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1

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3

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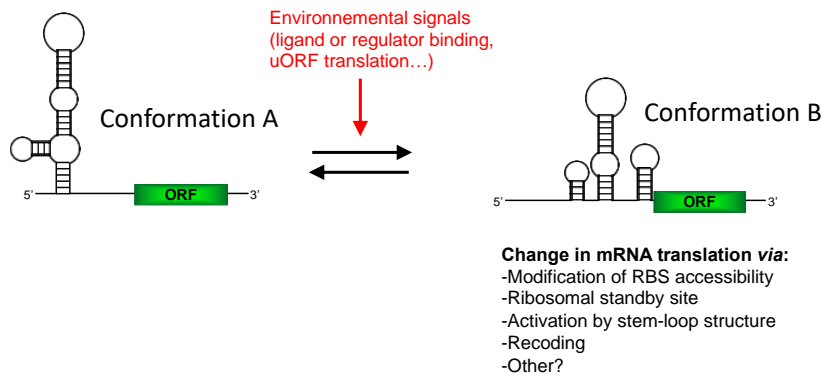
5 **Abstract**

6 Messenger RNA (mRNA) is no longer considered as a mere informational molecule whose sole function
7 is to convey the genetic information specified by DNA to the ribosome. Beyond this primary function,
8 mRNA also contains additional instructions that influence the way and the extent to which this
9 message is translated by the ribosome into protein(s). Indeed, owing to its intrinsic propensity to
10 quickly and dynamically fold and form higher order structures, mRNA exhibits a second layer of
11 structural information specified by the sequence itself. Besides influencing transcription and mRNA
12 stability, this additional information also affects translation, and more precisely the frequency of
13 translation initiation, the choice of open reading frame by recoding, the elongation speed and the
14 folding of the nascent protein. Many studies in bacteria have shown that mRNA secondary structure
15 participates to the rapid adaptation of these versatile organisms to changing environmental conditions
16 by efficiently tuning translation in response to diverse signals, such as the presence of ligands,
17 regulatory proteins or small RNAs.

18

19

20 **Graphical/Visual Abstract and Caption**



21

22 **mRNA secondary structure influences translation in bacteria and plays a key role in the rapid**
23 **adaptation of these organisms to their surroundings.**

24

25 **Introduction**

26 Bacteria are versatile organisms that are able to rapidly adapt to environmental changes, which
27 allows them to live in a wide range of different conditions. To this end, bacteria have a remarkable
28 capacity to rapidly fine-tune their cellular composition by regulating the combined activities of the
29 gene expression and quality control machineries. In particular, reshaping of the proteome occurs
30 through many regulatory steps that can target transcription, mRNA translation and protein stability.
31 By acting at a later step than transcription, especially during the initial phase of translation, post-
32 transcriptional regulation may allow a faster adaptation than purely transcriptional events. Thus, in
33 addition to being an essential step in gene expression, mRNA translation is also a key step in its control.
34 All phases of translation, including initiation, elongation, termination, and ribosome recycling
35 constitute checkpoints that can affect gene expression.

36 A large body of experimental evidence suggests that mRNA carries much more information than
37 just the coding sequence that is translated into proteins by the ribosomes and the aminoacyl-tRNAs.
38 In particular, RNA has the intrinsic propensity to dynamically fold and form higher order structures,
39 such as stem-loops (Brion & Westhof, 1997) or tertiary structures maintained by helical and/or
40 unpaired motifs, such as pseudoknots (Batey, Rambo, & Doudna, 1999; Pleij, Rietveld, & Bosch, 1985)
41 that are essential for its function. In mRNAs, a multitude of structural motifs determine the frequency
42 of translation initiation (Gualerzi & Pon, 2015), global and local elongation speeds (Wen et al., 2008;
43 Plotkin & Kudla, 2011; Chen et al., 2013), mRNA processing and degradation (Belasco, 2010; Nicholson,
44 2014; Briani, Carzaniga, & Dehò, 2016) and folding of the nascent protein (Komar, 2009; G. Zhang,
45 Hubalewska, & Ignatova, 2009; G. Zhang & Ignatova, 2011). In addition, since the pioneering work of

46 Yanofsky and colleagues on transcriptional attenuation (Yanofsky, 2007), it has been widely accepted
47 that alternative base pairing between mRNA segments plays a key role in the control of gene
48 expression. Considering that RNA secondary structures tend to be highly dynamic and undergo
49 conformational changes on a microsecond time scale (Crothers, Cole, Hilbers, & Shulman, 1974;
50 Pörschke, 1974), this is of great interest in terms of rapid mRNA conformational shifts and thereby
51 efficient adaptation to changing environments.

52 A combination of recent advances in quantitative methods to study mRNA structures, abundance and
53 translation on a genome-wide level *in vivo*, through biochemical, biophysical, genetic and
54 bioinformatic approaches has confirmed the strong impact of mRNA secondary structure on
55 translation efficiency and RNA stability or processing. In this review we will summarize our current
56 knowledge, based on both single-gene studies and high-throughput approaches, on the role of mRNA
57 secondary structure in modulating mRNA translation and in triggering specific translational events in
58 bacteria. While secondary structure also clearly impacts mRNA stability and processing, this will not
59 be discussed here as it is beyond the scope of this review.

60

61 **1. mRNA SECONDARY STRUCTURES AFFECT TRANSLATION INITIATION.**

62 In bacteria, translation initiation, that is the formation of a ternary complex in which the initiation
63 codon of the mRNA translation initiation region (TIR) is decoded by the anticodon of the initiator fMet-
64 tRNA in the P-site of the 30S ribosomal subunit, is the limiting step of translation of most mRNAs. As
65 such, initiation represents the target of the post-transcriptional regulation of a large number of
66 bacterial genes and for this reason constitutes the step where the influence of mRNA structure on
67 translation has been the most studied so far. Besides primary structure elements such as the initiation
68 codon and the Shine-Dalgarno (SD) sequence present in most bacterial mRNAs (Gualerzi & Pon, 2015)
69 or AU-rich stretches acting as recognition/binding sites for the RNA helix-unwinding ribosomal protein
70 S1 (Duval et al., 2013), secondary structure motifs contribute to shaping the TIR as the main variable
71 in translation initiation. So far, studies aimed to determine the influence of mRNA structure on
72 translation have shown that most mRNAs exhibit a lack of structure around the RBS and the start
73 codon, supporting the view that access to the TIR by the ribosome is of crucial importance for
74 translation initiation. However, and conversely, the TIR of other mRNAs exhibit a pronounced
75 secondary structure which limits translation. For instance, a hairpin structure encompassing the RBS
76 of the *sigG* mRNA encoding the late-acting sigma factor involved in *Bacillus subtilis* sporulation was
77 recently found to keep *sigG* translation relatively low, which participates to the proper timing of
78 sporulation (Mearls et al., 2018). Another example is provided by the structures that sequester the SD

79 regions of multiple toxin genes that are present in bacterial chromosomes as part of toxin-antitoxin
 80 modules. By limiting toxin production, these structures are important to prevent toxicity (Masachis &
 81 Darfeuille, 2018; Masachis et al., 2019). Furthermore, structural motifs are exploited by the cell to
 82 follow diverse strategies for regulation of translation initiation: they can either directly constitute the
 83 binding-site for regulators or build the structural scaffold required for adequate exposure of their
 84 binding-sites, or they can be modulated in response to diverse signals, such as temperature or pH, or
 85 binding of regulatory ligands, such as RNAs, proteins or metabolites.

86 **1.1. Secondary structures mediate recognition and binding of mRNA by trans-acting regulators.**

87 *1.1.1 Secondary structure motifs form binding sites for regulatory proteins.*

88 Regulation at the post-transcriptional level by trans-acting proteins was one of the earliest forms
 89 of gene control discovered. A wide range of secondary structure motifs present in bacterial mRNAs
 90 have been shown to play key roles in the control of gene expression by *trans*-acting proteins. This is
 91 due to the fact that such structures constitute *bona fide* binding-sites for repressor proteins. In most
 92 cases, they sterically outcompete the binding of the 30S ribosomal subunit to the ribosome binding
 93 site (RBS) (Figure 1). Two good examples of this simple regulatory mechanism are illustrated by the
 94 autoregulatory ribosomal proteins S8 and L20 of *Escherichia coli* (Cerretti, Mattheakis, Kearney, Vu, &
 95 Nomura, 1988; Gregory, Cahill, Thurlow, & Zimmermann, 1988; Guillier et al., 2002; Merianos, Wang,
 96 & Moore, 2004; Mangeol et al., 2011). A different mechanism is used by the repressor ribosomal
 97 proteins S4 and S15 of *E. coli* that decrease translation initiation of their own mRNAs by trapping the
 98 30S ribosomal subunit in an inactive complex (Philippe et al., 1993; Spedding & Draper, 1993).

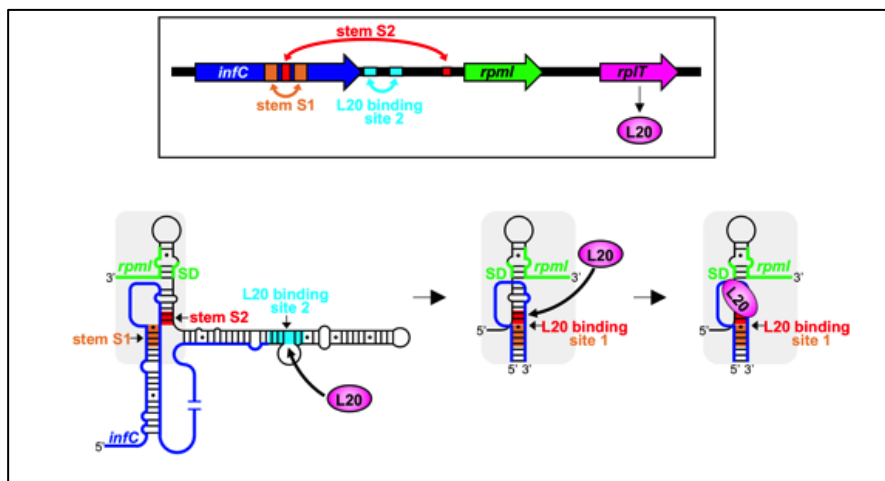


Figure 1. Schematic view of the two binding sites for repressor ribosomal protein L20 in the polycistronic *infC-rpmI-rplT* mRNA of *E. coli*. (Top) Schematic drawing of the mRNA. The *infC*, *rpmI* and *rplT* sequences are indicated by blue, green and purple arrows, respectively. Untranslated and intergenic sequences are colored in black. The sequences required for L20 binding are colored in orange for stem S1, red for stem S2 and cyan for L20-binding site 2. Double-headed arrows indicate base-pairing interactions. (Bottom left) Schematic

drawing of the secondary structure of the mRNA region containing the sequences forming the two L20-binding sites. Stems S1 and S2 and L20-binding site 2 are colored as indicated above. Sequences forming the pseudoknotted L20-binding site 1 are boxed in gray. (*Bottom middle*) L20-binding site 1 is formed by stacking of stem S2 on stem S1. (*Bottom right*) Scheme of L20-bound site 1 complex.

99

100 In other cases, secondary structures act in conjunction with sequence determinants for binding a
101 specific protein regulator. This has been reported for the CsrA protein and its homologues RsmA and
102 RsmE that bind to multiple GGA motifs of mRNAs and typically repress translation by outcompeting
103 with binding of the 30S ribosomal subunit (reviewed in (Babitzke, Lai, Renda, & Romeo, 2019)). Many
104 CsrA binding-sites are also present in several regulatory RNAs, allowing to titrate the protein when
105 these RNAs are expressed. A transcriptome-wide analysis of CsrA targets confirmed that the
106 consensus sequence for CsrA binding is preferentially located in the apical loop of hairpins,
107 highlighting the role of RNA structure in exposing RNA sequences (Holmqvist et al., 2016).

108 *1.1.2. Secondary structures shape mRNA scaffolds for proper recognition and binding by regulatory*
109 *small RNAs.*

110 RNA secondary structures also play a key role in mediating control by another class of major post-
111 transcriptional regulators in bacteria, the small RNAs (sRNAs). Regulation by sRNAs most often relies
112 on sequence complementarity between the sRNA and its target-mRNA(s) and, again, it is well
113 established that the structures of both the sRNA and the mRNA participate in the correct presentation
114 of the pairing regions. Indeed, complementary sequences in both mRNA and sRNA are only functional
115 when embedded in scaffolds that ensure they are mutually accessible. Early studies on antisense RNAs
116 involved in the control of plasmid copy number highlighted the role of ‘kissing’ loops in RNA-RNA
117 interactions, where the interacting regions of each RNA are found in the apical loops of helical
118 structures (Figure 2). Following this initial kissing, a more extended and stable base-pairing interaction
119 can take place such as a four-way junction in the case of *E. coli copA-copT* mRNA (Kolb et al., 2000;
120 Wagner, Altuvia, & Romby, 2002).

121

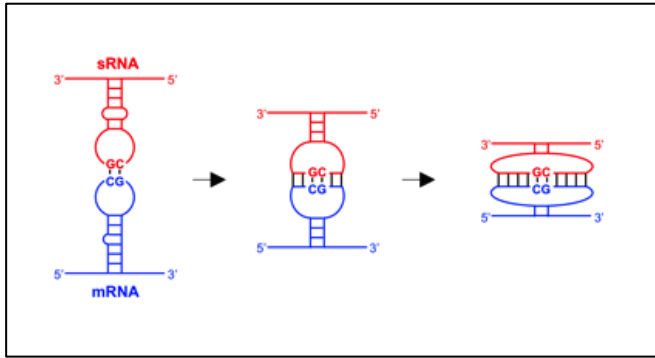


Figure 2. Schematic representation of a loop-loop interaction ('kissing') between a sRNA and its target mRNA. A loop-loop interaction (here two GC base pairs) between the sRNA (in red) and its target mRNA (in blue) initiates the formation of a helix nucleus. The nascent helix grows by addition of base pairs in both directions.

122 Not surprisingly, the importance of accessible pairing sites is also true for sRNAs with only partial
 123 complementarity to their targets, where sequences in loops or unstructured, often 5'-terminal
 124 regions, can be used for pairing (e.g.(Papenfort, Bouvier, Mika, Sharma, & Vogel, 2010; Romilly et al.,
 125 2012). In line with this, taking into account the accessibility of pairing-sites significantly improved the
 126 computational prediction of sRNA targets and RNA-RNA interactions (Beisel, Updegrove, Janson, &
 127 Storz, 2012; Busch, Richter, & Backofen, 2008; Mann, Wright, & Backofen, 2017; Miladi, Montaseri,
 128 Backofen, & Raden, 2018).

129 **1.2. Folding/unfolding of an mRNA secondary structure sequestering the RBS modulates its**
 130 **accessibility to the ribosome.**

131 *1.2.1 mRNA secondary structures act as temperature-sensing devices for temperature-dependent*
 132 *gene expression (RNA thermometers).*

133 The translation of several mRNAs whose TIRs encompass secondary structures around the RBS was
 134 found to be dependent on temperature, explained by the unfolding of RNA secondary structures to
 135 an open state at higher temperatures (Figure 3). These structures, known as RNA thermometers
 136 provide mRNAs with the ability to respond very rapidly to temperature shifts, because they control
 137 the translation of already existing or nascent mRNAs (Kortmann & Narberhaus, 2012). The existence
 138 of an RNA thermometer was first reported for the mRNA encoding the heat-shock alternative sigma
 139 factor RpoH in *E. coli* (Morita et al., 1999), where the formation of a secondary structure that hinders
 140 the RBS accessibility was found to be partially impaired when temperature increases. This results in
 141 unmasking of the RBS and activation of translation initiation, allowing synthesis of heat-shock
 142 proteins. Similar RNA thermometers were later found in other heat-shock response genes in diverse
 143 bacteria, but also in virulence genes of several pathogens (Loh, Righetti, Eichner, Twittenhoff, &
 144 Narberhaus, 2018). For instance, translation of the mRNA encoding the PrfA master virulence
 145 regulator of *Listeria monocytogenes* is up-regulated when temperature shifts from 30° to 37°C via the

146 melting of such a thermosensor, allowing expression of virulence genes in the host (Johansson et al.,
 147 2002). In both cases, an increase in temperature gradually shifts the equilibrium between the closed and
 148 open form of the RNA thermometer towards the open structure, in a zipper-like manner. The
 149 presence of such RNA thermometers in mRNAs illustrates a specific strategy that has evolved in
 150 bacteria to regulate the expression of temperature-dependent genes like those encoding virulence
 151 factors, heat shock and cold shock proteins (Kortmann and Narberhaus, 2012).

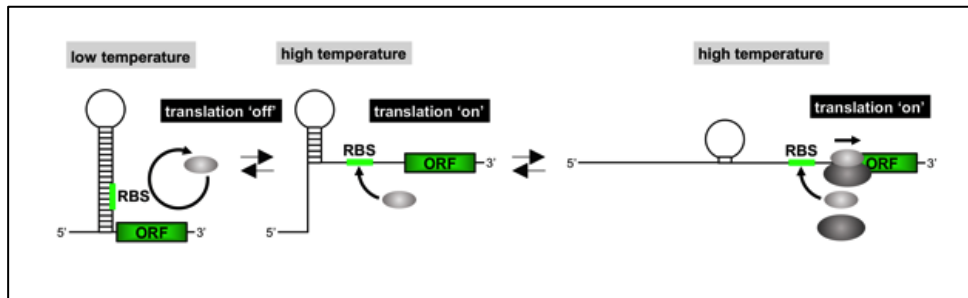


Figure 3. Mechanism of translation initiation control by environmental cues. An RNA thermometer (RNAT) is depicted in equilibrium between closed and open state. (*Left*) At low temperature, RNAT is closed and the RBS is masked, thus precluding 30S ribosomal subunit (in light gray) access to the mRNA (translation “off”) (*Middle and right*) As temperature increases, RNAT gradually melts and opens, making the RBS more accessible to the subunit (translation “on”) thus allowing translation to proceed upon binding of the 50S ribosomal subunit (in deep gray).

152 1.2.2. Secondary structures of mRNA act as translational coupling devices in the translation of
 153 polycistronic mRNAs.

154 In addition to environmental cues such as temperature, translating ribosomes themselves can
 155 disrupt mRNA secondary structures that mask the RBS of downstream genes, allowing initiation of
 156 their translation. Control of gene expression in the *E. coli* IF3 operon illustrates how two secondary
 157 structure motifs in a polycistronic mRNA, a long irregular stem-loop and a stem-loop involved in
 158 pseudoknot formation, mediate translational coupling, albeit using distinct molecular mechanisms.
 159 The *E. coli* IF3 operon contains three cistrons, *infC*, *rpmI* and *rplT*, encoding translation initiation factor
 160 3 and ribosomal proteins L35 and L20, respectively, in that order (see inset in Figure 1). The expression
 161 of *rpmI* and *rplT* is controlled at the translational level by L20, which directly represses the expression
 162 of *rpmI* and indirectly that of its own gene *via* translational coupling (Lesage, Truong, Graffe, Dondon,
 163 & Springer, 1990). Coupling of *rplT* expression to that of *rpmI* is governed by an inhibitory mRNA stem-
 164 loop structure formed by base-pairing interactions between the 3'-terminal region of the *rpmI* coding
 165 sequence and the RBS of *rplT* (Lesage et al., 1992). Disruption of this inhibitory stem-loop by ribosomes
 166 translating *rpmI* mRNA results in unmasking of the *rplT* RBS, which becomes accessible to incoming
 167 30S ribosomal subunit and initiator tRNA according to the standard model of bacterial translation
 168 initiation (Figure 4). Alternatively, it cannot be excluded that the 70S ribosome completing *rpmI*
 169 translation could scan the sequence surrounding the stop codon of *rpmI* for the presence of a nearby

170 accessible SD sequence, here that of *rpIT*, in order to reinitiate translation at *rpIT* RBS according to the
 171 “70S-scanning initiation” model (Yamamoto et al., 2016). Whatever the initiation mode, the coupled
 172 expression of *rpmI* and *rpIT* is a classical example of translational coupling in which translation is
 173 modulated through disruption of an inhibitory stem-loop by ribosomes translating the preceding open
 174 reading frame (ORF).

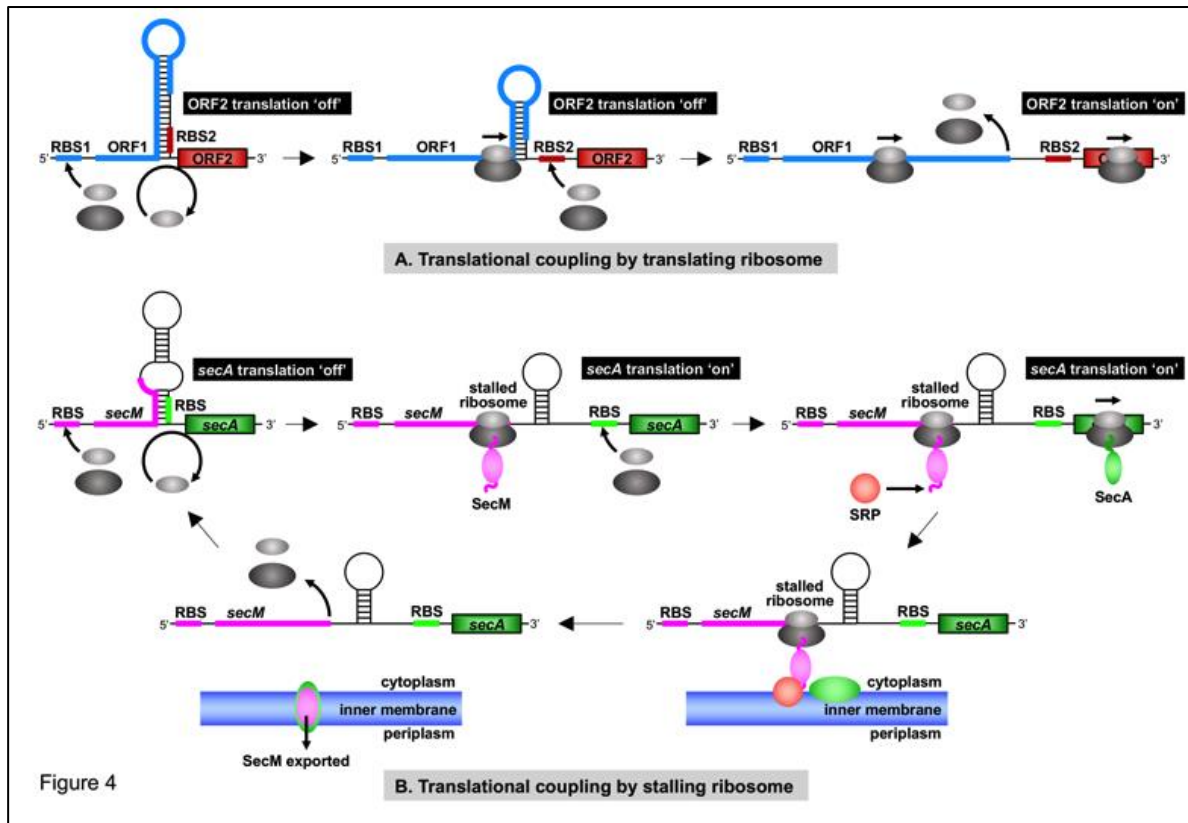


Figure 4. Mechanism of translation initiation activation by translational coupling. (A) Coupling by a translating ribosome. When ORF 1 is not translated by ribosomes (left), the RBS of ORF2 is masked with a stem-loop structure by base pairing with an anti-RBS sequence located within ORF1. As a result, 30S ribosomal subunit access to the mRNA is blocked (translation “off”). When ORF1 is translated (middle and right), the translating ribosome progressively opens the inhibitory secondary structure. As a result, the RBS of ORF2 becomes accessible to an incoming 30S ribosomal subunit (translation “on”). (B) Translational coupling of *secM* and *secA* expression by ribosome stalling on *secM* mRNA. When *secM* is not translated (upper left), the *secA* SD sequence is occluded in a stem-loop structure formed by the 3'-terminus of *secM* and the *secM*-*secA* intergenic region, leading to low initiation frequency of *secA* translation. When *secM* is translated, a ribosome stalls transiently at Pro166, located 12 nucleotides upstream of the stop codon (upper middle). During the time window of ribosome stalling, the stem-loop is disrupted and the *secA* SD sequence becomes accessible to incoming 30S ribosomal subunit. SRP binds to the peptide signal of SecM and co-translationally conveys the translating complex formed by the mRNA and the stalled ribosome tethered to the nascent SecM polypeptide to the membrane (lower right). As a result, SecA is synthesized close to the membrane where it assembles to the SecYEG channel to form the translocon. The *secM* translation arrest due to ribosome stalling is relieved by the mechanical ‘pulling force’ provided by the SecA-driven export of SecM from the cytoplasm to the periplasm through the translocon. As a result, SecM is dislodged from the translating complex (lower left) which in turn dissociates thus allowing reformation of the inhibitory stem-loop as the mRNA detaches from the ribosome (upper left). Consequently, duration of ribosome stalling, and thus induction of SecA synthesis, increases when SecM export is inhibited.

175

176 Translation of the *rpmI* ORF is itself modulated by translational coupling with *infC*. This involves a
177 secondary structure motif as well, but it proceeds *via* a more complicated mechanism than that
178 evoked for *rplT*. Translation of *rpmI* is governed by a pseudoknot formed between a stem-loop within
179 the *infC* coding region and a second sequence located far downstream that contains part of the RBS
180 of *rpmI* (Figure 1) (Chiaruttini, Milet, & Springer, 1996). Translation of IF3 necessarily first disrupts the
181 stem-loop and then the long range interaction with the *rpmI* RBS (Chiaruttini, Milet, & Springer, 1997).
182 Importantly, in addition to masking the *rpmI* RBS, the pseudoknot has a second function as a binding
183 site for L20, encoded by *rplT* (Guillier et al., 2002). Interestingly, L20 stabilizes the pseudoknot but
184 does not accelerate its formation, as shown in an *in vitro* study based on the use of optical
185 tweezers (Mangeol et al., 2011). As a consequence of pseudoknot disruption, not only is the *rpmI* RBS
186 unmasked but the binding of L20 is also impeded, resulting both in derepression of *rpmI* and in turn
187 that of *rplT*. This is a clear illustration of how ribosome-driven disruption of an mRNA secondary
188 structure involved in a tertiary interaction such as a pseudoknot can affect translation initiation of a
189 downstream gene.

190 A more sophisticated mechanism of translational coupling governs the control of translation of the
191 *secA* gene, encoding the protein translocation ATPase of *E. coli*. It has been shown that translation of
192 *secA* depends on that of the previous cistron, called *secM* (for secA modulation or secretion monitor)
193 (Murakami, Nakatogawa, & Ito, 2004; Nakatogawa, Murakami, & Ito, 2004). Coupling is governed by
194 a secondary structure formed by a base pairing interaction between the end of the *secM* coding region
195 and the *secA* RBS (Figure 4B). Importantly, the sequence of the SecM polypeptide between Phe150
196 and Pro166 causes the ribosome to stall transiently on mRNA. This stalling allows the unfolding of the
197 secondary structure by translating ribosomes and a basal level of *secA* translation. Defects in exporting
198 the SecM protein to the periplasm increase the stalling of the ribosomes and thereby ensure an up-
199 regulated synthesis of the SecA protein under these conditions. Hence, the duration of pausing, *i.e.*
200 the time that the repressive secondary structure is not formed, is feedback-controlled by the rate at
201 which the nascent SecM is secreted, which in turn depends on its signal peptide and interaction with
202 SecA and the translocon (Sarker & Oliver, 2002).

203 **1.3. An interplay between mutually exclusive mRNA secondary structures modulates ribosome** 204 **access to the RBS.**

205 Changes in RBS accessibility can also arise from the establishment of alternative secondary
206 structures in the vicinity of the RBS and other regions of the mRNA. Examples for this include
207 translational attenuation, as well as control of gene expression by riboswitches acting at the

208 translational level, or by trans-acting factors whose binding induces a structural remodeling of their
209 target mRNAs.

210 *1.3.1. A conformational switch between alternative mRNA secondary structures induced by ribosomes*
211 *translating a leader peptide affects translation initiation (translational attenuation).*

212 Conversely to the mechanism at work in translational coupling, where disruption of an upstream
213 inhibitory secondary structure by the ribosome activates the translation of a gene located
214 downstream (see above), it was found that translation of several bacterial genes involved in resistance
215 to antibiotics (e.g. erythromycin or chloramphenicol) depends on *blocking* the translation of a short
216 upstream ORF (uORF), that expresses a leader peptide (Horinouchi & Weisblum, 1980; Dubnau, 1985;
217 Lovett, 1996). Ribosome stalling during translation of the uORF allows the mRNA to fold into a
218 conformation favorable for translation of the downstream gene by exposing its RBS *via* the formation
219 of alternative secondary structure motifs (Figure 5). Remarkably, stalling depends not only on the
220 sequence of the leader peptide but also on binding of the cognate antibiotic to the ribosome, thereby
221 inducing expression of antibiotic resistance genes only in its presence. By analogy to ribosome-
222 mediated transcriptional attenuation, where ribosome stalling at uORFs controls transcription
223 termination by modulating mRNA structure *via* the formation of alternative stem-loops (Landick,
224 Carey, & Yanofsky, 1987), this mode of regulation is sometimes referred to as “translational
225 attenuation”.

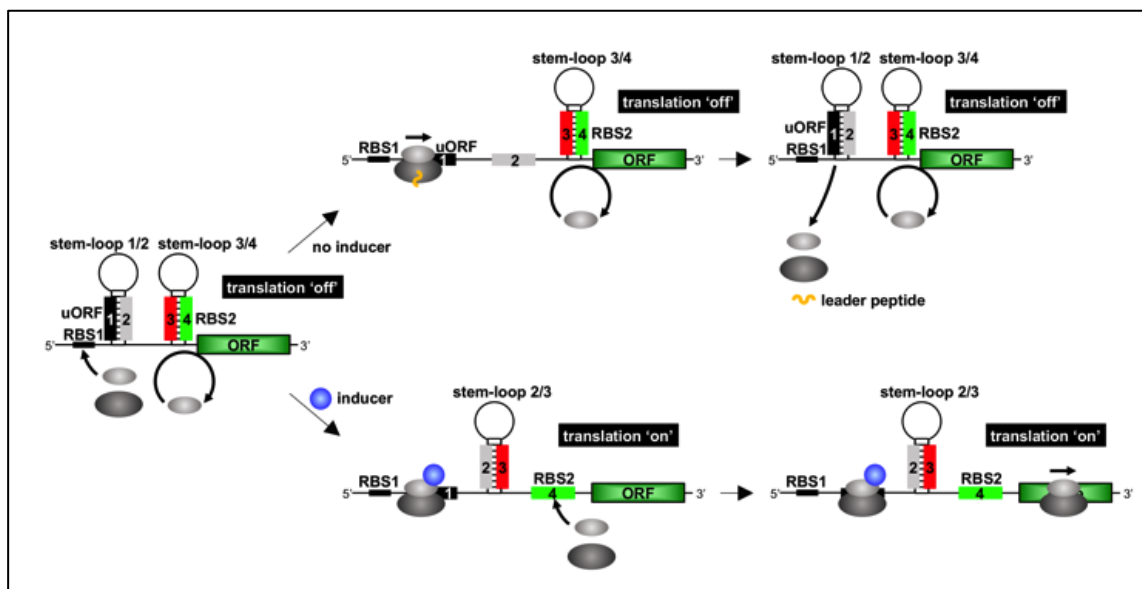


Figure 5. Control of translation initiation *via* rearrangement of mRNA secondary structure induced by ribosomes stalling on an upstream ORF (uORF). (Left and top left) In the absence of inducer, the 30S ribosomal subunit binds to RBS1, promoting synthesis of a leader peptide (in orange) from uORF by the ribosome. Stem-loop 1/2 is transiently unwound, while RBS2 is occluded within stem 3/4 and is not accessible to 30S ribosomal subunit (translation “off”). (Top right) Stem-loop 1/2 reforms once the ribosome has passed through, while RBS2 remains occluded within stem-loop 3/4 (translation “off”). (Bottom left) In the presence

of inducer, the inducer-bound ribosome stalls on uORF, triggering a structural switch by which disruption of stem-loop 1/2 leads to formation of alternative stem-loop 2/3 and consecutive unmasking of RBS2. (*Bottom right*) Binding of the 30S ribosomal subunit to RBS2 allows translation of the ORF to proceed (translation “on”).

226 More recently, it was found that ketolides, the newest generation of macrolides, could activate
227 expression of the *ermC* gene involved in macrolide resistance, even though they failed to promote
228 ribosome stalling in the uORF. Instead, ketolides induced a ribosomal frameshift during translation of
229 the uORF, resulting in translating ribosomes by-passing the uORF stop codon and thus covering the
230 intergenic region between the uORF and *ermC*. This prevented formation of the inhibitory structure
231 for *ermC* expression via a different molecular mechanism than ribosome stalling (Gupta, Kannan,
232 Mankin, & Vázquez-Laslop, 2013) and references therein).

233 *1.3.2. A conformational switch between alternative secondary structures is an essential feature of*
234 *mRNA riboswitches controlling translation initiation.*

235 Several mRNA riboswitches control translation initiation through an allosteric conformational
236 switch of the TIR caused by ligand binding to an aptamer domain (Figure 6). Depending on whether or
237 not the aptamer domain is bound by a ligand, the structure of TIR is shaped by mutually exclusive
238 secondary structures that can switch translation on or off. Riboswitch-control of translation was first
239 reported in *E. coli* with the binding of thiamine and adenosylcobalamin leading to conformational
240 switches in the *thiM* and *btuB* mRNAs encoding an enzyme involved in vitamin B1 biosynthesis and for
241 the outer membrane protein responsible for vitamin B12 transport, respectively (Nahvi et al., 2002;
242 Nou & Kadner, 2000; Tucker & Breaker, 2005; Winkler, Nahvi, & Breaker, 2002). In both cases
243 structural changes resulted in sequestration of the RBS and limited translation in presence of their
244 cognate ligands (Figure 6A).

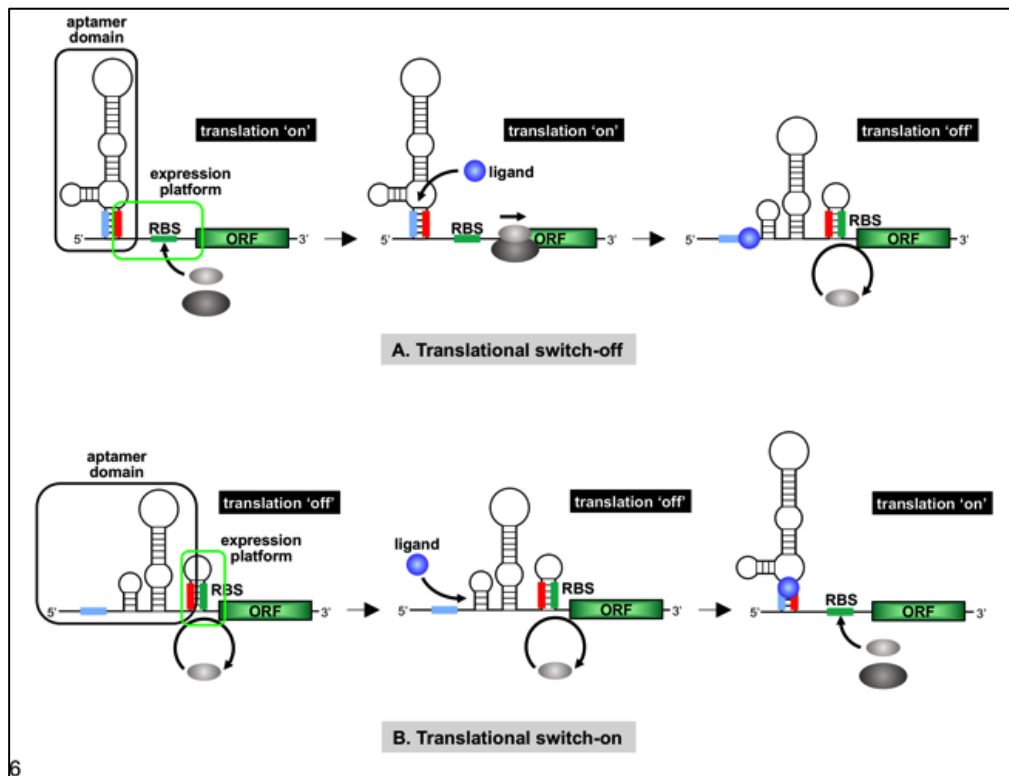


Figure 6. Mechanism of riboswitch-mediated control of translation initiation. A riboswitch is composed of an aptamer domain (outlined in black) that binds a small ligand and an expression platform (outlined in green) that controls gene expression. (A) (Left) In the absence of the ligand, an anti-RBS sequence (in red) is trapped in a secondary structure by pairing with an anti-anti-RBS sequence (in blue). (Middle) In this configuration, the RBS of the ORF (in green) is accessible to the 30S ribosomal subunit (in light gray) and translation is allowed to proceed (translation “on”) upon binding of the 50S ribosomal subunit (in deep gray). (Right) When bound to the aptamer domain, the ligand promotes and stabilizes the formation of an alternative structure where an anti-RBS sequence pairs with the RBS thus preventing the binding of the 30S ribosomal subunit (translation “off”). (B) (Left and middle) When the ligand is not bound to the aptamer domain, the RBS (in green) is trapped in a stem-loop by pairing with an anti-RBS sequence (in red), which prevents 30S ribosomal subunit binding (translation “off”). (Right) Binding of the ligand induces a conformational switch by which the anti-SD sequence is trapped by an anti-anti-RBS sequence (in blue). The RBS becomes accessible to 30S ribosomal subunit, thus leading to activation of translation initiation (translation “on”).

245

246 Riboswitch-dependent control of gene expression can also proceed through activation of
 247 translation initiation upon unmasking of the RBS (Figure 6B). This is the case for the adenine-sensing
 248 riboswitch located in the 5'UTR of the *add* mRNA, encoding adenosine deaminase involved in purine
 249 catabolism in the Gram-negative bacterium *Vibrio vulnificus* (Rieder, Lang, Graber, & Micura, 2007;
 250 Lemay et al., 2011). Although transcription and translation are coupled in bacteria, translation
 251 activation by the *add* riboswitch can be recapitulated *in vitro* using a pre-transcribed *add* mRNA, *i.e.*
 252 in the absence of coupling. This is certainly not a general rule, however, as transcriptional pausing
 253 plays a key role in RNA folding in bacteria in general. Consistent with this, several examples of
 254 regulation based on RNA conformational switches, including the *btuB* riboswitch mentioned above,
 255 require pausing of transcription at specific sites. This pausing presumably prevents the formation of

256 unwanted structures and, at least in the case of *btuB* mRNA, allows coordination of the folding of the
257 riboswitch domain involved in ligand binding with the regulation of gene expression (Perdrizet,
258 Artsimovitch, Furman, Sosnick, & Pan, 2012).

259 The property of riboswitches to control gene expression has been exploited to create functional
260 synthetic riboswitches, starting from a single small-molecule-binding RNA aptamer, to activate the
261 translation of heterologous mRNAs. For example, theophylline-responsive riboswitches were
262 synthesized that activate translation initiation of the *xyIR* gene, encoding the xylose transcriptional
263 repressor, and that of the *lacZ* gene in the Gram-positive bacterium *Bacillus subtilis* (Suess, Fink,
264 Berens, Stentz, & Hillen, 2004) and in the Gram-negative *E. coli* (Desai & Gallivan, 2004; Lynch, Desai,
265 Sajja, & Gallivan, 2007), respectively.

266 Most of the riboswitches that have been described so far function through a conformational switch
267 between two mutually exclusive states. However, in the case of the *add* riboswitch, the outcome of a
268 NMR-based study suggests that the regulation mechanism could be governed by three distinct
269 riboswitch conformations, that include two adenine-free conformations instead of only one (Reining
270 et al., 2013).

271

272 *1.3.3. A conformational switch between alternative mRNA secondary structures induced by trans-*
273 *acting factors affects translation initiation.*

274 As an alternative to sterically hindering the binding of the 30S ribosomal subunit to the RBS or
275 trapping the subunit in an unproductive complex with the mRNA as discussed above, binding of a
276 regulator outside of the TIR can induce a structural rearrangement of the mRNA, leading to either
277 positive or negative regulation. This can be achieved by RNAs or proteins.

278 **Secondary structure rearrangement induced by *trans*-acting RNAs.**

279 The translational control of the *rpoS* mRNA by multiple sRNAs in *E. coli* is a nice example of
280 translation activation mediated by alternative base-pairing, in this case involving the regulatory sRNA
281 itself. The RpoS sigma factor is involved in gene expression in stationary phase and in the bacterial
282 response and adaptation to multiple types of stress. Both the synthesis and the stability of the RpoS
283 protein are highly regulated, and at least three sRNAs activate its synthesis by directly pairing to the
284 5' UTR of *rpoS* mRNA. Although these three sRNAs, namely DsrA, RprA and ArcZ, have distinct
285 sequences, they pair imperfectly to the same region of the *rpoS* mRNA, upstream of the TIR. In the
286 unbound conformation, this site forms an imperfect intramolecular helix with the TIR of *rpoS*, thereby
287 limiting translation. As a result, the binding of DsrA, RprA or ArcZ sRNA to the *rpoS* 5' UTR prevents

288 formation of this inhibitory mRNA secondary structure and activates translation initiation (Figure 7A)
 289 ((Gottesman, 2019) for a recent review). Other examples of hairpin-competing mechanisms have been
 290 reported since then for other sRNAs that activate translation (Prevost et al., 2007; Fröhlich & Vogel,
 291 2009; Pappenfort, Espinosa, Casadesus, & Vogel, 2015). Furthermore, while a similar mechanism could
 292 in theory mediate inhibition by repressor sRNAs (Figure 7B), only very few examples of this kind have
 293 been reported so far to our knowledge. This is the case for instance for the SR1 sRNA in *Bacillus subtilis*
 294 whose binding to the coding sequence of the *ahrC* mRNA, expressing the transcriptional activator of
 295 the *rocABC* and *rocDEF* arginine catabolic operons, leads to a decrease in RBS accessibility *in vitro*
 296 (Heidrich, Moll, & Brantl, 2007). The formation of the SR1-*ahrC* duplex is facilitated by the CsrA
 297 protein, raising the question of the involvement of CsrA in the *ahrC* structural rearrangement (Müller,
 298 Gimpel, Wildenhain, & Brantl, 2019).

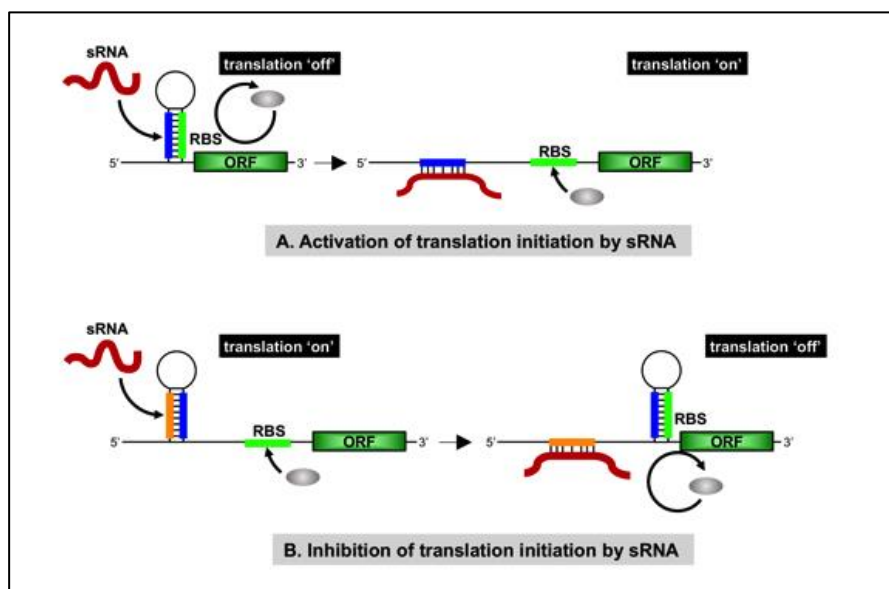


Figure 7. Regulation of translation initiation *via* rearrangement of mRNA structure by a regulatory small RNA. (A) Pairing of an sRNA (in brown) to an anti-RBS sequence (in blue) unmasks the RBS (in green) and promotes translation initiation of the ORF (translation “on”). (B) Pairing of an sRNA to an anti-anti-RBS sequence (in orange) can also induce a structural rearrangement leading to RBS occlusion by an anti-RBS sequence and thereby translation inhibition of the ORF (translation “off”).

299

300 Secondary structure rearrangement induced by *trans*-acting proteins.

301 Translation in bacteria can also be influenced, either positively or negatively, by a structural
 302 rearrangement of the mRNA that is induced by the binding of an activator or repressor protein (Figure
 303 8).

304 Such mechanism is used by the *E. coli* bacteriophage Mu to activate the translation of its *mom* gene
 305 by the phage Com protein. This allows Com to control the DNA-modification function of the Mom
 306 protein that provides protection of phage Mu DNA from the host restriction endonucleases. The Com
 307 and Mom proteins are encoded by two overlapping genes expressed from the bicistronic *com-mom*
 308 mRNA. The 5' proximal *com* gene encodes a 62-amino acid polypeptide that is required for translation
 309 of *mom*. Com binding to the *mom* TIR results in the destabilization of a stem-loop that is inhibitory for
 310 translation, thus rendering the *mom* SD sequence and GUG translation initiation codon accessible to
 311 the incoming ribosome and initiator tRNA (Wulczyn, Bolker, & Kahmann, 1989; Hattman, Newman,
 312 Murthy, & Nagaraja, 1991; Wulczyn & Kahmann, 1991).

313 Negative regulation of translation initiation may also be governed by an mRNA secondary structure
 314 shift induced by a repressor protein. In *Pseudomonas aeruginosa*, the post-transcriptional regulatory
 315 protein RsmA directly binds to sequences within the 5'UTR of the polycistronic *psl* mRNA, repressing
 316 the translation of genes necessary for synthesis of biofilm polysaccharide. It has been proposed that
 317 RsmA acts as a repressor by stabilizing a stem-loop structure in the mRNA that blocks ribosome access
 318 to the SD sequence of the proximal *pslA* gene due to base-pairing with an upstream anti-SD sequence
 319 (Irie et al., 2010). This would differ from the most common mechanism of repression by CsrA/RsmA
 320 proteins, whose binding to target-mRNAs typically overlaps the TIR and outcompetes the binding of
 321 the 30S ribosomal subunit.

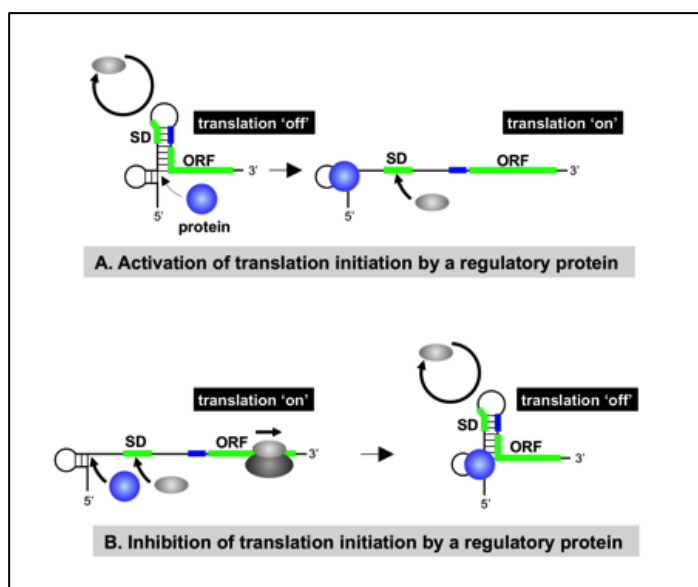


Figure 8. Control of translation initiation via rearrangement of mRNA secondary structure by a regulatory protein. (A) Binding of a regulatory protein to the mRNA induces a structural rearrangement that increases the SD accessibility and thereby activates translation initiation (translation “on”). (B) Binding of a regulatory protein to the mRNA induces a structural rearrangement that decreases the SD accessibility and thereby inhibits translation initiation (translation “off”).

323 1.4. mRNA secondary structures activate translation initiation.

324 Investigation of the mode of action of sRNAs that pair to the coding region of the *fepA* mRNA of *E.*
325 *coli*, encoding a receptor for iron-siderophore involved in iron uptake, allowed identification of a stem-
326 loop structure in the early coding sequence that activates its translation (Jagodnik, Chiaruttini, &
327 Guillier, 2017). This structure was found to promote the formation of an initiation complex between
328 the mRNA, the 30S ribosomal subunit and the initiator tRNA. Furthermore, this effect is largely
329 independent of the nucleotide sequence of this activating stem-loop (ASL), while the distance
330 between the translation start codon and this stem-loop is important for the activation. Similar
331 structures can be predicted in the early coding region of several mRNAs, and in at least one other case,
332 the predicted ASL was shown to activate gene expression. This second validated ASL is present in the
333 *bamA* mRNA, encoding one of the essential subunits of the BAM complex responsible for the assembly
334 of β -barrel proteins in the outer membrane of Gram-negative bacteria. As for the *fepA* ASL, the *bamA*
335 ASL promoted formation of a ternary complex between the mRNA, the 30S ribosomal subunit and the
336 initiator tRNA, independently of its nucleotide sequence. Importantly, the structure of this ASL is
337 predicted to be conserved in many species that are evolutionarily distinct from *E. coli*, despite a poor
338 conservation at the nucleotide level, suggesting that translation activation mediated by ASLs could be
339 a generally conserved mechanism. Interestingly, introducing structures at similar positions in
340 heterologous genes was previously found to increase gene expression in *E. coli* (Paulus, Haslbeck, &
341 Watzele, 2004). Although it is not yet known exactly how these ASLs promote gene expression, it has
342 been hypothesized that they may activate translation initiation by providing a “starting block” for the
343 30S ribosomal subunit, which prevents the subunit from “sliding” forward on the mRNA (Figure 9).
344 Considering that the 5’ edge of the ASL is located at position +19 of the ORF, which is exactly
345 contiguous to the 3’ edge of the RBS (Huttenhofer & Noller, 1994), the ASL would be ideally located
346 to block the subunit at an appropriate position for efficient translation initiation. Interestingly, such
347 an activation by a secondary structure is reminiscent of what has been described in eukaryotic systems
348 where recognition of a non-optimal AUG initiating codon is improved by the presence of a stem-loop
349 structure downstream (Kozak, 1990). However, secondary structures that activate translation are also
350 found upstream of the coding sequence. For example, a stem-loop structure located eight nucleotides
351 upstream of the initiation codon of bacteriophage T4 gene 25 mRNA, encoding a structural component
352 of the tail baseplate, has been shown to activate translation initiation. This stem-loop presumably acts
353 by bringing a SD sequence located 27 nucleotides upstream of the initiation codon, which is a very
354 unfavourable spacing for initiation, to a functional spacing of eleven nucleotides (Nivinskas, Malys,
355 Klaus, Vaiskunaite, & Gineikiene, 1999). The positive involvement of an upstream mRNA structure in
356 translation was also shown in the case of the toxin gene *tisB* in *E. coli*. As *tisB* RBS is poorly accessible,

357 its translation depends on the binding of the 30S ribosomal subunit to an upstream standby site
358 (Darfeuille, Unoson, Vogel, & Wagner, 2007) and a 5'-terminal hairpin was unexpectedly found to be
359 required for this process (Romilly, Deindl, & Wagner, 2019).

360

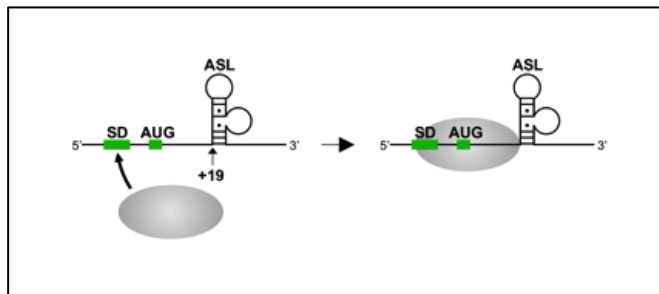


Figure 9. Activation of translation initiation of the *E. coli fepA* mRNA by a secondary structure located in the mRNA coding sequence. The activating stem-loop (ASL) located at position +19 of the coding sequence is shown in a model where it restricts the 30S ribosomal subunit (in light gray) to the appropriate position to initiate translation.

361

362 1.5. Contribution of high-throughput approaches

363 The findings described above are largely based on single-gene studies, but several large-scale
364 approaches have started to more generally address the relationship between mRNA structure and
365 translation. These results mostly confirmed the previously known correlation between low structure
366 in the TIR and the efficiency of translation initiation step.

367 Several studies investigating codon usage concluded that a major determinant of the effect of
368 codons at the beginning of ORFs is actually related to the ability of the early coding sequence to form
369 mRNA structures (Kudla, Murray, Tollervey, & Plotkin, 2009; Goodman, Church, & Kosuri, 2013; Boël
370 et al., 2016; Bhattacharyya et al., 2018). A poorly structured translation initiation region is also crucial
371 for translation of mRNAs that do not possess a Shine-Dalgarno sequence (Scharff, Childs, Walther, &
372 Bock, 2011).

373 Other recent studies used next-generation sequencing (NGS) to relate bacterial structuromes to a
374 concomitant analysis of mRNA levels and translation, by RNAseq and ribosome profiling, respectively.
375 Structuromes were either performed *in vitro* on total RNA (Del Campo, Bartholomaeus, Fedyunin, &
376 Ignatova, 2015), or *in vivo* using DMS (Burkhardt et al., 2017; Y. Zhang et al., 2018) or SHAPE reagents
377 (Mustoe et al., 2018). In the latest study, parallel structuromes were determined *in vitro* and upon
378 inhibiting translation with kasugamycin *in vivo* to assess the role of translation and other cellular
379 factors in determining mRNA structure. These studies converged on the finding that the region around

380 the RBS and the start codon of most mRNAs is poorly structured. In addition, a short structured region
381 7-12 nts upstream of the start codon was found only in the *in vitro* study, while the most unstructured
382 region started around nt -20, which was hypothesized to serve as an unspecific 30S ribosomal subunit
383 binding-site (Del Campo et al., 2015). This is consistent with the ribosome standby site model
384 proposed by de Smit and Van Duin and the observation that unstructured regions located on the 5'-
385 side of structured RBS promote translation initiation, *e.g.* in the mRNA for the MS2 coat protein or the
386 *E. coli* TisB toxin (de Smit & van Duin, 2003; Darfeuille et al., 2007; Sterk, Romilly, & Wagner, 2018). At
387 this stage however, from a general point of view, no significant structural difference between the
388 region centered on nt -20 and the -7-12 region was observed in the *in vivo* structurome studies, and
389 further experiments will be required to assess the validity of this hypothesis for more genes.

390 **2. mRNA SECONDARY STRUCTURES AFFECT TRANSLATION ELONGATION.**

391

392 **2.1. The coding sequence of the mRNA not only encodes the primary structure of a protein but** 393 **also contains additional information that influences translation elongation rates in bacteria.**

394 After the initiation step, the ribosome proceeds into the elongation step, catalyzing the formation
395 of peptide bonds between amino acids added in an order dictated by the coding sequence of the
396 mRNA. While the control of translation initiation determines how often an mRNA is translated,
397 controlling translation elongation tunes the speed at which it is translated. It has long been recognized
398 that elongation of the polypeptide chain does not proceed at constant speed (Pedersen, 1984;
399 Varenne, Buc, Llobes, & Lazdunski, 1984; Sørensen, Kurland, & Pedersen, 1989; Andersson & Kurland,
400 1990). Thus, even though initiation is the step at which bacterial translation is mostly regulated,
401 regulation during the elongation step may also impact gene expression and, in addition to its primary
402 function as a conveyor of the genetic message, the mRNA may contain other genetic instructions that
403 affect the way the message is translated.

404 A first layer of additional information is provided by the primary structure of the mRNA itself, more
405 precisely by the redundancy of the genetic code. The non-random use of synonymous codons, also
406 called “codon bias”, and the availability of the respective isoacceptor tRNAs may have important
407 implications for translation. Codon bias can modulate elongation speed since rare codons, which code
408 for low-abundance tRNAs, delay the progression of the ribosome along the mRNA (Curran & Yarus,
409 1989; Berg & Kurland, 1997; Plotkin & Kudla, 2011). While it is generally accepted that the codon bias
410 contributes to translation efficiency (and accuracy) by adjusting the elongation rate, how this occurs
411 is still intensively debated (Quax, Claassens, Söll, & van der Oost, 2015) and references therein). In
412 addition to a role of codon bias in determining translation speed and ribosomal density at key steps,

413 thereby ensuring optimal translation (Tuller, Waldman, Kupiec, & Rupp, 2010) or protein folding
414 (Komar, 2009; G. Zhang et al., 2009; Spencer, Siller, Anderson, & Barral, 2012), it has also been
415 reported, as stated above, that limiting RNA structure at the 5' region of coding sequences rather than
416 codon rarity alone contributed to the effect of codon usage on translation (Kudla et al., 2009;
417 Goodman et al., 2013; Boël et al., 2016; Bhattacharyya et al., 2018).

418 **2.2. Translational pausing “noise” caused by the intrinsic propensity of RNA to fold into secondary** 419 **structure.**

420 Besides codon usage, a second layer of information that shapes translation in bacteria is provided
421 by the secondary structure adopted by the mRNA. As stated for translation initiation, secondary
422 structures located at the RBS and the very beginning of the coding sequence are important modulators
423 of translation efficiency. This may be true for elongation as well, because translating ribosomes face
424 the potential thermodynamic and kinetic barriers of RNA secondary structure motifs or even highly
425 structured domains like pseudoknots (Takyar, Hickerson, & Noller, 2005; Qu et al., 2011). It has been
426 shown that mRNA secondary structures indeed influence the translation elongation rate *in vitro*. This
427 influence was nicely illustrated by elegant optical-tweezer experiments showing that the secondary
428 structure of mRNA determines ribosome pausing, and thus the overall rate of translation, and,
429 importantly, that destabilization of hairpins decreases the duration of pauses without affecting
430 translocation time (Wen et al., 2008). Similarly, an *in vitro* study using single molecule fluorescence
431 resonance energy transfer (FRET) to measure reaction rates for specific steps during elongation
432 highlighted the role played by stem-loops and pseudoknots in slowing down or even halting the
433 movement of the ribosome on mRNA (Chen et al., 2013). The movement of the translating ribosome
434 along the mRNA during elongation necessarily disrupts these structures. Consistently, it has been
435 shown that pausing or braking of ribosomes at RNA helices is very transient due to their remarkable
436 ability to unwind downstream helices of very high stability (Takyar et al., 2005).

437 In addition to the helicase activity of the ribosome, the coupling of transcription and translation in
438 bacteria ((Proshkin, Rahmouni, Mironov, & Nudler, 2010) and references therein) suggests that the
439 first ribosome has the benefit of an unstructured mRNA for translation. However, as the time needed
440 to form a simple hairpin helix is much faster than ribosome association to the mRNA, i.e. an RNA helix
441 folds in the millisecond range (Crothers et al., 1974; Pörschke, 1974) while initiation frequency is in
442 the second range (Kennell & Riezman, 1977), hairpins should have enough time to refold between the
443 subsequent (uncoupled) translating ribosomes. Thus, although extent of mRNA secondary structure
444 measured *in vitro* certainly overestimates the degree to which they occur *in vivo*, cellular mRNAs are
445 structured to some degree. In line with this, transcriptome-wide chemical probing approaches have

446 shown that a lower fraction of *E. coli* mRNA secondary structures persists *in vivo* compared to that
447 detected *in vitro* or upon inhibition of translation (Burkhardt et al., 2017; Mustoe et al., 2018). This
448 highlights the importance of energy-dependent processes at work during elongation, *e.g* the
449 unwinding of mRNA helices by ATP-dependent helicases or translating ribosomes, or passive events,
450 such as the association of RNA binding proteins, in shaping mRNAs in the cell. In addition, it has been
451 proposed that the intrinsic propensity of RNA to rapidly form secondary structures within coding
452 sequences is cancelled out by selection of codons for high-abundant tRNAs that are translated faster
453 and in general re-equilibrate the overall translation speed (Gorochowski, Ignatova, Bovenberg, &
454 Roubos, 2015). Nevertheless, persistent secondary structures that cause ribosomes to slow or stall on
455 mRNA have probably been selected for functional reasons that are yet unknown in most cases and
456 deserve further investigation to define their roles in bacteria. So far, only a small sub-set of these
457 structures have been characterized whose role is to transiently stall ribosomes translating *E. coli*
458 mRNAs encoding membrane proteins, most likely to regulate integration of these proteins into the
459 membrane (Del Campo et al., 2015).

460 Interestingly, ribosome profiling data point to strong variations in the translation efficiency among
461 the different genes of a polycistronic mRNA, allowing for instance to reach the stoichiometry of the
462 different components of multi-protein complexes (Li, Burkhardt, Gross, & Weissman, 2014). While
463 more experiments are needed to understand the underlying molecular mechanism in most cases, it is
464 likely that mRNA secondary structures could be involved in this process and could act at the translation
465 initiation and/or elongation steps to fine-tune protein synthesis.

466 A combination of ribosome profiling and *in vivo* structuromes also revealed different possible roles
467 for mRNA secondary structures in regions outside of the RBS. Using DMS-based approaches, it has
468 been proposed that mRNAs present a similar degree of structure within a given ORF and that this ORF-
469 wide structure is an important determinant of translation (Burkhardt et al., 2017; Y. Zhang et al.,
470 2018). In contrast, SHAPE-based data did not identify a significant correlation between the structure
471 of the coding sequences and their translation, except for the TIR region (Mustoe et al., 2018). This
472 study instead revealed structures within ORFs that could play an important role in translation, by
473 mediating translational coupling or serving as regulatory elements. Several differences in the
474 experimental process could explain these different conclusions, among which are the probing method
475 and the overall sample preparation procedure, the criteria used for the bioinformatics approaches, as
476 well as the set of mRNAs considered in the final analysis. More generally, the use of high-throughput
477 approaches to determine RNA structure may not be optimal for poorly abundant RNAs. In addition,
478 because structurome analysis questions the structure of the whole population of RNA, it will provide

479 in most cases an average of the structures that can be adopted by a given region of an RNA molecule.
480 In other words, alternative foldings as well as different structures that could correspond to different
481 RNA isoforms may not necessarily be discriminated in these approaches. The co-transcriptional folding
482 of RNA will also not be addressed by the most classical structurome analyses. However, despite these
483 current limitations, there is no doubt that the generalization of these powerful approaches in the years
484 to come will provide significant advances of our understanding of translation regulation on a global
485 scale in diverse organisms.

486 **2.3. Stem-loop structures govern the recoding of the mRNA.**

487 In general, the role of mRNA secondary structures in translation elongation is still much less
488 understood than its role in translation initiation. In some mRNAs, an additional layer of information is
489 embedded in the mRNA coding sequence that specifies a change in how the genetic code is read out
490 by the ribosome. In some cases, this information alters the reading frame, while in other cases, the
491 codon usage is altered. This phenomenon, called "recoding", is determined by specific instructions,
492 called "recoding signals", embedded in the mRNA (Gesteland, Weiss, & Atkins, 1992).

493 *2.3.1. Recoding by frameshifting of the coding sequence.*

494 Some mRNAs carry special sequence information and structural elements in their mRNAs that
495 enable ribosomes to slip either backward or forward, hence shifting the reading frame. This event,
496 termed "Programmed ribosomal frameshifting" (PRF), goes well beyond bacteria, since organisms
497 from all three kingdoms and viruses use frameshifting to regulate gene expression (Atkins, Loughran,
498 Bhatt, Firth, & Baranov, 2016). In the case of a -1 frameshift, ribosomes slip in the 5' direction on
499 mRNAs by one nucleotide, whereas +1 frameshifting results in forward, or 3', slippage by one
500 nucleotide. In rare cases the ribosome can also shift by -2, -4, +2, +5, or even +6 nucleotides (Weiss,
501 Dunn, Atkins, & Gesteland, 1987; Lainé, Thouard, Komar, & Rossignol, 2008; Fang et al., 2012; Yan,
502 Wen, Bustamante, & Tinoco, 2015). A classical example of bacterial programmed frameshifting is
503 provided by the translation of the *dnaX* gene of *E. coli* (Figure 10A) encoding two subunits of DNA
504 polymerase III, namely proteins τ and γ (Blinkowa & Walker, 1990; Flower & McHenry, 1990;
505 Tsuchihashi & Kornberg, 1990). Protein γ is shorter than τ due to a programmed -1 frameshift
506 provoked by the tandem slippage of the peptidyl-tRNA and the aminoacyl-tRNA occupying the
507 adjacent P and A ribosomal sites, respectively. As a result, the new frame terminates at a UGA stop
508 codon located two bp downstream of the 'slippery' sequence. The frameshift event is very efficient,
509 since it reprograms fifty per cent of the ribosomes, thus ensuring that both proteins are produced in
510 a 1:1 ratio. The frameshift signals are a stimulatory SD-like sequence located few nucleotides upstream
511 of the slippery site, a heptanucleotide slippery sequence at which ribosomes shift to the -1 frame and

512 a stimulatory stem-loop structure located downstream of the slippery site (Figure 10). It is generally
 513 accepted that the stem-loop provokes ribosome pausing on the slippery sequence decreasing the rate
 514 of ribosomal translocation (Kim et al., 2014). However, the intimate mechanism by which the
 515 downstream stimulatory stem-loop promotes efficient frameshifting has not emerged yet.

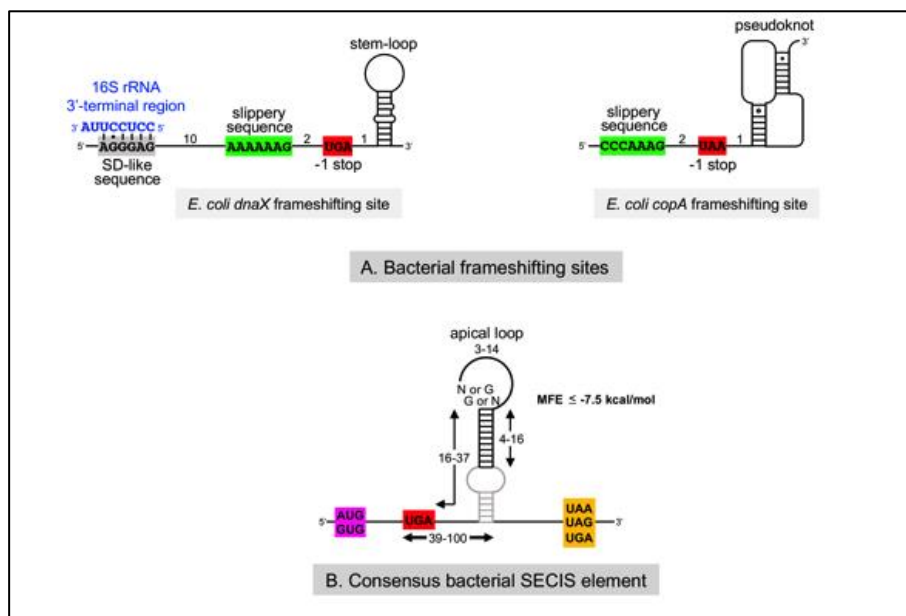


Figure 10. Modulation of mRNA recoding by specific secondary structures. (A) Models of the slippery sites of *E. coli dnaX* and *copA* genes, based on (Larsen, Wills, Gesteland, & Atkins, 1994; Meydan et al., 2017), respectively. Frameshift sites and downstream stop codons in the -1 frame are boxed in green and red, respectively. The upstream SD-like sequence present on *dnaX* mRNA is boxed in gray and is shown in base pairing interaction with the 3'-terminus of 16S rRNA (in blue). The numbers indicate the number of nucleotides separating the indicated sites. (B) Consensus bacterial SECIS element based on (Y. Zhang & Gladyshev, 2005). The start codon (AUG or GUG) of the gene subjected to recoding is boxed in purple while the recoded UGA stop codon and the UAA, UAG and UGA stop codons specifying arrest of translation following recoding are boxed in red and orange, respectively. The numbers are as in (A).

516

517 More recently, it has been shown that expression of the *copA* gene in *E. coli*, encoding both the
 518 834-amino acids copper transporter CopA and the 70-amino acid copper chaperone CopA(Z),
 519 undergoes recoding by PRF. Translation of CopA(Z) results from highly efficient -1 frameshift at a
 520 slippery sequence, which is stimulated by the presence of a downstream pseudoknotted stem-loop
 521 (Meydan et al., 2017). The -1 frame terminates at a UAA stop codon located two bp downstream of
 522 the slippery sequence. It should be noted that ribosome profiling data points to an abrupt drop in
 523 ribosome density immediately after the 70th codon, supporting the view that a large fraction of
 524 ribosomes shift to the -1 frame and terminate translation of *copA* after the 70th codon. The
 525 conservation of the co-occurrence of a slippery sequence together with a downstream pseudoknot at
 526 the appropriate distance in a range of bacterial species strongly suggests that co-expression of the

527 transporter and its chaperone is a beneficial trait allowing the proper regulation of copper
528 homeostasis.

529 2.3.2. Recoding the coding sequence by altering codon usage

530 In *E. coli*, the coding sequences of a few genes coding for selenoproteins, mostly oxido-reductases,
531 end with an opal UGA codon that co-translationally directs the incorporation of selenocysteine instead
532 of acting as a translation termination codon (Zinoni, Birkmann, Leinfelder, & Böck, 1987). This UGA
533 recoding process requires a *cis*-acting mRNA element called SECIS (selenocysteine insertion
534 sequences), consisting of 40-nucleotide sequence on average that form a stem-loop structure (Zinoni,
535 Heider, & Böck, 1990). The sequence forming the SECIS element is located immediately downstream
536 of the recoded UGA codon. A number of *tran*-acting elements are also required for selenocysteine
537 incorporation, including the specific minor tRNA^{Sec}, which is a specialized tRNA containing a UCA
538 anticodon (Leinfelder, Zehelein, Mandrand-Berthelot, & Böck, 1988) and a special elongation factor
539 protein, called SelB, which replaces the function of elongation factor EF-Tu (Forchhammer, Leinfelder,
540 & Böck, 1989). SelB exhibits extensive sequence homology to EF-Tu and has been shown to bind
541 specifically to both Sec-tRNA^{Sec} and the SECIS element *in vitro* (Fourmy, Guittet, & Yoshizawa, 2002).
542 The bacterial SECIS stem-loop element is defined by characteristic nucleotide sequences and
543 secondary structure base-pairing patterns. The essential region for selenocysteine insertion is located
544 in its apical loop, which includes the binding site for SelB. Once bound to the SECIS element, SelB in
545 turn recruits Sec-tRNA^{Sec} to the ribosomal A-site and mediates elongation at the in-frame UGA by
546 competing with release factor RF2 (Mansell, Guévremont, Poole, & Tate, 2001). Therefore, the SECIS
547 element acts as a binding site for SelB rather than a roadblock to slow the movement of the ribosome
548 along the mRNA.

549 3. A ROLE FOR mRNA SECONDARY STRUCTURES IN TRANSLATION TERMINATION?

550 Compared to their role in regulating translation initiation and elongation, our current knowledge
551 of the function performed by mRNA secondary structure motifs in translation termination lags by far
552 behind. Recently, a global *E. coli* mRNA structure analysis performed *in vitro*, coupled to deep-
553 sequencing and ribosome profiling detected an enrichment of ribosome density upstream of a
554 persistent mRNA secondary structure located 4-8 nucleotides upstream of the ochre UAA termination
555 codon (Del Campo et al., 2015). It was suggested that this secondary structure slows the translating
556 ribosome to assist the appropriate positioning of the ribosomal A-site for accurate decoding of the
557 UAA codon. However, SHAPE analysis of the *E. coli* structurome *in vivo* did not identify such a structure
558 (Mustoe et al., 2018), and its existence and precise role thus remain to be investigated.

559

560 **Conclusion**

561 Multiple studies, conducted either at the single-gene level or on a genome-wide scale, indicate that
562 increased bacterial translation efficiency correlates mostly with a reduced propensity of mRNA to form
563 secondary structures, especially in the TIR. Specific mRNA secondary structures allow the regulation
564 of translational events like ribosome binding, translational coupling and recoding, as well as mRNA
565 decay. However, an understanding of the effects of mRNA secondary structures on termination and
566 ribosome recycling, collisions between translating ribosomes in polysomes, coupling between
567 transcription and translation in bacteria or co-translational protein folding and quality control lags well
568 behind. Similar to the incorporation of selenocysteine *via* SelB, whether ribosomal or non-ribosomal
569 factors can promote translation of mRNAs displaying different structural content, *e.g.* by recruiting or
570 targeting ribosomes to specific mRNAs, is also an important and still poorly addressed question.
571 Finally, research has mostly focused on the impact of secondary RNA structures so far, and the role of
572 tertiary RNA structures such as triple helices, loop-loop interactions, or pseudoknots just to name a
573 few, will have to be determined as well in the future.

574 The ascent of genome-wide approaches based on NGS technologies applied to ribosome profiling
575 and structure analyses has allowed scientists to address new questions regarding the relationship
576 between mRNA structure and translation. While some of the first results may initially appear
577 contradictory, more ribosome profiling and structurome data, from different growth conditions and
578 the use of more standardized steps in sample preparation and data analysis between different
579 laboratories, will be key not only to understanding the current differences, but more generally to
580 address the future questions in the field. The combination of these genome-wide approaches has
581 certainly proved to be a very powerful tool to begin to unravel and map structural features in mRNA
582 that are involved in translational regulation or recoding; nevertheless, the intimate details of the
583 molecular mechanisms governing these events can only be dissected following investigation
584 conducted on a single gene-scale.

585

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