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Review

Bacterial helicases in post-transcriptional control ☆

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ABSTRACT

Among the five superfamilies of helicases involved in RNA and DNA metabolism, superfamily 2 and superfamily 5 include bacterial RNA-helicases. These enzymes have been shown to be involved in ribosome biogenesis and post-transcriptional gene regulation. Here, we focus on bacterial regulatory mechanisms that are mediated by RNA helicases belonging to superfamily 2, which includes DEAD-box and DEAH-box helicases. Some of these helicases are part of bacterial degradosomes and were shown to unwind RNA duplexes. We will review examples where these enzymes have been implicated in translatability and metabolic stability of bacterial transcripts. This article is part of a Special Issue entitled: The Biology of RNA helicases — Modulation for life.

1. Introduction

Five superfamilies of helicases are involved in RNA and DNA metabolism from bacteria to man [1]. Only superfamily 2 and superfamily 5 include bacterial RNA-helicases implicated in regulation of gene expression. Superfamily 5 is represented by transcription factor Rho known for its role in transcription termination [2]. Here, we focus on superfamily 2 that includes DEAD-box and DEAH-box helicases, some of which play important roles in post-transcriptional regulation, i.e. in mRNA translation and turnover. For a review on the role of the latter enzymes in ribosome assembly we refer to the article by I. Iost (in this issue).

The *Escherichia coli* genome encodes five DEAD-box helicases: DeaD/CsdA, RhlB, RhlE, DbpA and SrmB. The DEAD-box family proteins contain nine conserved sequence motifs, including the Q motif that is unique to this set of superfamily 2 helicases [3] and the Asp-Glu-Ala-Asp (DEAD) motif, which coined their name [4]. The DEAD motif has been shown to be essential for ATPase and/or the RNA unwinding activity of several members, including the *E. coli* RhlB helicase [5]. It has further been shown that the DeaD/CsdA, RhlE, DbpA and SrmB helicases display ATPase and unwinding activity in the presence of RNA duplexes with either 5′ or 3′ extensions, and that the C-terminal part of DeaD/CsdA is required for its function at 15 °C [6,7]. However, while all four enzymes can unwind RNA duplexes with long single stranded extensions, only RhlE can unwind RNA duplexes with short or even without extensions [6].

Several models for helicase action have been discussed by Bizebard et al. [6]. As the latter helicases can unwind RNA substrates with 5′ or 3′ extensions it is possible that unwinding occurs bi-directionally. However, as RhlE does not require extensions for ATPase stimulation and unwinding, this questions a simple "inch-worm model" in which helicase translocation from either end of the RNA duplex leads to unwinding. It has been suggested that the helicases DeaD/CsdA, RhlE and SrmB could translocate not only on ssRNA but also on dsRNA, and that translocation on dsRNA causes unwinding. However, as the unwinding activity of these helicases dropped severely with duplex length [6] this model seems to be less attractive. Finally these authors [6] considered that helicases may interact statically with dsRNA, and that ATP hydrolysis then results in local unwinding of the RNA duplex.

In brief, the following functions have been assigned to the five *E. coli* DEAD-box helicases. DeaD/CsdA and SrmB have been implicated in ribosome biogenesis. Each factor associates with ribosomal subunits, and the absence of either protein causes ribosome assembly defects [8,9]. Both factors were initially identified as multicopy suppressors of ribosomal protein gene mutants [10,11]. For instance, multicopy *dead/csdA* expression restored both ribosomal proteins S1 and S2 on 30S subunits at the non-permissive temperature in an *E. coli rpsB*^{ts} (encodes ribosomal protein S2) strain [12]. In addition, and as outlined below, DeaD/CsdA has further been implicated in RNA degradation [13,14] and translational regulation [15].

DbpA interacts with 23S ribosomal RNA (rRNA) [16], and the ATPase and helicase activities of the enzyme have been shown to be stimulated through interaction with helix 92 of 23S rRNA [16–18].

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RhlB is a major component of the "degradosome", an RNA degrading complex involved in RNA turnover and processing [5,19].

Like DeaD/CsdA and RhlB, RhlE has been shown to be associated with the "degradosome" [20]. In addition, RhlE appears to play a role in the interconversion of ribosomal RNA-folding intermediates that are further processed by DeaD/CsdA or SrmB during ribosome maturation [21]. In the following, we will focus on posttranscriptional regulatory mechanisms that occur mainly in *E. coli*, and which have been shown or suggested to be mediated by bacterial DEAD-box helicases.

2. DEAD-box helicases in translation initiation: Enhanced translation (initiation) through destabilization of structured RNA?

As mentioned above, the *deaD*/*csdA* helicase gene was first discovered as a multicopy suppressor of a temperature-sensitive mutant in *rpsB* [11]. Later, the DeaD/CsdA protein was re-discovered among cold-shock proteins that are induced upon a temperature downshift of *E. coli* cells from 37 °C to 15 °C [15]. These authors demonstrated that the protein displays RNA unwinding activity and renamed it CsdA (cold shock DeaD-box protein A). It was shown that DeaD/CsdA was predominantly associated with ribosomes at low temperatures [15], which may be explained by its role in ribosome biogenesis [22].

In some studies DeaD/CsdA was implicated in directly acting on mRNA by destabilizing secondary structures, which impede ribosome binding to the translation initiation regions. Jones et al. [15] showed that de-repression of the heat shock genes *dnaK* and *groEL* usually observed after temperature-downshift followed by longer exposure (3 h) to low temperature (15 °C) was reduced in a *deaD*/*csdA* mutant. The *rpoH* mRNA encoding the heat-shock sigma factor σ^{32}, which is required for transcription of these heat shock genes, contains extensive secondary structures, which partially sequester the translation initiation determinants [23,24]. It was therefore speculated that the down-regulation of heat shock gene expression at 15 °C in the absence of DeaD/CsdA results from an impaired translation of the structured *rpoH* mRNA. In other words CsdA was suggested to be required at low temperature for destabilization of secondary structures in *rpoH*, and thus for efficient translation (initiation). As the synthesis of other proteins, e.g. EF-Tu, was unimpaired in the *deaD*/*csdA* mutant under the same conditions, it seems less likely that the observed lack of de-repression of heat shock genes is attributable to defective ribosomes observed in *deaD*/*csdA*$^-$ strains [9]. Nevertheless, no follow-up studies have been performed to delineate the molecular mechanism of DeaD/CsdA action on *rpoH* translation.

Very recently, Tamura et al. have shown that disruption of the *deaD* gene can partly restore cell growth of an *E. coli rne* mutant lacking functional RNase E [25]. Although the actual mechanism of suppression is unknown, one of the authors' interpretations is that DeaD/CsdA might be implicated in translation of mRNAs encoding toxic polypeptides such as toxin components of toxin–antitoxin systems. As these mRNAs are likely stabilized and their level is elevated in the *rne* mutant, the authors speculated that DeaD/CsdA-mediated stimulation of translation might, in turn, increase the concentration of the cognate toxic polypeptides thereby inhibiting cell growth, whereas *deaD* inactivation might reverse this effect [25].

Similar to *E. coli*, the *crhB* and *crhC* genes encoding the DEAD-box helicases CrhB and CrhC are upregulated in the cyanobacterium *Anabena* sp. PCC7120 upon temperature downshift [26]. However, follow-up studies as to the function of these helicases have not been performed. The synthesis of another cyanobacterial DEAD-box helicase CrhR of *Synechocystis* sp. PCC6803 is autoregulated [27], and is induced under a variety of other stress conditions including cold shock [26]. Inactivation of the *crhR* gene resulted in severe effects on physiology and morphology at normal growth temperature as well as under cold stress [28]. Similar to *E. coli*, the observed effects may be rooted in the inability of the *crhR* mutant to express the heat shock genes *groEL1/2* during cold acclimation. In contrast to *E. coli*, where DeaD/CsdA was suggested to affect heat shock gene expression *via* the regulation of *rpoH* translation, in *Synechocystis* sp. PCC6803 the CrhR helicase was reported to stabilize the *groEL1/2* transcripts [29]. Thus, the question whether the observed stabilization results from enhanced translation (initiation) of these mRNAs remains open.

The experiments described above did not allow a direct conclusion as to whether the DEAD-box helicases indeed stimulate translation (initiation) by altering secondary structures. So far, this question has been addressed in only a few experiments. It has been reported that the incorporation of labeled methionine in *E. coli* S30 extracts programmed with structured phage MS2 RNA was increased in the presence of DeaD/CsdA [30]. More recently, it was shown that ectopic over-expression of the *dead*/*csdA* gene in *E. coli* increased the translation of a *cat* mRNA harboring an inhibitory secondary structure, sequestering the Shine and Dalgarno sequence [31]. In addition, translation of the same *cat* mRNA construct was strongly decreased at 25 °C in a *deaD*/*csdA* mutant strain when compared with the wild-type strain or with the *deaD*/*csdA* mutant strain growing at 37 °C. As the presence of DeaD/CsdA at low temperature apparently counteracted the predicted higher stability of the stem–loop structure 5′ of the *cat* start codon, the authors [31] concluded that the DeaD/CsdA helicase destabilizes the inhibitory secondary structure and thereby enhances translation (initiation) (Fig. 1A). While this is possible, direct experimental proof is lacking. Although the unwinding activity of DeaD/CsdA has been demonstrated *in vitro* with RNA duplexes, more sophisticated experiments, e.g. *in vitro*/*in vivo* RNA structural probing experiments in the presence and absence of DeaD/CsdA with mRNAs containing inhibitory secondary structures, are warranted to validate a function of bacterial DEAD-box helicases in restructuring of translation initiation regions.

2.1. Involvement of DeaD/CsdA in riboregulation

Bacterial *trans*-acting small regulatory RNAs (sRNAs) are generally synthesized in response to certain growth or stress conditions [32]. The majority of sRNAs in *E. coli* and several other bacteria act as anti-sense RNAs by negatively controlling gene expression. They prevent ribosome access to the mRNA through base-pairing with, or in the vicinity of the ribosome binding site. As a result, the respective mRNA undergoes rapid decay [32]. In contrast, several sRNAs were found to act as translational activators (reviewed in [33]). They base pair with target mRNAs to open intramolecular inhibitory RNA secondary structures, which otherwise block ribosome binding.

A well-studied model system in *E. coli* is the translational activation of *rpoS* mRNA, encoding the stationary phase sigma-factor, RpoS, by the sRNA DsrA at low growth temperature (25 °C) [34,35]. The ribosome binding site of *E. coli rpoS* mRNA is sequestered by an intramolecular RNA secondary structure. Translational activation of *rpoS* mRNA at low temperature and during exponential growth includes duplex formation between *rpoS* and DsrA, which is mediated by the RNA chaperone Hfq [36], and a concomitant re-direction of RNase III cleavage in the 5′-untranslated region of *rpoS* upon DsrA·*rpoS* annealing [37]. In this way, DsrA-mediated regulation does not only activate *rpoS* translation by disrupting the inhibitory secondary structure but also stabilizes the *rpoS* transcript. As only minor structural changes occur upon Hfq binding to *rpoS* mRNA prior to DsrA·*rpoS* annealing [38], the action of this RNA chaperone appears to be insufficient to promote efficient unfolding of the inhibitory secondary structure in *rpoS* at low growth temperature. Recently, DeaD/CsdA was identified as an ancillary factor required for low temperature activation of RpoS synthesis by Hfq/DsrA. The lack of RpoS synthesis observed in the *dead*/*csdA* mutant strain at low growth temperature could be attributed to a lack of duplex formation between *rpoS* and DsrA [39]. A model for DsrA–Hfq mediated translational activation of *rpoS* mRNA entails binding of the Hfq–DsrA complex