Functional and molecular analysis of *Escherichia coli* strains lacking multiple DEAD-box helicases

KEVIN L. JAGESSAR and CHAITANYA JAIN
Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, Florida 33136, USA

ABSTRACT

DEAD-box RNA helicases are enzymes that unwind RNA duplexes and are found in virtually all organisms. Most organisms harbor multiple DEAD-box helicases, suggesting that these factors participate in distinct aspects of RNA metabolism. To define the individual and collective contribution of the five DEAD-box helicases in the bacterium *Escherichia coli* (*E. coli*), nonpolar deletion mutants lacking single or multiple DEAD-box genes were constructed. An analysis of the single-deletion strains indicated that the absence of either the DeaD or SrmB RNA helicase causes growth and/or ribosomal defects under typical laboratory growth conditions. The analysis of strains lacking multiple DEAD-box genes showed cumulative growth defects at low temperatures. A strain deleted for all five DEAD-box genes was also constructed for these studies, representing the first time all DEAD-box genes have been removed in any organism. Additional investigations revealed that the growth and ribosomal defects of such a DEAD-box deficient strain can be sharply attenuated under alternative conditions, indicating that the defects caused by a lack of DEAD-box genes are modulated by growth context.

Keywords: DEAD-box proteins; *Escherichia coli*; RNA processing; ribosome assembly

INTRODUCTION

Different classes of RNA molecules play a variety of functions in the cell, which include important roles in translation, gene regulation, and the transfer of genetic information. The function of RNA is critically dependent upon its ability to adopt productive secondary and tertiary conformations. For that purpose, cells contain a number of factors that promote correct RNA folding or resolve incorrect structures. One prominent class of such factors includes RNA helicases, which regulate RNA structure through their ability to unravel duplex RNA into single strands using energy derived from nucleotide triphosphates (Cordin et al. 2006; Linder 2006).

The known RNA helicases can be grouped into five superfamilies that share a number of characteristic motifs (Gorbalenya et al. 1989; Tanner and Linder 2001). Among these, the majority of RNA helicases are members of the DEAD-box family, which belongs to the SF2 superfamily of RNA helicases. DEAD-box RNA helicases are characterized by the presence of nine sequence motifs distributed over 350–400 amino acids (Gorbalenya et al. 1989; Rocak and Linder 2004; Cordin et al. 2006). These motifs are variously involved in binding substrate RNA, nucleotide triphosphate (NTP), magnesium ion, or in NTP-coupled unwinding of duplex RNA. Apart from a canonical RNA duplex unwinding activity, several DEAD-box helicases possess additional functions, including RNA chaperone, RNA annealing, strand exchange, and protein displacement activity (Jankowsky et al. 2001; Fairman et al. 2004; Yang and Jankowsky 2005; Rajkowitsch et al. 2007). Such properties indicate that DEAD-box proteins can be even more versatile than was previously appreciated.

Escherichia coli is a well-established model organism for studying RNA metabolism that harbors five DEAD-box RNA helicases: DbpA, RhlB, SrmB, DeaD (CsdA), and RhlE (Kalman et al. 1991; Iost and Dreyfus 2006). DbpA interacts specifically with helix 92 of 23S ribosomal rRNA (rRNA), an interaction that promotes its ATPase and helicase activity (Diges and Uhlenbeck 2001). RhlB is an integral component of the "RNA degradosome," an RNA degrading complex that includes two ribonucleases (RNases), ribonuclease E and polynucleotide phosphorylase (Coburn et al. 1999; Khemici and Carpousis 2004; Khemici et al. 2005). SrmB and DeaD are implicated in ribosome biogenesis as assembly factors and the absence of either confers

Reprint requests to: Chaitanya Jain, Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL 33136, USA; e-mail: cjain@med.miami.edu; fax: (305) 243-3955.

Article published online ahead of print. Article and publication date are at http://www.rnajournal.org/cgi/doi/10.1261/rna.2015610.

a cold-sensitive growth phenotype (Charollais et al. 2003, 2004). No role for RhlE has been established, though it was recently shown to modulate the function of SrmB and DeaD (Jain 2008).

DEAD-box proteins are present in virtually all organisms, which support the notion that these proteins are required in key RNA metabolic processes. In many model organisms, several DEAD-box proteins have also been found to be essential. For example, each of the two DEAD-box genes in *Mycoplasma genitalium* (*M. genitalium*), a bacterium with a reduced ~0.6 Mb genome, is required for viability (Glass et al. 2006). Similarly, of the 26 DEAD-box genes in *Saccharomyces cerevisiae* (*S. cerevisiae*), 18 are essential (Linder et al. 2000). In contrast, none of the five *E. coli* genes is individually required for growth (Iost and Dreyfus 2006). This raises the possibility that either the *E. coli* DEAD-box proteins do not perform any essential cellular function, or that they possess redundant functions that allow individual DEAD-box genes to be deleted without dramatic consequences. To address these issues, we constructed a suite of *E. coli* mutant strains that lack different combinations of DEAD-box proteins. A phenotypic and molecular analysis of these strains is described below.

RESULTS AND DISCUSSION

To study the consequences of removing DEAD-box genes, isogenic strains containing DEAD-box gene deletions were created in strain MG1655*. MG1655* is a direct derivative of MG1655, the first sequenced *E. coli* strain, which unlike the latter, contains a wild-type allele for the Ribonuclease PH gene (Blattner et al. 1997). Deletion alleles of helicase genes, obtained from the Keio collection of *E. coli* gene deletion strains (Baba et al. 2006), were transferred into MG1655* by P1 transduction and gene deletion was confirmed by PCR after each round.

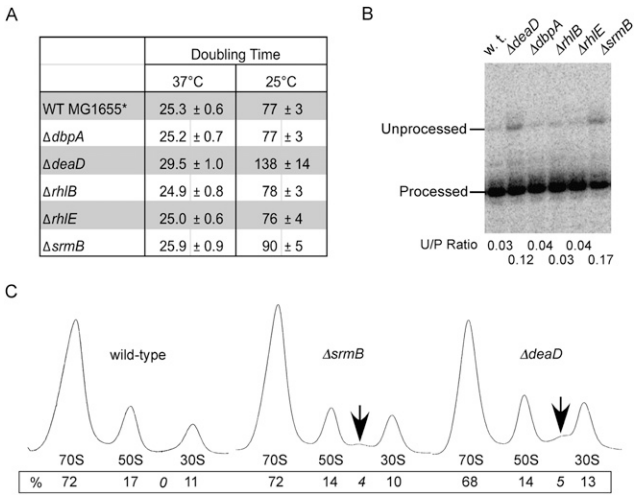


FIGURE 1. Characterization of single DEAD-box gene-deletion strains. (*A*) Doubling time measurements. Saturated cultures of the wild-type strain, MG1655*, and its derivatives containing DEAD-box gene deletions were subcultured into rich medium, and grown at 37°C or 25°C with periodic measurements of cell density within the exponential growth phase. Cell doubling times and standard deviations were calculated based on eight to 10 replicates. (*B*) 23S rRNA processing. Total RNA was isolated from MG1655* or DEAD-box gene-deletion strains grown in rich medium at 37°C. Processing of 23S rRNA at the 5′ end was analyzed by primer extension using a labeled oligonucleotide complementary to 23S rRNA. The position of the processed 5′ end and a precursor that contains seven unprocessed nucleotides is indicated. The ratio of unprocessed to processed RNAs (U/P) is also indicated for each sample. (*C*) Ribosome analysis. Cell lysates were prepared from MG1655* or from *ΔdeaD* and *ΔsrmB* strains grown at 37°C in rich medium. Ribosome profiles were generated following ultracentrifugation of clarified cell extracts on a 14%–32% sucrose gradient. The positions of the 70S ribosomes and of the 50S and 30S subunits are indicated. An increased density at ~40S observed in *ΔdeaD* and *ΔsrmB* mutants, which corresponds to 50S precursors, is indicated by an arrow. A quantitation of the normalized amounts of 70S ribosomes, the individual subunits and the precursors (indicated in italics), is shown at the *bottom*. The precursors are assumed to be present at negligible levels in the wild-type strain.

Analysis of single-deletion strains

A first set of mutant strains examined contained single nonpolar deletion mutations in each of the five DEAD-box genes. An initial analysis of these strains was carried out by measuring growth rate in rich medium at 37°C. Strains containing *ΔdbpA*, *ΔrhlB*, *ΔrhlE*, or *ΔsrmB* deletions grew, as well as the wild-type strain, but the *ΔdeaD* strain consistently grew with a 4–5 min longer generation time as compared to the wild-type strain (Fig. 1A). Similar growth rate measurements were also performed at 25°C to test for a low-temperature growth phenotype. These measurements showed a marked reduction in the growth rate of the *ΔdeaD* strain as compared to the wild-type strain and a small reduction for the *ΔsrmB* strain. These results are consistent with a cold-sensitive phenotype reported previously for the *ΔdeaD* and *ΔsrmB* strains (Charollais et al. 2003, 2004). Additional experiments indicated that the growth defects of *ΔdeaD* and *ΔsrmB* strains were even more pronounced at lower temperatures (data not shown). To rule out the possibility that the observed defects were not caused by changes in the expression of a gene abutting *deaD* or *srmB*, the corresponding deletion strains were transformed with *deaD* or *srmB* plasmids, respectively. In each case, growth rates comparable to the wild-type strain were observed at 25°C (data not shown).

Defects in processing of 23S ribosomal RNA (rRNA) at both the 5′ and 3′ ends have been observed in *deaD* and *srmB* strains at low temperatures (Srivastava and Schlessinger 1990; Charollais et al. 2003, 2004), but no such defects have been previously reported at 37°C. The processing defects in the former case include the accumulation of precursors containing three to seven unprocessed nucleotides at the 5′ end and seven to nine unprocessed nucleotides at the 3′ end. To explore the possibility that similar processing

defects can also occur at 37°C, total RNA was isolated from a wild-type strain and each of the single-deletion strains grown at 37°C, and 5′-end processing of 23S rRNA was analyzed by primer extension. Two mutants showed increased levels of rRNA precursors. In the *ΔdeaD* strain, the fraction of rRNA containing seven unprocessed nucleotides at the 5′ end was found to be 0.12 as compared to 0.03 in a wild-type strain (Fig. 1B). A *ΔsrmB* deletion strain also showed significant accumulation of unprocessed RNA, with an unprocessed to processed rRNA ratio of 0.17 (Fig. 1B). The latter result was unexpected because the *ΔsrmB* strain, unlike the *ΔdeaD* strain, showed no associated growth defect at 37°C (Fig. 1A). Additional analysis of the 3′ end of 23S rRNA indicated that the levels of 3′-end precursors in *ΔdeaD* and *ΔsrmB* strains were similarly elevated three- to fourfold over the wild-type strain (data not shown).

The increased levels of unprocessed RNA in *ΔdeaD* and *ΔsrmB* strains suggested that there could be additional ribosomal defects as well. To investigate this possibility, ribosomal profiles were generated from wild-type, *ΔdeaD*, and *ΔsrmB* strains grown at 37°C. Each strain showed the expected peaks corresponding to 70S ribosomes and to the 30S and 50S ribosomal subunits. However, the mutant strains also displayed a noticeable accumulation of aberrant particles that sediment at around 40S and account for 4%–5% of the ribosomal particles (Fig. 1C). In addition, the levels of 50S particles, as compared to the 30S subunits, were reduced slightly, consistent with the 40S particles harboring precursors of the 50S subunits (Charollais et al. 2003, 2004). A similar profile has been recently reported for a *ΔdeaD* strain grown at 37°C (Peil et al. 2008). The profiles from the mutant strains were qualitatively similar to those observed at low temperatures; conditions under which the accumulation of the 40S particles is accentuated in the mutant strains (Charollais et al. 2003, 2004). We conclude that DeaD and SrmB contribute to 50S ribosomal subunit biogenesis not only at low temperatures, but also at 37°C, near the optimum temperature for *E. coli* growth.

Construction and analysis of multiple-deletion strains

An important question concerning DEAD-box helicases is whether cellular defects become more pronounced as DEAD-box helicases are successively deleted, and specifically, whether it is possible to delete all five DEAD-box helicase genes in *E. coli*. To address these questions, a series of multiple-deletion strains was constructed (Fig. 2A). Noting that only *deaD or srmB* deletions gave rise to significant defects, double mutant strains lacking each of these genes (*ΔdΔs* and *ΔsΔd*) were constructed first. Three triple-deletion mutant strains lacking one additional helicase were then made, followed by three quadruple-deletion mutants that contained only one functional DEAD-box gene (*dbpA*, *rhlB*, or *rhlE*). Finally, a quintuple mutant (*Δ5*) strain that lacks all five DEAD-box helicase genes was successfully constructed. Because of the possibility that suppressor mutants might have arisen if any of the mutant strains were to exhibit a significant growth defect, each multiply mutated strain was constructed independently a minimum of two times, and in particular, the *Δ5* strain was made four times. PCR verification to confirm that all five DEAD-box genes were deleted in one isolate of the *Δ5* strain is shown in Figure 2B.

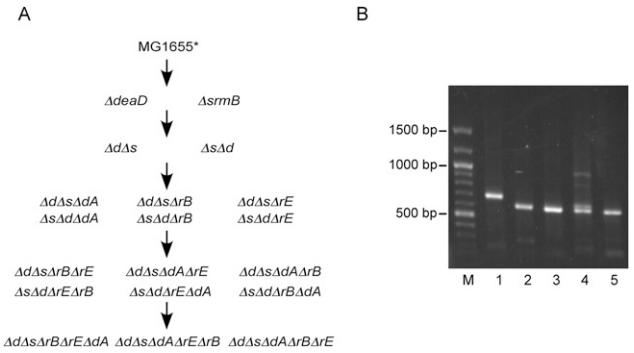


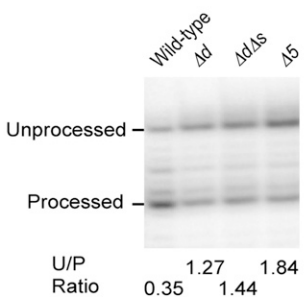
FIGURE 2. Construction of multiply mutated strains. (*A*) Strain construction strategy. Strains deleted for up to five DEAD-box genes were constructed sequentially. The starting single-deletion strains lacked either *deaD* or *srmB*. Two double-deletion strains (*ΔdΔs* or *ΔsΔd*; *d* = *deaD*, *s* = *srmB*) lacking both genes were created from either single mutant. Duplicate isolates of three triple mutant strains were derived from *ΔdΔs* or *ΔsΔd*. Next, two isolates for each of the three quadruple mutant strains were made from the triple mutant strains. Finally, three independent isolates of the quintiple mutant strain (*Δ5*) were derived from these quadruple mutants. *dA* = *dbpA*; *rB* = *rhlB*; and *rE* = *rhlE*. For each multiply deleted isolate, the order in which sequential deletions were introduced is from *left* to *right*. A fourth *Δ5* isolate (not shown), in which the *ΔdeaD* deletion was introduced last, was made via a different route (*ΔdAΔrBΔrEΔsΔd*). The successive transfer of deletion alleles at each step was confirmed by PCR genotyping of all strains. (*B*) PCR verification of DEAD-box gene deletion. A *Δ5* strain isolate was subjected to colony PCR using primer sets that hybridize 250–500 base pairs (bp) upstream of and 100–250 bp downstream from each DEAD-box gene. Lanes *1–5* correspond to PCR using primer sets for *dbpA*, *deaD*, *rhlB*, *rhlE*, or *srmB*, respectively. The expected sizes of the PCR products are 0.69 kilobases (kb) for the *ΔdbpA* deletion allele and 0.60 kb for each of the other four deletion alleles. M = 100 bp DNA molecular weight marker. None of these products was obtained when DNA from strains containing wild-type alleles was used instead (data not shown).

To determine the consequences of deleting multiple DEAD-box genes, first, growth rates were measured for the multiple-deletion strains in rich medium at 37°C. As controls, the wild-type and *ΔdeaD* strains were also included. Growth measurements revealed that each of the multiple mutants grew more slowly than a wild-type strain, but none grew significantly slower than the singly mutated *ΔdeaD* strain (Fig. 3A). These results suggested that the absence of *deaD* is primarily responsible for the growth defects of the multiply mutated strains at 37°C. Consistent with this inference, we found that combined deletion of the other four helicases genes (*dbpA*, *rhlB*, *rhlE*, and *srmB*) from MG1655* increased the cell doubling time by just ~20% as compared to the increase observed for the singly

A

	Doubling Time	
	37°C	25°C
WT MG1655*	26.9 ± 0.3	72 ± 1
Δ*deaD*	33.6 ± 0.8	140 ± 4
Δ*deaD*, Δ*srmB*	34.5 ± 1.5	141 ± 5
Δ*deaD*, Δ*srmB*, Δ*rhlE*	33.1 ± 0.6	146 ± 7
Δ*deaD*, Δ*srmB*, Δ*rhlB*	35.1 ± 1.6	155 ± 7
Δ*deaD*, Δ*srmB*, Δ*dbpA*	33.1 ± 0.9	160 ± 7
Δ*deaD*, Δ*srmB*, Δ*rhlE*, Δ*rhlB*	35.2 ± 0.8	160 ± 8
Δ*deaD*, Δ*srmB*, Δ*rhlE*, Δ*dbpA*	34.5 ± 1.0	166 ± 7
Δ*deaD*, Δ*srmB*, Δ*rhlB*, Δ*dbpA*	34.5 ± 0.7	172 ± 7
Δ*deaD*, Δ*srmB*, Δ*rhlB*, Δ*rhlE*, Δ*dbpA*	35.2 ± 0.7	181 ± 10

B



C

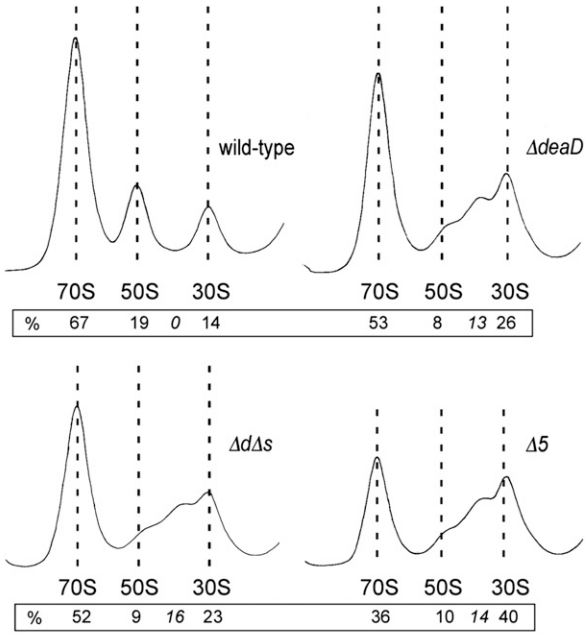


FIGURE 3. Characterization of multiply mutated strains. (*A*) Growth rate measurements. Multiply deleted strains were grown in rich medium at 37°C or 25°C, and cell density was measured during exponential phase growth. Mean cell-doubling times and standard deviations are based on eight to 12 cultures using two independent isolates of each multiple-deletion strain or four isolates of the *Δ5* strain (Fig. 2A). No statistically significant growth rate differences were found between different isolates of any mutant strain, suggesting an absence of growth suppressors. For comparison purposes, doubling times of the wild-type and *ΔdeaD* strains were also measured. (*B,C*) RNA and ribosomal analyses. Four strains: wild-type, *ΔdeaD*, *ΔdΔs*, and *Δ5* were grown at 25°C, and separately harvested for RNA or ribosomal analysis. (*B*) RNA was isolated and analyzed by primer extension using a 23S rRNA-specific labeled oligonucleotide (Materials and Methods). The positions of unprocessed and processed RNA ends, as well as the relative amounts of the two products (U/P), are indicated. (*C*) Cell extracts were analyzed by sucrose density gradient ultracentrifugation. The positions of 70S ribosomes and the ribosomal subunits (50S and 30S) are shown. These particles, as well as precursors of the 50S subunit that sediment at 40S, were quantified as described for Fig. 1C.

deleted *ΔdeaD* mutant (data not shown). Next, growth rate measurements were performed in rich medium at 25°C. Under these conditions, a progressive trend of increasing doubling times with successive gene deletion was observed (Fig. 3A). These observations indicated that there are additive effects of deleting multiple helicase genes on growth at low temperatures. In particular, the cell doubling time for the *Δ5* strain (181 min) was significantly longer than for the *ΔdeaD* strain (140 min). The growth phenotypes were corroborated by rRNA processing and ribosome analysis of four strains: wild-type, *ΔdeaD*, *ΔdΔs*, and *Δ5*. Primer extension to measure 5′-end processing indicated an increasing ratio of unprocessed to processed 23S rRNA at 25°C, ranging from 0.35 in the wild-type strain to 1.84 in the *Δ5* strain (Fig. 3B). Similarly, ribosomal analysis indicated significant defects in the mutant strains (Fig. 3C). In particular, each mutant strain displayed reduced amounts of 70S ribosomes in relation to 30S subunits, an accumulation of 40S precursors and decreased levels of 50S subunits. These defects are symptomatic of an increasing failure of the mutant cells to convert 40S precursors into 50S subunits and to form functional ribosomes. The magnitude of the ribosomal defects was least for the *ΔdeaD* mutant, slightly greater for the *ΔdΔs* double mutant, and greatest for the *Δ5* mutant. Additional ribosome profiling experiments indicated that the ribosomal defect observed in a strain lacking *dbpA*, *deaD*, and *srmB*, three DEAD-box genes with an established role in ribosome interaction or assembly, is less severe than in a *Δ5* strain (data not shown), indicating that the remaining two helicases, RhlB and RhlE, also contribute to ribosome biogenesis when DbpA, DeaD, and SrmB are absent. Overall, these analyses demonstrate cumulative low-temperature growth defects as a consequence of deleting multiple DEAD-box genes in *E. coli*.

The defects of the *Δ5* strain are growth-condition dependent

To further explore the consequences of deleting DEAD-box genes, growth rates of the single mutant strains were measured under several different conditions. Although no differences were observed between a wild-type strain and

those containing *ΔdbpA*, *ΔrhlB*, *ΔrhlE*, or *ΔsrmB* deletions (data not shown), surprisingly, we noted that in minimal medium at 37°C, the *ΔdeaD* strain grew just as rapidly as the wild-type strain. This contrasts with a 15%–20% increase in the doubling time for the *ΔdeaD* strain in rich medium at 37°C (Figs. 1A, 3A). To extend these observations, growth of the wild-type and *Δ5* strains was also compared. As shown in Figure 4A, the doubling times of wild-type strain, the *ΔdeaD* strain, and the *Δ5* strain in minimal medium at 37°C were statistically indistinguishable, indicating a lack of any substantial growth defect.

To ascertain whether the effects on growth in minimal medium extend to lower temperatures, the wild-type and *Δ5* strains were grown at 25°C. Under these conditions, the doubling time for the *Δ5* strain was 25% greater than for a wild-type strain (Fig. 4A). This contrasts with a much greater 150% increase in rich medium at 25°C (Fig. 3A). Thus, the attenuation of growth defects for a *Δ5* strain in minimal medium extends to low temperatures as well.

To determine whether a lack of growth defect for the *Δ5* strain in minimal medium is correlated with ribosomal integrity, ribosome profiles of the *Δ5* strain grown in rich or minimal medium at 37°C were compared. The *Δ5* strain ribosomal profile was found to be aberrant both in minimal and rich mediums, though slightly less so in the latter case (Fig. 4B). We also did not observe any significant differences in the extent of the 23S rRNA processing defects exhibited by the *Δ5* strain in either of the two media (data not shown). Therefore, growth in minimal medium abolishes the growth defects of a *Δ5* strain but not the ribosomal defects. These observations are reminiscent of the *ΔsrmB* mutant, which shows a ribosomal defect in rich medium at 37°C, but no defect in growth (Fig. 1).

It has been proposed that many DEAD-box helicases are especially important for RNA metabolism at low temperatures, a condition under which incorrect RNA duplexes become stabilized and would require greater assistance of these unwinding factors to restore correct RNA folding (Noble and Guthrie 1996; Mohr et al. 2002). Conversely, elevated temperatures could be a condition under which the defects caused by a lack of DEAD-box helicases become minimized. To test this prediction, the wild-type and *Δ5* strains were grown at 37°C, 41°C, or 45°C in rich medium, and ribosome profiles were generated. Whereas, the latter strain shows an expected ribosomal defect at 37°C, with a significant accumulation of 40S particles and reduced levels of 50S subunits (Fig. 4B), these defects were slightly reduced at 41°C and considerably so at 45°C (Fig. 4C). Therefore, a relatively modest increase in growth temperature was sufficient to alleviate the ribosomal defects associated with a loss of DEAD-box helicases. These observations were extended using RNA analysis to compare the extent of 23S rRNA processing at different temperatures. These analyses showed a progressive reduction in the amount of unprocessed RNA in the *Δ5* strain as the growth temperature was increased (Fig. 4D). Specifically, the ratio of unprocessed to processed rRNA in the *Δ5* strain was reduced from 0.46 at 37°C to 0.15 at 45°C. The comparable numbers for the wild-type strain over this temperature range were 0.07–0.08. Overall, these data indicate that the defects associated with the absence of helicases are sensitive to the environmental conditions experienced by cells during growth.

DEAD-box helicases have been implicated in a number of important cellular processes. However, in most instances, the

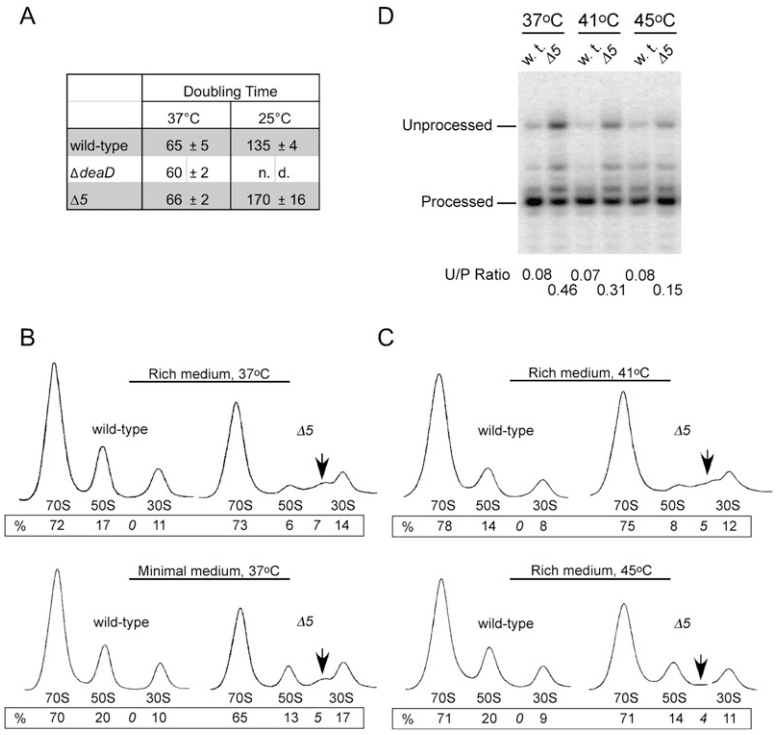


FIGURE 4. Analysis of mutant strains in minimal medium and at elevated temperatures. (*A*) Cell doubling times for wild-type, *ΔdeaD*, and *Δ5* mutants in minimal medium at 37°C or 25°C. Mean and standard deviations are based on four cultures per strain. n.d. = not determined. (*B,C*) Ribosome profiles of the wild-type and *Δ5* strains. The positions of 70S ribosomes and ribosomal subunits (50S and 30S) are indicated, and the location of 40S precursor particles is indicated by an arrow. Profiles were generated and quantified as described for Fig. 1C. (*B*) Ribosome profiles of the strains grown in rich or minimal medium at 37°C. (*C*) Ribosome profiles of the strains grown in rich medium at 41°C or 45°C. (*D*) Analysis of 23S rRNA processing in wild-type and *Δ5* strains grown at 37°C, 41°C, or 45°C in rich medium. The positions of the processed and unprocessed 5′ ends are shown. The ratios of the unprocessed to processed RNAs (U/P), averaged over five independent experiments, are indicated.

precise functions of DEAD-box helicases inside the cell are not known. As most organisms contain multiple DEAD-box helicases, it suggests that different DEAD-box helicases could possess distinct functions. In that event, the analysis of single mutants, coupled with the use of appropriate assays, could help to delineate their individual cellular contributions. However, it is also possible that different sets of DEAD-box helicases could share common or overlapping functions. To identify such related helicase functions, it would then be necessary to analyze multiple mutants. Here, we present the findings derived from an analysis of single- and multiple-deletion mutant strains.

We observed that singly mutated strains lacking DeaD or SrmB exhibit defective rRNA processing and ribosomal maturation, not only at low temperatures as previously described, but also at 37°C in rich medium. Under these conditions, 23S rRNA by itself can account for 50% of total cellular RNA by weight (Nomura et al. 1984). Hence, both SrmB and DeaD are involved in a major metabolic process. In addition, a series of multiple-deletion strains was constructed to ascertain whether the viability of the single-deletion strains could be explained by the presence of overlapping functions shared by different helicases. These included the successful construction of a strain lacking all five DEAD-box helicases, demonstrating that a lack of DEAD-box helicase activity does not cause inviability. However, as our analysis shows, under certain conditions, such as at low temperatures, the loss of all five DEAD-box helicases causes a greater defect as compared to the loss of any subset of these factors (Fig. 3). These observations indicate a collective role of these enzymes for maintaining optimal cellular fitness.

In addition, we observed that many defects associated with the loss of the DEAD-box helicases can be alleviated by growing helicase-deficient strains under alternative conditions. One such condition was minimal medium, whereby growth rate differences between a wild-type and *Δ5* strain were abolished. Another condition was growth at elevated temperatures, which resulted in considerable attenuation of the ribosomal defects of a *Δ5* strain. These examples highlight the role of environmental conditions in regulating the mutant phenotype. Using a similar analogy, we speculate that a rigorous search may help to identify conditions under which the role of DEAD-box helicases, either collectively or individually, become much more significant than under the limited set of conditions tested here.

MATERIALS AND METHODS

Strain construction

MG1655* (F^- LAM^-) was the wild-type strain used in this study. Strains containing marked DEAD-box helicase gene deletions were obtained from the Keio collection (Baba et al. 2006), and the deletions were transduced into MG1655* using P1 phage. Subsequently, a kanamycin-resistance (kan^R) cassette used for selection was removed by FLP-mediated excision to leave behind a nonpolar deletion (Datsenko and Wanner 2000). Each gene deletion was confirmed by PCR using cells or genomic DNA as a template. The primers sets used for verification of the deletion alleles are as follows:

ΔdbpA: 5′-GTAACAAAAGCAATTTTTCCGG-3′ and 5′-GGTTA ACGGGGGTGATCTGGCG-3′;
ΔdeaD: 5′-GATACGCATTGTTGGAATTATCGC-3′ and 5′-GCA GAGAAAACATCCCTGCGCC-3′;
ΔrhlB: 5′-GGCGATCATTTTGCACGGACCGC-3′ and 5′-GCGTT TTATTCGCGTCAGCGTCTGG-3′;
ΔrhlE: 5′-GTCATGGCAGGATTATTCATCGC-3′ and 5′-GGCTA TAGAGAAGTAACGTCTCGG-3′; and
ΔsrmB: 5′-GCCCATGCGCCCAATAAAATACC-3′ and 5′-GTGT GTTGAAGAGAGTTTTGTGGC-3′.

Growth studies

To measure cell doubling time, saturated cultures were diluted 50-fold into 5 mL of rich medium (LB medium supplemented with 0.2% glucose and 1 mM $MgSO_4$) or minimal medium (1X M9 salts, 0.2% glucose, and 1 mM $MgSO_4$). Cultures were incubated with shaking to an OD_{600} of ~0.2. One-milliliter aliquots were then removed at regular intervals to measure cell density during exponential phase growth, and doubling times were calculated by regression of the data points obtained.

Ribosome and RNA analysis

Strains were grown in 40 mL of rich or minimal medium and harvested at an A_{600} of 0.2–0.5. Cells were lysed by sonication, and ribosome profiles were generated by sucrose density gradient ultracentrifugation, as described previously (Jain 2008). Each ribosome profile experiment was repeated three to eight times. Quantitation of the different ribosomal particles is based on averages derived from two to three matching sets. For RNA isolation, cells were grown under the conditions specified and RNA was extracted from using the hot-phenol method (Aiba et al. 1981). Primer extension reactions to assay 5′-end maturation of 23S rRNA were carried out using a radioactively labeled oligonucleotide primer that anneals 35–55 nucleotides from the 5′ end of mature 23S rRNA, as described previously (Slagter-Jäger et al. 2007). The primer extension products were separated on a 6% polyacrylamide–8 M urea sequencing gel, dried, and visualized using a Molecular Dynamics Storm 840 PhosphorImager.

ACKNOWLEDGMENTS

We thank Dr. Murray Deutscher and Dr. Marc Dreyfus for comments on the manuscript. The studies described here were supported by a grant from the National Institutes of Health (GM18735).

Received December 1, 2009; accepted April 2, 2010.

REFERENCES

Aiba H, Adhya S, de Crombrugghe B. 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. *J Biol Chem* **256:** 11905–11910.

Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol Syst Biol* **2:** 2006.0008. doi: 10.1038/msb4100050.

Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, et al. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277:** 1453–1474.

Charollais J, Pflieger D, Vinh J, Dreyfus M, Iost I. 2003. The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. *Mol Microbiol* **48:** 1253–1265.

Charollais J, Dreyfus M, Iost I. 2004. CsdA, a cold-shock RNA helicase from *Escherichia coli*, is involved in the biogenesis of 50S ribosomal subunit. *Nucleic Acids Res* **32:** 2751–2759.

Coburn GA, Miao X, Briant DJ, Mackie GA. 1999. Reconstitution of a minimal RNA degradosome demonstrates functional coordination between a 3′ exonuclease and a DEAD-box RNA helicase. *Genes Dev* **13:** 2594–2603.

Cordin O, Banroques J, Tanner NK, Linder P. 2006. The DEAD-box protein family of RNA helicases. *Gene* **367:** 17–37.

Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci* **97:** 6640–6645.

Diges CM, Uhlenbeck OC. 2001. *Escherichia coli* DbpA is an RNA helicase that requires hairpin 92 of 23S rRNA. *EMBO J* **20:** 5503–5512.

Fairman ME, Maroney PA, Wang W, Bowers HA, Gollnick P, Nilsen TW, Jankowsky E. 2004. Protein displacement by DExH/D 'RNA helicases' without duplex unwinding. *Science* **304:** 730–734.

Glass JI, Assad-Garcia N, Alperovich N, Yooseph S, Lewis MR, Maruf M, Hutchison CA 3rd, Smith HO, Venter JC. 2006. Essential genes of a minimal bacterium. *Proc Natl Acad Sci* **103:** 425–430.

Gorbalenya AE, Koonin EV, Donchenko AP, Blinov VM. 1989. Two related superfamilies of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. *Nucleic Acids Res* **17:** 4713–4730.

Iost I, Dreyfus M. 2006. DEAD-box RNA helicases in *Escherichia coli*. *Nucleic Acids Res* **34:** 4189–4197.

Jain C. 2008. The *E. coli* RhlE RNA helicase regulates the function of related RNA helicases during ribosome assembly. *RNA* **14:** 381–389.

Jankowsky E, Gross CH, Shuman S, Pyle AM. 2001. Active disruption of an RNA-protein interaction by a DExH/D RNA helicase. *Science* **291:** 121–125.

Kalman M, Murphy H, Cashel M. 1991. rhlB, a new *Escherichia coli* K-12 gene with an RNA helicase-like protein sequence motif, one of at least five such possible genes in a prokaryote. *New Biol* **3:** 886–895.

Khemici V, Carpousis AJ. 2004. The RNA degradosome and poly(A) polymerase of *Escherichia coli* are required in vivo for the degradation of small mRNA decay intermediates containing REP-stabilizers. *Mol Microbiol* **51:** 777–790.

Khemici V, Poljak L, Toesca I, Carpousis AJ. 2005. Evidence in vivo that the DEAD-box RNA helicase RhlB facilitates the degradation of ribosome-free mRNA by RNase E. *Proc Natl Acad Sci* **102:** 6913–6918.

Linder P. 2006. Dead-box proteins: A family affair—active and passive players in RNP-remodeling. *Nucleic Acids Res* **34:** 4168–4180.

Linder P, Gasteiger E, Bairoch A. 2000. A comprehensive web resource on RNA helicases from the baker's yeast *Saccharomyces cerevisiae*. *Yeast* **16:** 507–509.

Mohr S, Stryker JM, Lambowitz AM. 2002. A DEAD-box protein functions as an ATP-dependent RNA chaperone in group I intron splicing. *Cell* **109:** 769–779.

Noble SM, Guthrie C. 1996. Identification of novel genes required for yeast pre-mRNA splicing by means of cold-sensitive mutations. *Genetics* **143:** 67–80.

Nomura M, Gourse R, Baughman G. 1984. Regulation of the synthesis of ribosomes and ribosomal components. *Annu Rev Biochem* **53:** 75–117.

Peil L, Virumae K, Remme J. 2008. Ribosome assembly in *Escherichia coli* strains lacking the RNA helicase DeaD/CsdA or DbpA. *FEBS J* **275:** 3772–3782.

Rajkowitsch L, Chen D, Stampfl S, Semrad K, Waldsich C, Mayer O, Jantsch MF, Konrat R, Blasi U, Schroeder R. 2007. RNA chaperones, RNA annealers and RNA helicases. *RNA Biol* **4:** 118–130.

Rocak S, Linder P. 2004. DEAD-box proteins: The driving forces behind RNA metabolism. *Nat Rev Mol Cell Biol* **5:** 232–241.

Slagter-Jäger JG, Puzis L, Gutgsell NS, Belfort M, Jain C. 2007. Functional defects in transfer RNAs lead to the accumulation of ribosomal RNA precursors. *RNA* **13:** 597–605.

Srivastava AK, Schlessinger D. 1990. Mechanism and regulation of bacterial ribosomal RNA processing. *Annu Rev Microbiol* **44:** 105–129.

Tanner NK, Linder P. 2001. DExD/H box RNA helicases: From generic motors to specific dissociation functions. *Mol Cell* **8:** 251–262.

Yang Q, Jankowsky E. 2005. ATP- and ADP-dependent modulation of RNA unwinding and strand annealing activities by the DEAD-box protein DED1. *Biochemistry* **44:** 13591–13601.