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REVIEW

How does sub-cellular localization affect the fate of bacterial mRNA?

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Abstract Recently a number of seminal studies have revealed that both sequence and spatio-temporal factors govern RNA decay in bacteria, which is crucial for regulation of gene expression. Ribonucleases have been described that not only exhibit sequence preferences, but also are sub-cellularly localised. Furthermore, the RNA itself is distributed in an organised manner and does not diffuse freely or randomly within the bacterial cells. Thus, even within the sub-micrometer distances of the bacterial intra-cellular space, the positions of the enzymes and their substrates are kept in check. Adding to this complexity is the secondary structure and sequence specificity that many, perhaps all, ribonucleases exhibit, including those that are responsible for "general" RNA degradation. In this review, the implications of these novel findings are discussed and specific examples from *Staphylococcus aureus* are analysed.

Keywords Bacteria · RNA degradation · Sub-cellular localisation · RNase Y · RNase E · CshA helicase

Introduction

One of the main physiological differences between bacteria and eukaryotes, is that bacteria frequently lack membrane-defined organelles. This, combined with their smaller size, originally led to the general assumption that all molecules could freely diffuse in the bacterial cytosol, unless they were bound to the membrane or the chromosome. However, this view has been severely challenged in the last decades, especially with the advent of high-resolution light-microscopy and various fluorescent proteins fusions, which have revealed that many "cytosolic" proteins are actually localized to specific regions of the cell.

However, it is not only proteins that are localized, but also their substrates, and an intriguing example of this are the mRNAs. These molecules are transcribed at their gene of origin, i.e., at a discrete location inside the cell, and are thereafter bound and translated by ribosomes until they are degraded with typical half-lives of 1–10 min. However, several of the key ribonucleases that perform the mRNA decay are associated with the inside of the membrane, and as a consequence, the localization of each mRNA molecule determines whether it can be degraded.

Surprisingly for such a central function as mRNA decay, the machinery is not conserved, or even similar, in all bacteria (Laalami et al. 2014). Instead, each genus or family appears to have adopted its own specific combination from the pool of bacterial ribonucleases, both for RNA degradation, but also for maturation of essential RNA molecules such as ribosomal RNA and tRNA (Ow and Kushner 2002; Britton et al. 2007; Linder et al. 2014). Nevertheless, membrane association appears to be a recurring theme, since RNase E and RNase Y, both of which are endoribonucleases that serve as assembly-points for other enzymes that participate in RNA decay, are membrane bound (Hunt et al. 2006; Khemici et al. 2008; Lehnik-Habrink et al. 2011; Roux et al. 2011; Murashko et al. 2012; Mackie 2013). RNase E can be found in many model organisms, chief of which is *Escherichia coli*, where a wealth of information about this enzyme has been accumulated over the years. In contrast, the recently discovered and evolutionary unrelated

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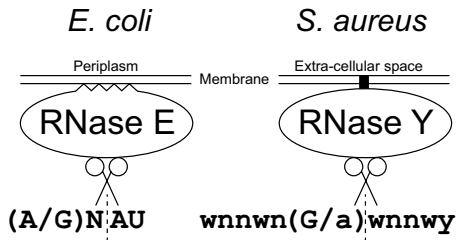

Fig. 1 Cartoon showing some of the key differences between the membrane bound endoribonucleases RNase E and RNase Y. The *zig-zag line* represents the internal amphipatic helix which anchors the *E. coli* RNase E to the inside of the inner membrane. The *thick black line* indicates the N-terminal hydrophobic helix which anchors the *S. aureus* RNase Y to the inside of the membrane. At the *bottom* are shown the consensus endonucleolytic cleavage sequences for the two enzymes (Mackie 2013; Khemici et al. 2015), where *upper case* denote high conservation and lesser conservation is in *lower case*. *N* any base, *W* A or U, *Y* C or U

RNase Y has mainly been studied in Firmicutes, but can for example also be found in δ- and ε-proteobacteria (Laalami et al. 2014).

In *E. coli* RNase E is essential, and performs the decay-initiating endoribonucleolytic cleavage on virtually all mRNAs (Clarke et al. 2014). Each individual mRNA must therefore migrate from its locus of transcription in the nucleoid to the membrane before it can be cleaved by RNase E (Fig. 1). Such a change in localisation is consistent with the realisation in the last decade that coupled transcription and translation appears to be the exception rather than the rule; however, the actual rate with which the mRNA diffuses away from its point of origin is still highly debated (Montero Llopis et al. 2010; Bakshi et al. 2012). In the α-proteobacterium *Caulobacter crescentus*, the mRNAs are surprisingly translated in clusters near the location of their encoding chromosomal loci, after which they appear to be degraded. This is less paradoxal than it seems, because in this organism, RNase E lacks an amphipatic helix to associate it with the membrane and it is not essential (Christen et al. 2011; Voss et al. 2014; Aït-Bara and Carpousis 2015). This would in principle permit RNase E-dependent decay-initiation anywhere in the cytosol, except that the *C. crescentus* RNase E is not free, but is localised to specific foci defined by the highly organised *C. crescentus* chromosome instead (Montero Llopis et al. 2010). This localisation presumably extends to the various RNase E-associated degradosome components as well (Voss et al. 2014), and the flow of mRNA is therefore still a crucial factor in *C. crescentus*.

RNase Y is evolutionary unrelated to RNase E, but the concept appears at first glance to be strikingly similar, with endoribonucleolytic activity and a membrane binding domain, albeit with an N-terminal hydrophobic helix instead of an internal amphipatic helix (Fig. 1) (Hunt et al. 2006; Shahbabian et al. 2009; Lehnik-Habrink et al. 2011). Additionally, similar to RNase E, RNase Y from *Bacillus subtilis* interacts with a number of other enzymes that have been linked to RNA decay (Lehnik-Habrink et al. 2011), although in *Staphylococcus aureus* this seems limited to the key degradation RNA helicase CshA (Roux et al. 2011; Giraud et al. 2015). However, in contrast to RNase E in *E. coli*, RNase Y is neither essential in *B. subtilis* nor in *S. aureus*, and deleting the gene only gives significant growth defects in the former (Redder and Linder 2012; Figaro et al. 2013; Khemici et al. 2015).

RNase Y selectivity

In terms of RNA decay, it is striking that only about a hundred open reading frames have their RNA half-lives significantly extended in an RNase Y mutant of *S. aureus* (Khemici et al. 2015), which shows that RNase Y cannot be the major initiator of RNA decay, but instead suggests that RNase Y is rather selective in its target choice.

Where does this selectivity come from? Our lab recently discovered a preference for a guanosine immediately upstream of the *S. aureus* RNase Y cleavage site (Khemici et al. 2015); however, this is obviously not enough to exclude a majority of the transcriptome from being cleaved, since all transcripts contain guanosines. Instead it seems probably that the sub-cellular localisation of RNase Y plays a significant role in limiting its activity, but that leads to the question of whether certain RNA molecules are more likely to move to the membrane than others, and which factors would regulate this.

The signal recognition particle will recognise the N-terminal amino acids of nascent proteins and activate the secretion pathway, which will transport the nascent polypeptide chain and the translating ribosome to the membrane, and with it, the mRNA that is being translated (Elvekrog and Walter 2015). This universally conserved mechanism ensures that mRNAs that encode exported or membrane-bound proteins are actively transported to a subcellular location where they in principle could be cleaved by RNase Y. To find out whether such Membrane Protein Encoding (MPE) mRNAs are more likely to be targets of RNase Y, the RNase Y dependent RNA decay data (Khemici et al. 2015) was combined with the TMHMM transmembrane and signal-peptide prediction algorithm (Krogh et al. 2001) to reveal potential correlations (Table 1). Note that for this analysis, each open reading frame is treated as a mono-cistronic transcript, whereas in the cell, an entire poly-cistronic transcript can be transported towards the membrane if it encodes even a single membrane protein. However, taking the results at face value, only 10 %

Table 1 RNase Y is not rate-limiting for the decay of the majority of MPE mRNAs

	RNase Y independent decay[a]	RNase Y dependent decay[a]
Non-MPE mRNAs[b]	1052	60
MPE mRNAs[b]	294	33

[a] Only protein-coding RNAs that pass the quality control criteria in (Khemici et al. 2015) are included

[b] As predicted by TMHMM v2.0 software, using a score of 18 as cut-off (Krogh et al. 2001)

(33/327) of the *S. aureus* MPE mRNAs are significantly stabilised in an RNase Y mutant, but this is much higher than the 5 % (60/1112) found for non-MPE mRNAs. On the other hand, 90 % of the MPE mRNAs are not dependent on RNase Y for the rate-limiting step in their degradation, and it is therefore difficult to imagine that the type of encoded protein is a major determining factor.

Importance of the RNase Y membrane anchor

The above-mentioned findings seem to indicate that the membrane-localisation of RNase Y serves a minor role. However, in *S. aureus*, a deletion of the RNase Y gene only carries a slight fitness cost, whereas a mutant with an anchorless RNase Y—an N-terminally truncated protein that has no membrane anchor—has a significantly longer doubling time (Khemici et al. 2015). Moreover, in a mutant deleted for the CshA helicase, which normally over-produces haemolysins and is growth-inhibited at 25 °C, the removal of the membrane anchor results in an almost complete reversal of the *ΔcshA* phenotypes (Khemici et al. 2015). Furthermore, this dramatic effect could be linked directly to the membrane location of RNase Y, and not to any allosteric effects of removing the N-terminal domain, because a re-anchoring of the anchorless RNase Y via dimerization with an enzymatically dead (but membrane-bound) RNase Y almost completely removes the suppression of the *ΔcshA* phenotypes (Khemici et al. 2015) (Fig. 2). Therefore, it seems that RNase Y activity is curtailed by its membrane localisation, and an anchorless RNase Y enzyme presumably gains access to RNA molecules which are normally protected from RNase Y cleavage by being localised away from the membrane. Indeed, preliminary results from our lab are consistent with a general shortening of RNA half-lives in the anchorless RNase Y mutant, and it is possible to imagine that this could rescue the RNA decay defects previously observed in the CshA mutation (Giraud et al. 2015), even though the wild-type RNase Y and CshA are rate-limiting for two virtually non-overlapping subsets of transcripts (Khemici et al. 2015). If

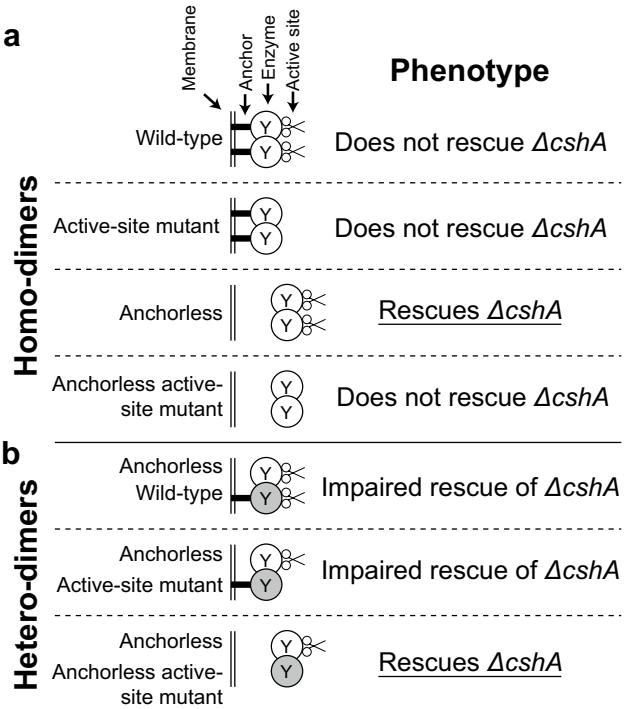

Fig. 2 Membrane localisation of RNase Y is the key factor for suppressing *ΔcshA* phenotypes. Cartoon adapted from (Khemici et al. 2015) which summarises the effects that wild-type and mutants of RNase Y have, when the CshA RNA degradation helicase is deleted in *S. aureus*. RNase Y interacts with itself, at the very least as dimers (*as drawn*), although higher multimers cannot currently be excluded (Lehnik-Habrink et al. 2011). *White* and *grey circles* indicate protein expressed from the chromosome and a plasmid, respectively. **a** The chromosomal RNase Y allele was mutated to either remove the N-terminal membrane anchor and/or the enzymatic activity of RNase Y. Only an enzymatically active anchorless mutant will rescue the *ΔcshA* strain. **b** When membrane-anchored RNase Y proteins are expressed from a plasmid (shown in *grey*), they can re-anchor the chromosomally encoded anchorless RNase Y, to impair the rescue of the *ΔcshA* strain, irrespective of enzymatic activity. However, an anchorless active-site mutant has no such effect

RNase Y is indeed membrane anchored in order to limit its activity and further its selectivity, then the next step should be to uncover the features of an RNA that determines its movement within the cell, and thereby its potential for being degraded.

Perspectives

The target selection by the bacterial RNA decay machineries is clearly multi-factorial, and might differ significantly between even relatively closely related species. However, it is also clear that certain concepts, such as membrane association, occur again and again across evolution, and thus it is not futile to apply knowledge gained from one organism in order to understand another. Nevertheless, although we

owe an enormous debt to the massive and diligent work performed in *E. coli*, and would have been nowhere without it, the *E. coli*-centric approach that has until recently been prevalent, probably only revealed a fraction of the story. One of the most important advances in recent years, for the field of bacterial RNA turnover, is therefore that efficient genetic tools have become available for multiple bacteria from a variety of phyla and classes.

I expect that the systemic understanding of bacterial RNA degradation will be greatly advanced in the near future, due to (1) the availability of super-resolution microscopy, permitting visualisation on scales that are appropriate for the small size of bacteria, (2) the possibility of detecting individual RNA molecules in situ, by a variety of methods, (3) recent progress in global chemical and enzymatic probing of RNA structures (Del Campo and Ignatova 2015), and (4) the development of transcriptome-wide methods adapted for examination of RNA decay and cleavage-site identification (Redder 2015). However, the challenge will be to combine data from these diverse experimental setups into unified models, and to correlate/verify these models with biochemical and genetic experiments.

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References

Aït-Bara S, Carpousis AJ (2015) RNA degradosomes in bacteria and chloroplasts: classification, distribution and evolution of RNase E homologs. Mol Microbiol 97:1021–1135. doi:10.1111/mmi.13095

Bakshi S, Siryaporn A, Goulian M, Weisshaar JC (2012) Superresolution imaging of ribosomes and RNA polymerase in live *Escherichia coli* cells. Mol Microbiol 85:21–38. doi:10.1111/j.1365-2958.2012.08081.x

Britton RA, Wen T, Schaefer L et al (2007) Maturation of the 5′ end of *Bacillus subtilis* 16S rRNA by the essential ribonuclease YkqC/RNase J1. Mol Microbiol 63:127–138. doi:10.1111/j.1365-2958.2006.05499.x

Christen B, Abeliuk E, Collier JM et al (2011) The essential genome of a bacterium. Mol Syst Biol 7:528. doi:10.1038/msb.2011.58

Clarke JE, Kime L, Romero AD, McDowall KJ (2014) Direct entry by RNase E is a major pathway for the degradation and processing of RNA in *Escherichia coli*. Nucleic Acids Res 42:11733–11751. doi:10.1093/nar/gku808

Del Campo C, Ignatova Z (2015) Probing dimensionality beyond the linear sequence of mRNA. Curr Genet. doi:10.1007/s00294-015-0551-5

Elvekrog MM, Walter P (2015) Dynamics of co-translational protein targeting. Curr Opin Chem Biol 29:79–86. doi:10.1016/j.cbpa.2015.09.016

Figaro S, Durand S, Gilet L et al (2013) *Bacillus subtilis* mutants with knockouts of the genes encoding ribonucleases RNase Y and RNase J1 are viable, with major defects in cell morphology, sporulation, and competence. J Bacteriol 195:2340–2348. doi:10.1128/JB.00164-13

Giraud C, Hausmann S, Lemeille S et al (2015) The C-terminal region of the RNA helicase CshA is required for the interaction with the degradosome and turnover of bulk RNA in the opportunistic pathogen *Staphylococcus aureus*. RNA Biol 12:658–674. doi:10.1080/15476286.2015.1035505

Hunt A, Rawlins JP, Thomaides HB, Errington J (2006) Functional analysis of 11 putative essential genes in *Bacillus subtilis*. Microbiology 152:2895–2907. doi:10.1099/mic.0.29152-0

Khemici V, Poljak L, Luisi BF, Carpousis AJ (2008) The RNase E of *Escherichia coli* is a membrane-binding protein. Mol Microbiol 70:799–813. doi:10.1111/j.1365-2958.2008.06454.x

Khemici V, Prados J, Linder P, Redder P (2015) Decay-Initiating Endoribonucleolytic Cleavage by RNase Y Is Kept under tight control via Sequence Preference and Sub-cellular Localisation. PLoS Genet 11:e1005577. doi:10.1371/journal.pgen.1005577

Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580. doi:10.1006/jmbi.2000.4315

Laalami S, Zig L, Putzer H (2014) Initiation of mRNA decay in bacteria. Cell Mol Life Sci 71:1799–1828. doi:10.1007/s00018-013-1472-4

Lehnik-Habrink M, Newman J, Rothe FM et al (2011) RNase Y in *Bacillus subtilis*: a Natively disordered protein that is the functional equivalent of RNase E from *Escherichia coli*. J Bacteriol 193:5431–5441. doi:10.1128/JB.05500-11

Linder P, Lemeille S, Redder P (2014) Transcriptome-wide analyses of 5′-ends in RNase J mutants of a gram-positive pathogen reveal a role in RNA maturation, regulation and degradation. PLoS Genet 10:e1004207. doi:10.1371/journal.pgen.1004207

Mackie GA (2013) RNase E: at the interface of bacterial RNA processing and decay. Nat Rev Microbiol 11:45–57. doi:10.1038/nrmicro2930

Montero Llopis P, Jackson AF, Sliusarenko O et al (2010) Spatial organization of the flow of genetic information in bacteria. Nature 466:77–81. doi:10.1038/nature09152

Murashko ON, Kaberdin VR, Lin-Chao S (2012) Membrane binding of *Escherichia coli* RNase E catalytic domain stabilizes protein structure and increases RNA substrate affinity. Proc Natl Acad Sci USA 109:7019–7024. doi:10.1073/pnas.1120181109

Ow MC, Kushner SR (2002) Initiation of tRNA maturation by RNase E is essential for cell viability in *E. coli*. Genes Dev 16:1102–1115. doi:10.1101/gad.983502

Redder P (2015) Using EMOTE to map the exact 5'-ends of processed RNA on a transcriptome-wide scale. Methods Mol Biol 1259:69–85. doi:10.1007/978-1-4939-2214-7_5

Redder P, Linder P (2012) New range of vectors with a stringent 5-fluoroorotic acid-based counterselection system for generating mutants by allelic replacement in *Staphylococcus aureus*. Appl Environ Microbiol 78:3846–3854. doi:10.1128/AEM.00202-12

Roux CM, DeMuth JP, Dunman PM (2011) Characterization of components of the *Staphylococcus aureus* mRNA degradosome holoenzyme-like complex. J Bacteriol 193:5520–5526. doi:10.1128/JB.05485-11

Shahbabian K, Jamalli A, Zig L, Putzer H (2009) RNase Y, a novel endoribonuclease, initiates riboswitch turnover in *Bacillus subtilis*. EMBO J 28:3523–3533. doi:10.1038/emboj.2009.283

Voss JE, Luisi BF, Hardwick SW (2014) Molecular recognition of RhlB and RNase D in the *Caulobacter crescentus* RNA degradosome. Nucleic Acids Res 42:13294–13305. doi:10.1093/nar/gku1134