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The interplay of Hfq, poly(A) polymerase I and exoribonucleases at the 3' ends of RNAs resulting from Rho-independent termination

A tentative model

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Abbreviations: PAP I, Poly(A) polymerase; PNPase, Polynucleotide phosphorylase; RNase II, Ribonuclease II; RNase R, Ribonuclease R; sRNA, small regulatory RNA

Discovered in eukaryotes as a modification essential for mRNA function, polyadenylation was then identified as a means used by all cells to destabilize RNA. In *Escherichia coli,* most accessible 3' RNA extremities are believed to be potential targets of poly(A) polymerase I. However, some RNAs might be preferentially adenylated. After a short statement of the current knowledge of poly(A) metabolism, we discuss how Hfq could affect recognition and polyadenylation of RNA terminated by Rho-independent terminators. Comparison of RNA terminus leads to the proposal that RNAs harboring 3' terminal features required for Hfq binding are not polyadenylated, whereas those lacking these structural elements can gain the oligo(A) tails that initiate exonucleolytic degradation. We also speculate that Hfq stimulates the synthesis of longer tails that could be used as Hfq-binding sites involved in non-characterized functions of Hfq-dependent sRNAs.

Introduction

Polyadenylation is a universal post-transcriptional modification, which profoundly affects the activity and fate of RNA. First discovered in eukaryotes, where it contributes to export of RNA to the cytoplasm and promotes mRNA stability and translation, polyadenylation was then demonstrated to have an RNA destabilizing function that is conserved in bacteria, organelles and nuclei.[1-12] In prokaryotes, it is widely accepted that oligo(A) tails primarily expedite degradation of short-structured mRNA decay intermediates and control the turnover of several non-coding RNAs that regulate plasmid replication and maintenance, viral lysogeny and translation efficiency.[13-20] In addition, poly(A) assisted decay also has been shown to be involved in the quality control of precursors of a defective and a wild-type tRNAs, to affect gene expression and to compensate for a deficiency in the main pathway of RNA decay orchestrated by RNase E.[21-25]

In *Escherichia coli*, all types of RNA are polyadenylated by poly(A) polymerase I (PAP I) encoded by the *pcnB* gene;[26-35] these RNAs include mRNAs, mature and precursor tRNAs and rRNAs, decay intermediates and by-products of processing as well as small regulatory non-coding RNAs (sRNAs) and viral RNAs. Nearly all mRNAs were shown to be adenylated at the 3'-extremities, which can result from transcription termination, processing and/or exonucleolytic nibbling.[28,29,34,36] Besides this set of data leading to the widely accepted idea that any accessible 3' end of an RNA can be polyadenylated, there are clues that PAP I is able to discriminate between RNA substrates. Indeed, global analysis of polyadenylated mRNAs showed that stimulation of polyadenylation of individual mRNAs ranges from 2–50 times when PAP I is overexpressed.[29] Moreover, the structures of the 5' and 3' extremities of the RNA were reported to affect its efficiency. In particular, 3' ends resulting from rho-dependent transcription termination were reported to be preferentially polyadenylated by PAP I.[29,35,37-39] By analogy with the situation in eukaryotic cells, where the activity of PAP I depends on a complex machinery that includes a poly(A) binding protein,[40] we have demonstrated that the Hfq protein, which exhibits a very high affinity for A-rich sequences, stimulates the polyadenylation activity in *E. coli*.[36,41-44] In contrast, another poly(A) binding protein, ribosomal protein S1, has no effect on this reaction.[45] At that time, Hfq was known as a cellular factor involved in the replication of a viral RNA.[46] Its function in the control of gene expression was just beginning to be investigated[47,48] and neither its structure nor its mode of action were elucidated. We scrutinize here how the properties of Hfq, which are now well-characterized, may account for the current understanding of poly(A) metabolism and functions.

Our knowledge of poly(A) metabolism and poly(A) dependent degradation of RNA in *E. coli* was gained from the study of actors of poly(A) metabolism as well as by a limited set of transcripts such as *rpsO*, *rpsT*, *ompA*, *lpp,* RNA I of ColE1 and

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few other regulatory RNAs where the abundance or stability was dependent on polyadenylation.[2,3,9,13-16,18,49-53] We present below a rough outline of the poly(A) dependent mechanism of RNA degradation as it is known to date.

Poly(A) synthesis. The current model postulates that the poly(A) tails detected in bacteria result from the equilibrium between the activity of PAP I and the activity of 3'–5' exoribonucleases, which attack RNA 3' extremities (see ref. 3 for a review). The fraction of oligoadenylated molecules range from 0.011–40% depending on the RNA, and poly(A) tails ranging from 1–50 nucleotides have been described.[39] Ten percent of *rpsO* transcripts harbor short oligo(A) tails of 1–5 nucleotides in a wild-type strain.[36] The slow lenghtening of poly(A) tails in vivo, which most probably results from the low intracellular concentration of PAP I, explains at least in part the limited size of oligo(A) tails.[29,54]

In spite of the fact that all the 3' extremities may be recognized and elongated by PAP I, different studies reported that poly(A) tails were often detected downstream of secondary structures of Rho-independent transcription terminators, which stabilize RNAs. This is the case of RNA I, which controls ColE1 plasmid replication and of *rpsO, ompA* and *lpp* mRNAs.[8,13,29,34,39] The presence of a few unpaired nucleotides downstream of the hairpin (at least two) is required for the RNA to be polyadenylated.[38,55] PAP I is a distributive enzyme.[56] It only polymerizes the addition of A residues in vivo but it can also incorporate C residues in vitro but with a lower efficiency. GTP and UTP are not PAP I substrates.[44]

Exoribonucleases that degrade RNA from the 3' end recognize single-stranded extremities. It was demonstrated both in vivo and in vitro that oligo(A) tails are efficiently recognized and degraded by the major exoribonucleases, which are implicated in RNA catabolism; these include RNase II, PNPase and RNase R.[49,57-61] Oligo(A) tails are longer in cells when PNPase and RNase II are inactive.[8,57,62-65] Moreover, efficient degradation of RNA by these three exoribonucleases can only occur if 3' terminal stable secondary structures are followed by single-stranded sequences, which are used as recognition sites. PNPase degrades transcripts harboring a Rho-independent transcription terminator provided it exhibits 10-12 3' terminal unpaired nucleotides.[66] Secondary structures impeding PNPase progression are more efficiently degraded when the ribonuclease is associated with the RhlB helicase within the degradosome complex.[59,67] In contrast, RNase II, which is the most active ribonuclease in the cell as evaluated by its ability to degrade oligo(A)[68] is unable to go through secondary structures.[66,69,70] It nibbles single-stranded stretches longer than 9–10 nucleotides.[54,66] Finally, RNase R, the less abundant of these three exoribonucleases, can degrade structured RNA harboring a 3' single-stranded extension with a minimum of seven nucleotides without being slowed down by annealed nucleotides.[71,72] These properties allow us to understand on the one hand why oligo(A) tail addition by PAP I downstream of terminator hairpins allow PNPase and RNase R to degrade folded transcripts and on the other hand why RNase II, which shortens poly(A) tails, protects RNAs against the attack of both PNPase and RNase R.[16,64,73,74] We also have observed that primary transcripts harboring Rho-independent terminators may be nibbled by RNase II, which reduces the number of unpaired uridine residues downstream of the 3' hairpin of the *rpsO* mRNA.[36,54,75] These extremities may then be readenylated by PAP I. Exoribonucleases and PAP I can act distributively or processively depending on the length of the oligo(A) extension. The distributive synthesis of short tails, namely the dissociation of the enzyme from oligo(A) after the addition of each nucleotide, implies that the adenyl residues that have just been polymerized can be immediately removed by exoribonucleases that are able to bind to the accessible 3' RNA extremities. In addition, in vitro experiments reveal that longer oligo(A) tails (more than 10 As downstream of the terminal Us) are very rapidly degraded processively by exoribonucleases while short oligo(A) tails (1–5 As) are degraded slowly and through a distributive reaction.[54,75] As a consequence, short oligo tails (1–5 As downstream of the Us) result from the dynamic equilibrium mediated by distributive enzymes while longer oligo(A) extensions are hydrolysed processively and more rapidly. We also have demonstrated that oligo(A) tail synthesis also becomes progressively processive in the presence of Hfq.

Hfq stimulates poly(A) synthesis. Oligo(A) tails are shorter and less abundant in cells deficient for Hfq.[36,39,43] Consistently, Hfq stimulates poly(A) synthesis by PAP I in vitro.[39,43,76] Hfq affects the synthesis rate when tails reach about 20 nucleotides in length. The elongation is slightly quicker at first becoming strongly stimulated when tails reach about 30–35 nucleotides. The reaction becomes processive in the presence of Hfq; processivity can be detected as soon as tails reach about 5–10 As. Tails of several hundred As are rapidly synthesized processively in vitro in the presence of Hfq. Preferential binding of Hfq to 3' oligo(A) extensions presumably accounts for the speed and the processivity of the reaction.[43,44] The correlation between the low affinity for poly(C) and the failure to stimulate PAP I-mediated poly(C) synthesis reinforces the idea that Hfq must bind at the 3' end of RNA in order to stimulate elongation by PAP I.[44] Moreover, the fact that Hfq does not affect ADP polymerization by PNPase implies that it does not impair access to the 3'OH extremity of oligo(A) tails. The many poly(A) Hfq complexes detected when protein concentration increases suggest that Hfq sequentially binds every 14 residues to form complexes looking like perls on a string.[42,75] Although, Hfq was reported to bind preferentially to pre-existing oligo(A)-Hfq complexes,[77] the cooperativity of Hfq binding remains controversial.[42,75] Besides the evidence presented above that Hfq stimulates poly(A) synthesis, there are some clues that its binding at the 3' end of non-adenylated RNAs terminated by Rho-independent terminators impairs polyadenylation.

Hfq recognizes the 3' ends of Rho-independent terminators. Hfq was indeed reported to bind Rho-independent terminators harboring a 3' stretch of Us; Hfq affinity decreases when the stretch of Us downstream of the terminal hairpin is shortened.[39] Moreover, locations of polyadenylation sites in vivo suggest that 3' ends of the *rpsO* mRNA are more efficently adenylated when Hfq is inactive thereby indicating that Hfq inhibits poly(A) synthesis downstream of the hairpin of the Rho-independent terminators.[36] In the case of the *rpsO* transcript, oligo(A) tails appended

3' to the terminator hairpin increase Hfq binding, which is 15 times more efficient when 18 As are added downsteam of the U6C-OH of the terminator.[44] These observations suggest that Hfq can bind both the 3' terminal oligo(A) of polyadenylated RNAs and the stretch of Us following the terminal hairpin of Rho-independent terminators. Intriguingly, they also indicate that the consequences of Hfq binding are different: polyadenylation is stimulated in the first case while elongation of the RNA is inhibited in the latter one. It must be pointed out here that enhanced polyadenylation due to a 5' terminal mono-P extremity[37,44] does not result from Hfq binding, which is not affected by the phosphorylation status of the 5' RNA extremity.[44]

Hfq has various effects on RNA stability. These investigations suggest that Hfq could either destabilize RNA when it stimulates synthesis of poly(A) tails or protect RNA from exoribonucleases when it binds at their 3' end.[78] It was in fact observed that RNAs whose stability depends upon polyadenylation are stabilized when Hfq is inactive; this is the case of the *rpsO*, *rpsT*, *ompA* and *lpp* transcripts.[39,43] However, this stabilizing effect is pretty weak, and it has been attributed to translation activation as respect to the *rpsO* and *rpsT* mRNAs.[79] In addition, the stabilizing effect was not observed in the case of RNA I of ColE1 plasmids, whose decay is dramatically dependent on polyadenylation.[13] While poly(A) tail lengths decrease slighly, RNA I stability is not modified in the Hfq mutant and the copy number of pBR322 is not affected (Hajnsdorf unpublished data). In contrast, many sRNAs, also terminated by a Rho-independent transcription terminators, are stabilized by Hfq, which prevents ribonuclease attacks. Several sRNAs that were efficiently degraded exonucleolytically by PNPase in the absence of Hfq become resistant to this nuclease when Hfq is present.[80] For example, the MicA sRNA, whose degradation by PNPase is facilitated by poly(A), is stabilized by Hfq, which, in this case, protects the RNAs against ribonucleases instead of stimulating synthesis of destabilizing tails.[18,80,81] Similarly, the SraL sRNA (also named RyjA), whose degradation is poly(A) dependent, is stabilized by Hfq.[18] Paradoxically, SraL does not co-immunoprecipitate with Hfq,[82] which contrasts with the majority of sRNAs.[82-86] One of them, SgrS, forms a stable complex with Hfq, which presumably prevents exonucleolytic degradation.[87] Hfq also protects the oligo(A) tails of *rpsO* transcript from degradation by PNPase and RNase II.[75] Moreover, Hfq also impairs endonucleolytic cleavages. Its binding just upstream of the 3' terminal hairpins protects the *rpsO* and *cspA* transcripts from cleavage by RNase E.[75,88] In the case of *rpsO,* this cleavage is rate-limiting for decay.[89] Hfq also protects several sRNAs from RNase E.[18,90] In contrast, polyadenylation does not affect the stability of the CsrC sRNA, which is mostly degraded by endonucleases.[18] CsrC does not form a stable complex with Hfq,[85] which does not affect its stability.[18] This series of observations highlight the different roles played by Hfq in RNA metabolism. It forms very strong complexes with most sRNAs and some mRNAs while some fail to be co-immunoprecipitated with Hfq.[83-85] In respect to RNA stability, it can either destabilize RNA fragments and some mRNAs probably through stimulation of poly(A) synthesis,[3] or have a stabilizing effect that presumably results from the formation of complexes, which may reduce the access of ribonucleases to the RNA.[90] In contrast, Hfq also facilitates the coordinated degradation of sRNAs and of their associated mRNA targets.[90-92] In an effort to rationalize these observations, we postulate below that the accessibility of RNA to the exonucleolytic degradation machinery depends, at least in part, on the interactions of Hfq with the structural features of Rho-independent terminators, which have been recently demonstrated to play a major part in Hfq-dependent sRNA-mediated regulation of gene expression.

Molecular and functional interactions of Hfq with the 3' end of RNAs. Recent data showed that the binding of Hfq to the 3' terminal structural features resulting from Rho-independent termination is required for the regulatory function of sRNA[78,87] (**Fig. 1A**). In addition to the 3' terminal U-stretch and the stable hairpin of the terminator (t on **Fig. 1A**), formation of a stable Hfq-RNA complex requires a second single-stranded U-rich sequence located upstream of the terminator and, in several cases, a second hairpin between this U-rich sequence and the terminator. Moreover, current models based on the solved crystal structure of Hfq-RNA complexes and RNA-binding properties of Hfq mutants postulate that Hfq exhibits three different RNA-binding surfaces that can interact with the 3' ends of polyadenylated and non-adenylated Rho-independent terminators.[77,93-95] The proximal domain, located on the internal rim of the ring formed by the six identical protomers, establishes strong interactions with the 3' terminal U-stretch of Rho-independent terminators. Importantly, the 3'-OH terminus is masked in this complex.[95] The distal site, located at the other face of the ring, recognizes repetitions of A-R-N triplets (for Adenine, Purine and A, U, G or C), and it strongly binds a A15 oligoribonucleotide.[93,94] Finally, the lateral site located on the outer rim of the ring is involved in the binding of U-rich sequences and base-paired elements of sRNAs.[77]

PAP I selectively adenylates RNA extremities that are not recognized by Hfq. It was noticed that sRNAs that do not co-immunoprecipitate with Hfq lack the 3' terminal Hfq-binding site depicted above.[95] The same holds true for the 3' regions of sRNAs and mRNAs, which were shown to be polyadenylated in vivo. In effect, the polyadenylated SraG and SraL sRNAs, which were not isolated as stable Hfq-RNA complexes, miss the U-rich sequence upstream of the Rho-independent terminator hairpin (**Table 1**).[19,85,95] In addition, their 3' terminal U-stretch is presumably masked because it anneals with the stretch of As laying immediately upstream of the terminator hairpin (**Fig. 1B**).[95] Similarly, other sRNAs (RNA I, Oop, CsrC, RNA OUT, Sok, CopA)[15,16,19,73,96,97] and mRNAs, such as *rpsO*, *lpp*, *rpsT* and *ompA*[39,62,98,99] that are terminated by Rho-independent terminators, can be polyadenylated in vivo probably because they lack accessible structural features required for binding of Hfq at the 3' end (**Table 1**). The strong affinity of Hfq for polyadenylated *rpsO* mRNA compared with the non-adenytated transcript[75] indeed confirms that the 3' terminal U-stretch is weakly bound by Hfq. The *cspA* mRNA whose 3' extremity can both be polyadenylated and form a stable Hfq-RNA complex confirms that Hfq binding and polyadenylation are not exclusive.[76] Interestingly, internal base-pairing masks the U-rich singled-standed stretches of nucleotides located in the 3' UTR of the *cspA* transcripts (**Table 1**).[88] The 3' terminal Us could either

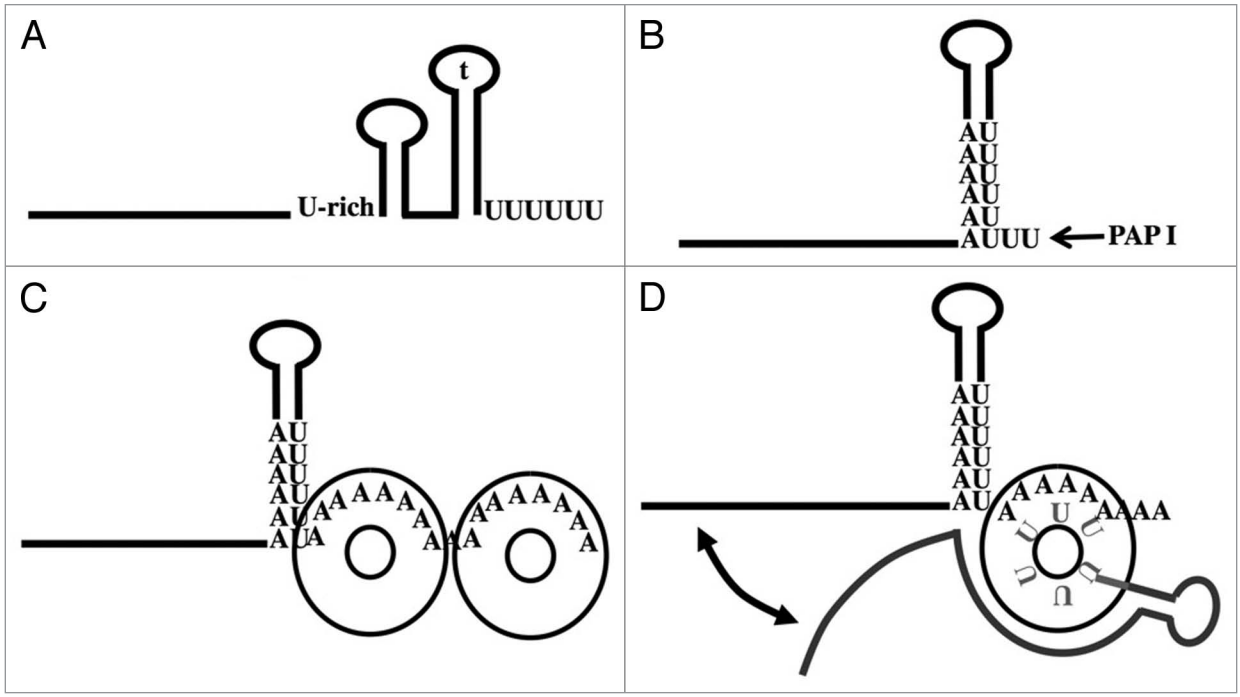

Figure 1. Structural features and potential interactions at the 3′ end of adenylated RNAs terminated by Rho-independent terminators. (**A**) The four structural elements involved in Hfq binding[87] are described in the text. (**B**) RNAs that are polyadenylated by PAP I contain an oligo(A) or an A-rich sequence located upstream of the hairpin of the terminator which can hybridize with the 3′ terminal single standed U-stretch recognized by Hfq. (**C**) Processive elongation of oligo(A) tails in the presence of Hfq presumably generates stable oligo(A)-Hfq complexes. Several Hfq probably bind side by side on the oligo(A) tail. The number of As interacting with the distal RNA-binding surface of Hfq on the scheme is arbitrary. See the text for the description of this hypothetical complex. (**D**) The 3′ end of polyadenylated RNA can in principle also bind an Hfq-sRNA complex. The terminal U-stretch of the sRNA and the oligo(A) tail can bind simultaneously to the proximal and the distal-binding surfaces located on the opposite faces of the hexamer. The U-stretch hidden by the protein is in light gray. The sRNA shown in dark gray also interacts with the lateral surface of Hfq. There are indications that a sRNA and a mRNA can compete for binding at the same site.[114] We propose in the text that such complexes may favor the interaction of the sRNA with potential target sites located in 3′ UTR close to the terminator.

form a stable complex with Hfq, implying also an upstream U-rich region when they are both single-stranded, or be accessible and polyadenylated when the 3' UTR is folded. Similarly, melting of the A-U base pairs that mask the terminal U-stretch of the *rpsO* Rho-independent terminator may facilitate the binding of Hfq at the 3'end of the mRNA (**Table 1**). This could explain why polyadenylation of the *rpsO* mRNA is affected by Hfq in vivo.[36] The 3' region of some mRNAs, *e.g., flgL* of *Salmonella typhimurium* that exhibits a typical potential Hfq binding site, co-immunoprecipitate with Hfq (**Table1**).[85] In the case of the *dapB* gene of *Salmonella typhimurium* co-immunoprecipitation of the 3' region of the mRNA with Hfq reflects the synthesis of the DapK sRNA from a promoter located at the end of the *dapB* coding sequence.[84] A similar origin was proposed for the RyeF sRNA in *E. coli* and *S. typhimurium*.[83,84] These data indicate that Hfq interacts in the same way with the 3' ends of mRNAs and sRNAs. Indeed, the 3' end of the *cspA* mRNA forms a complex with the proximal RNA-binding surface of Hfq, which also interacts with the 3' terminal U-stretch of the RybB sARN.[76,95] Consistently, a large part (35%) of the predicted Rho-independent terminators of *Salmonella* co-immunoprecipitate with Hfq.[84] The RybD sRNA characterized in *E. coli* and *S. typhimurium* presumably belongs to this category.[83,84] The observations above suggest that the 3'ends of RNAs resulting from Rho-independent transcription termination affect the fate of RNA that can either form a stable functional complex with Hfq or be attacked by the oligo(A)-dependent exonucleolytic degradation machinery. A speculative model taking in account the molecular events occurring at the 3' ends of these transcripts is presented below.

The role of Hfq in RNA polyadenylation: A mechanistic speculation. RNAs terminated by Rho-independent terminators harboring the structural motifs required for Hfq binding probably rapidly establish strong interactions with the proximal and lateral sites of Hfq.[77] In the case of sRNA, these complexes probably facilitate annealing of the seeding sequence with the complementarity sequence of the target mRNA, which may be made accessible through an association with the distal sites on the other face of Hfq.[100,101] Similarly, mRNAs resulting from Rho-independent termination, whose 3' ends associate with the proximal site of Hfq, are presumably not accessible to exoribonucleases and PAP I.[77] Some of these stabilized 3' terminal mRNA fragments are probably regulatory RNAs as proposed for RybD.[84] In contrast, 3' fragments of mRNAs generated by endonucleases that cannot be bound by Hfq are presumably marked for degradation. This is the case of *rpsO*, *rpsT*, *lpp*, *ompA* mRNAs and of the SraL,[18] SraG,[19] RNA I,[13] Oop,[15] Sok,[16] RNA OUT[73] and CopA[14] sRNAs that are all polyadenylated and mostly degraded by a oligo(A)-dependent exonucleolytic process (see above). It is interesting to point out that most polyadenylated sRNA mentioned above (RNA I, RNA OUT,

Table 1. The 3' ends of polyadenylated RNAs

Polyadenylated mRNAs
rpsO ---**UAA***UUUCUUGCGAGUUUC*AGAAAAGGGGCCUGAGUGGCCCCUUUUUUC
cspA ---**UAA***UCUCUGCUU*AAAAGCACAGA*AUCU*AAGAUCCCUGCCAUUUGGCGGGGAUUUUUUU
lpp ---**UAA***UAGUACCUGUG*AAGUGAAAAAUGGCGCACAUUGUGCGCCAUUUUUUUU
ompA ---**UAA***GUUCUCGUCUGGU*AGAAAAACCCCGCUGCUGCGGGGUUUUUUUU
rpsT ---UCAACAAACUGGCU**UAA**UCGCCAAUUUGCUGAAGC*UUUGU*GAAAAAGCCCGCGCAAGCGGGUUUUUUU
Polyadenylated sRNAs
RNA I pAUUUGGUAUCUGCGCUCUGCUGAAGCCAGUUACCUUCGGAAAAAGAG*UUGGUAG* *CUCUU*GAUCCGGCAAACAAACCACCGCUGGUAGCGGUGGUUUUUUUGUU
CopA p*UUU*AAGUGGGCCCCGGUAAUCUUUUCGUACUCGCCAAAGUUGAAGAAGAUUAUCGGGGUUUUUGCUU
Oop pppGUUGAUAGAUCCAGUAAUGACCUCAGAACUCCAUCUGGAUUUGUUCAGAACGCUCGGUUGCCGCCGGGCGUUUUUUA
Sok pppGACUAGACAUAGGGAUGCCUCGUGGUGGUUAAUGAAAAUUAACUUACUACGGGGCUAUUUCCUU
RNA OUT pppUCGCACAUCUUGUUGUCUGAUUAUUGAUUUUUCGCGAAACCAUUUGAUCAUAUGACAAGAUGUGUAUCC
SraL ---GAUAGAGAGAAAGACAAAGACCGGAAAACAAACUAAAGCGCCCUUGUGGCGCUUUAGUUU
SraG ---A*UUAGUUU*CCAGUGAUUGCUGCCGUCAGCUUGAAAAAAGGGGCCACUCAGGCCCCCUUUUCU
CsrC ---CCCGUUAAGGGUUAAGAGUCAGGAAAAAAGGCGACAGAGUAAUCUGUCGCCUUUUUUCUU
GlmY ---GC*UUAUU*CCAUAACAAAGCCGGGUAAUUCCCGGCUUUGUU
Hfq complexes
SgrS ---G*UAUU*GGUGUAAAAUCACCCGCCAGCAGAUUAUACCUGCUGGUUUUUUUU
flgL ---**UAA**CGCC*UCUUUUUG*AAACAUAUCACGAAACUGGAUAUGUUUUGUCUGCCCGCGCCAUCCACCCCGGCGCGGGCAUUUUUUUA

Sequence data are from references quoted in the text and from databases. SgrS and *flgL* are taken as examples of an sRNA and mRNA forming stable complexes with Hfq.[85] The 3' terminal oligo (U) sequences and the complementary stretches of As are underlined. Secondary structures are shaded un gray (light gray for the terminator and dark gray for the upstream hairpin). Termination codons of mRNAs are in bold, ppp and p at the 5' ends means that the full-length or a processed sRNA are shown, respectively.

Oop, CopA and Sok) are cis-acting regulators that do not require Hfq for annealing the complementary target transcribed from the opposite DNA strand. Polyadenylation is one of the parameters that control their activity. Biological activity of the GlmY sRNA also depends on polyadenylation, which controls its stability.[17] Consistent with the hypothesis above, GlmY miss a strong putative 3' terminal Hfq-binding site (**Table 1**).[95] However, in this case, polyadenylation takes place at an endonucleolytic processing site just upstream of the terminator.

It is reasonable to assume that oligo(A) tails less than 10 As in length, which are too short to form stable complexes with Hfq,[42,77] are nevertheless long enough to be used as "toe-holds" by exoribonucleases able to carry out the degradation of structured RNAs (see above). Because PNPase dissociates when it encounters secondary structures the current model postulates that the oligo(A) "toe-holds" are repetitively degraded and resynthesized until the base-paired nucleotides are removed.[7,52,66] RNase R is also acting in the oligo(A)-dependent degradation of the *rpsO* mRNA and several structured RNA fragments.[57,58] In contrast, RNase II activity, which overpasses PNPase and RNase R activities in the cell,[68] efficiently prevents oligo(A) tail extension.[54,66] Thereby, it presumably moderates the amount of RNA that has to be degraded by the poly(A) dependent machinery of degradation and prevents the formation of oligo(A) tails long enough for Hfq binding.[52,61,64] However, the growth-rate could stimulate their appearance; it has been reported that PAP I expression, as well as Oop RNA and *lpp* mRNA polyadenylation, increase when the growth rate slows down.[102]

The impact of these long tails on RNA metabolism remains mysterious. One can imagine that Hfq, which stimulates poly(A) synthesis and strongly binds tails that it protects against ribonucleases, plays a major part in poly(A) metabolism. Oligo(A) tails longer than 14 As most likely form stable complexes with Hfq.[42,44,75,77,93,94] The interaction would involve the distal face of Hfq that is capable of interacting with consecutive "A-R-N" triplets. Several Hfq molecules presumably bind side by side on long poly(A) (**Fig. 1C**) so that four protomers of each Hfq interacts with 12 A residues. The observation that oligo(A) tails as long as 5–10 As are sufficient to transform PAP I into a processive enzyme suggests that the interaction of two triplets of the oligo(A) tail with two Hfq protomers is sufficient to impact polyadenylation activity.[43] That polymerization progressively becomes processive upon oligo(A) elongation supports the idea that the fixation of several Hfq molecules facilitate PAP I access to the 3' RNA extremity. How Hfq stimulates PAP I activity is unknown. An interaction between PAP I and Hfq has been reported,[39] suggesting that Hfq may act by increasing the local concentration of PAP I. Such a complex could also account for the processivity of the reaction. Polynucleotide phosphorylase could associate with this complex and as a consequence coordinate degradation and RNA elongation.[39] It is worth remembering here that PNPase was proposed to synthesize long heterogeneous tails at the 3' ends of transcripts.[103] In contrast to short tails used as "toe-holds" by exoribonucleases, long oligo(A) tails are strong Hfq-binding sites that likely protect RNAs against exonucleolytic degradation.[75] In the case of *rpsO*

mRNAs, Hfq could bind simultaneously to the oligo(A) tail via its distal site and the U-rich sequence recognized by RNase E just upstream of the terminator hairpin through its lateral surface.[75,77] Such an interaction could explain why Hfq protects the transcripts from RNase E processing in vitro. Two Hfq hexamers bound independently on both sides of the hairpin would also protect the RNA from endo- and exoribonuclease attacks.[75] It is worth noting here that simultaneous interaction of Hfq with the 3' extremity and an internal segment of the Qß RNA has been reported.[104,105] It is also appealing to speculate that Hfq bound to oligo(A) tails still has accessible proximal and lateral RNA-binding surfaces that can be occupied by sRNAs (**Fig. 1D**). Such oligo(A) tail-Hfq-sRNA complexes may favor sRNA annealing with complementary sequences located on the oligoadenylated mRNA.[101,106] The locations of facilitating oligo(A) sequences in the vicinity of sRNA target sites on the mRNA suggests that oligo(A) tails may contribute to the recognition of hypothetical sRNA target sites located at the 3' end of the mRNA. Moreover, as demonstrated for sRNA-mRNA, interactions occurring upstream or in coding sequences formation of such Hfq-sRNA-mRNA complexes may end up in RNase E cleavage.[91,92,107] However, there is no indications that the RNase E cleavage rate limiting for decay of the *rpsO* mRNA, occurring just upstream of the Rho-independent terminator, is activated by Hfq in vivo. In contrast, the fact that poly(A) dependent decay is only operational when RNase E is inactive suggests that a direct, or sRNA-mediated, RNase E-Hfq complex interacting with the 3' extremity of the mRNA could switch off poly(A) dependent decay of the *rpsO* mRNA and account for the coordinated activities of the RNase E and poly(A) dependent decay pathways.[24,108,109] One can also imagine that oligo(A) tails would also compete with the repetition of "A-R-N" motifs for binding to the distal RNA-binding surface of Hfq.[87,100,106] Indeed, it is now recognized that the intracellular concentration of Hfq is limiting in respect to the concentration of its potential partners. An exchange mechanism based on the progressive binding of competing RNAs to the multiple sites of the RNA-binding surfaces of Hfq has been proposed to explain the paradoxical rapid dissociation of strong Hfq-RNA complexes in the presence of a competing RNA.[110-113] Hfq-sRNA complexes could interact with oligo(A) tails in the absence of target mRNAs. This might be a means used by the cell to store sRNAs that could then be rapidly delivered to their cognate mRNA. Finally, one could also speculate that long tails bound by Hfq are synthesized when free Hfq or unpartnered Hfq-sRNA complexes, harboring an unoccupied distal site that is able to bind oligo(A) sequences are present in the cell. Under these conditions, short tails contributing to the degradation of tightly folded 3' RNA extremities might be processively elongated and generate Hfq docking sites that are degraded when Hfq becomes limiting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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