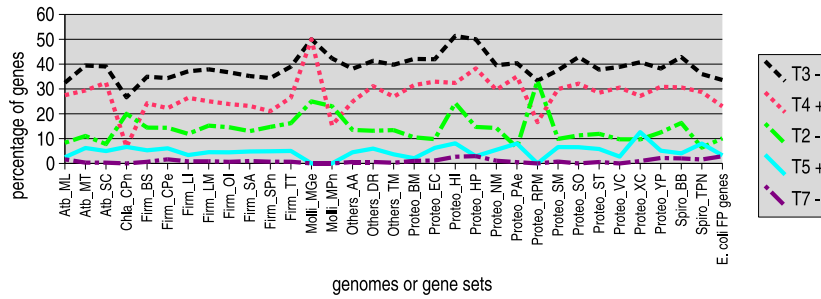


Figure 5: Typical secondary structures identified in the mRNA 5' terminal region, for genes harbouring putative strong $\sigma 70$ promoters - comparison of average frequencies between 32 prokaryotic genomes. The various conformations are described in Table 1. See Figure 2 for species nomenclature; *E. coli* FP genes: genes harbouring known functional promoters in *E. coli* genome; the top-down order in the legend reflects the frequency decrease through *T3* to *T7*; + and - symbols indicate the presumed favourable or unfavourable influence on mRNA stability.

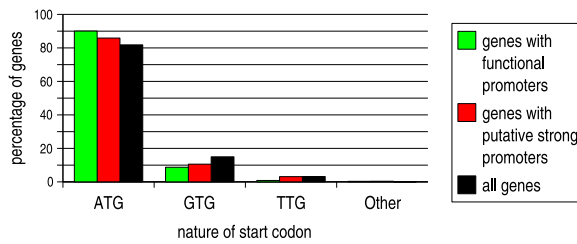


Figure 6: Nature of start codon for specific gene sets of *Escherichia coli* - Comparison between all 3772 genes coding for m-RNAs, 583 genes harbouring functional $\sigma 70$ promoters and 255 genes harbouring potentially strong $\sigma 70$ promoters with UP elements.

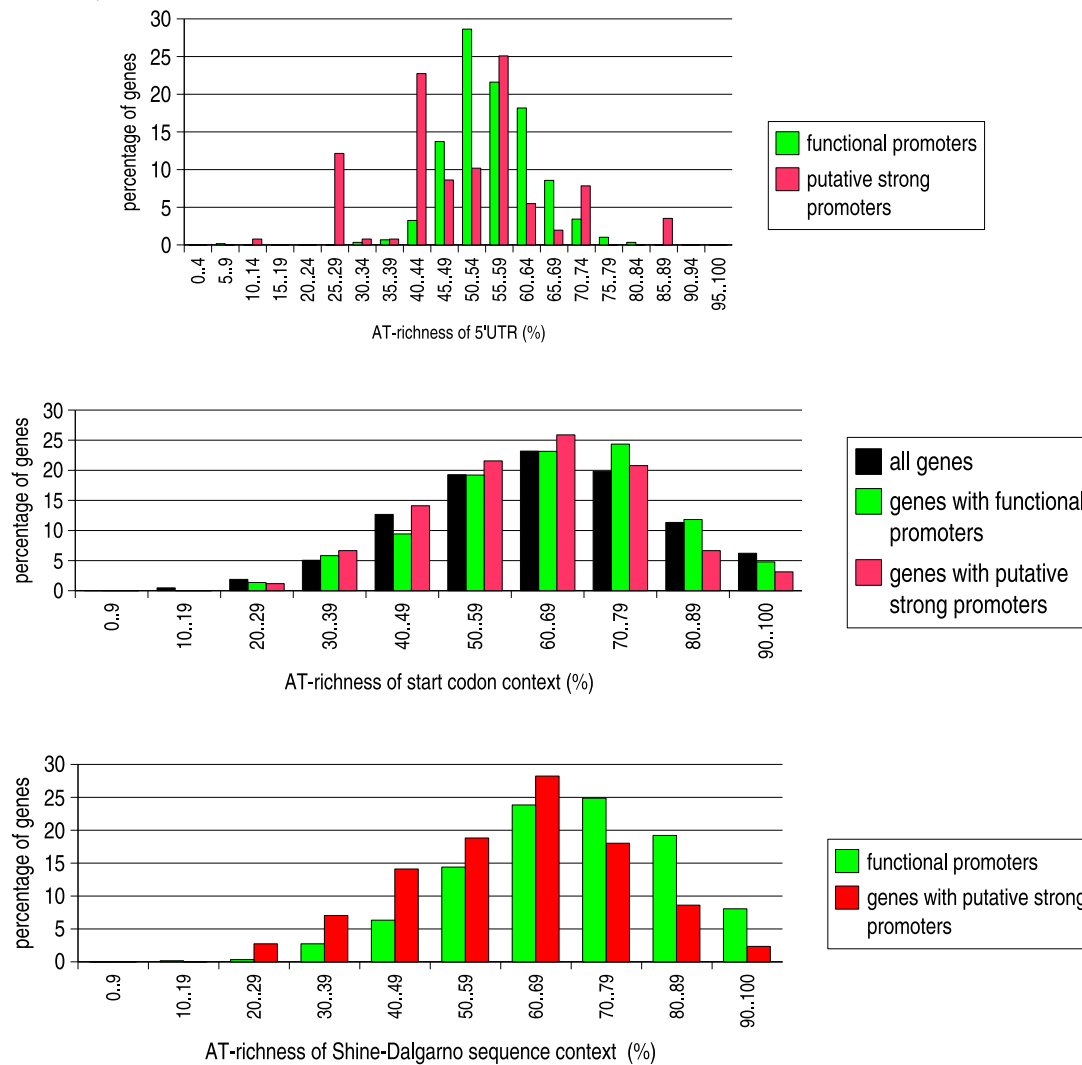


Figure 7: Distribution of AT-percentages relative to the 5'UTRs and the regions flanking translation signals, for specific gene sets of *Escherichia coli* - Comparison between 583 genes harbouring functional $\sigma 70$ promoters (FP genes) and 255 genes harbouring potentially strong $\sigma 70$ promoters (PSP genes). AT-richnesses relative to the regions flanking the start codon are also provided for the 3772 genes encoding proteins.

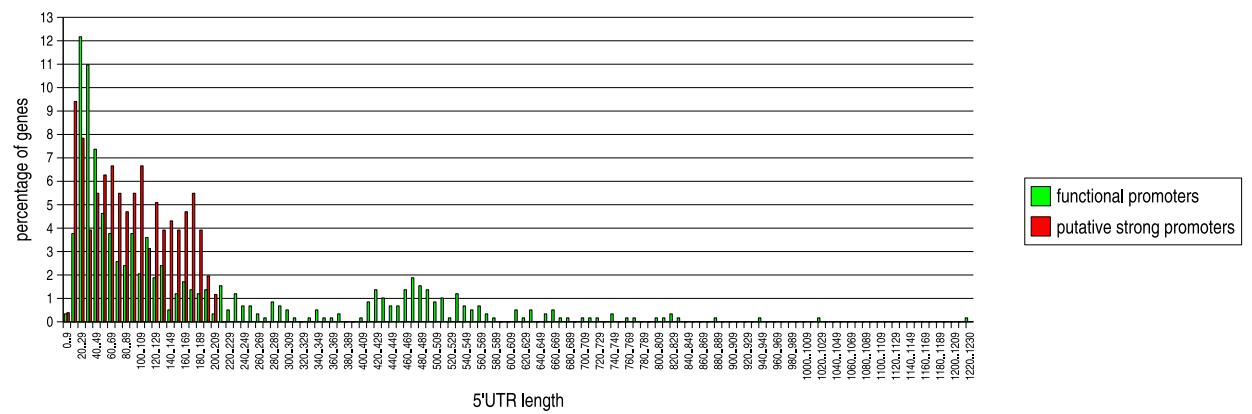


Figure 8: Distribution of 5'UTR lengths, for specific gene sets of *Escherichia coli* - Comparison between 583 genes harbouring σ_{70} functional promoters and 255 genes harbouring putative strong σ_{70} promoters.

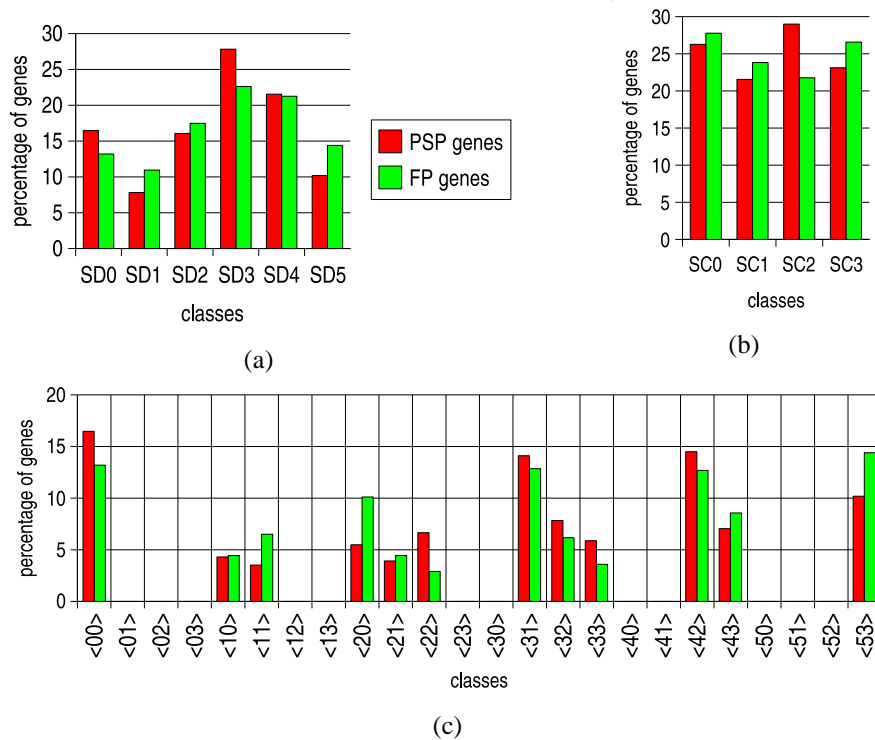


Figure 9: Interference between the translation signals and the mRNA 5' terminal secondary structure, for specific gene sets of *Escherichia coli* - Comparison between 583 genes harbouring $\sigma 70$ functional promoters and 255 genes harbouring putative strong $\sigma 70$ promoters. Interference means that the translation signal considered partially overlaps or is totally contained in a stem. (a) interference with Shine-Dalgarno sequence: each class through *SD0* to *SD5* corresponds to the number of nucleotides of the Shine-Dalgarno sequence which are involved in a stem. (b) interference with start codon: each class through *SC0* to *SC3* corresponds to the number of nucleotides of the start codon involved in a stem. (c) The classes are labelled ;#SD #CS_i, where #SD and #SC are the numbers of nucleotides in the Shine-Dalgarno sequence and the start codon respectively involved in a stem.

Genome-comparative computational approach for investigating prokaryotic ORF expression potentialities, in relation with potentially high transcription

Christine Sinoquet[†], Sylvain Demey[†], Frédérique Braun[‡]

Abstract

To synthesize a protein of interest, biotechnological techniques associate a strong promoter and a synthetical 5' Un-Translated Region (UTR) optimized to enhance gene expression. Besides, in prokaryotic genomes, transcription and translation are temporally and spatially related events. Therefore, it is worth investigating whether potentially high transcription is associated or not with high translation potentiality. Secondly, since bacteria share common characteristics related to transcription (binding sites); a cross-genome comparison of 5' UTRs' features is of importance for insights into genome organization.

We implemented BACTRANS², a software platform dedicated to the identification of genes harbouring putative strong promoters in prokaryotic genomes. Through a complementary software suite, we analysed such genes identified in 32 bacterial genomes with respect to various characteristics related to translation: nature of start codon, location of Shine-Dalgarno sequence upstream of the start codon, AT-richness in the regions flanking translation signals, interference of secondary structure with translation signals, possible influence of mRNA 5' terminal secondary structure on mRNA stability. In particular, regarding AT-richness and interference with secondary structure, we show that putative strong promoters are characterized by a typical bias common to the known functional promoters of *Escherichia coli* genome. Generally speaking, all five criteria related to translation indicate that putative strong promoters are not favoured over known functional promoters. On the other hand, the large-scale genome-comparison implemented highlights the similarity of the 5' UTR characteristics between genes harbouring putative strong promoters.

Platform BACTRANS² is available at <http://www.sciences.univ-nantes.fr/lina/bioserv/BacTrans2/>. The present work required implementing many more softwares devoted to the analysis of mRNA translatability in relation with potentially transcription. The source code is freely distributed to academic users upon request to the authors. The annotation files relative to the 32 genomes studied are available at http://www.sciences.univ-nantes.fr/lina/bioserv/BacTrans2/putative_strong_promoters.