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**deaD**, a New *Escherichia coli* Gene Encoding a Presumed ATP-Dependent RNA Helicase, Can Suppress a Mutation in *rpsB*, the Gene Encoding Ribosomal Protein S2

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We have cloned and sequenced a new gene from *Escherichia coli* which encodes a 64-kDa protein. The inferred amino acid sequence of the protein shows remarkable similarity to eIF4A, a murine translation initiation factor that has an ATP-dependent RNA helicase activity and is a founding member of the D-E-A-D family of proteins (characterized by a conserved Asp-Glu-Ala-Asp motif). Our new gene, called *deaD*, was cloned as a gene dosage-dependent suppressor of temperature-sensitive mutations in *rpsB*, the gene encoding ribosomal protein S2. We suggest that the DeaD protein plays a hitherto unknown role in translation in *E. coli*.

Cellular processes in which RNA plays an essential role are likely to involve proteins whose function is the unwinding of RNA-RNA (or RNA-DNA) duplexes. Until recently only a few RNA helicases have been characterized biochemically. These include the *Escherichia coli* transcription termination factor Rho (7), the simian virus 40 large T antigen (53), the eukaryotic translation initiation factor eIF4A (45), and the nuclear protein TIF1/2 (35), which shares similarity with both eIF4A and the simian virus 40 large T antigen. Recently a number of gene products have been identified which, on the basis of their homology with eIF4A, are presumed to be ATP-dependent RNA helicases. These gene products, which make up the so-called D-E-A-D family of proteins (34), are thought to function primarily in events that occur posttranscriptionally. This growing family includes the following: the *Drosophila vasa* gene product (28) and the murine sperm-specific protein PL10 (32), both of which are thought to be involved in temporal or tissue-specific mRNA translation; the yeast proteins Prp5 (14) and Prp-16 (11) and yeast mitochondrial protein MSS116 (54), which participate in pre-mRNA splicing; and the yeast translation initiation factors TIF1/2 (35), and SPB4 (49) and *E. coli* SrmB (41), which play roles in mRNA translation and ribosomal assembly, respectively.

The *E. coli* ribosome is the prototypical ribonucleoprotein particle. As with other ribonucleoprotein particles, there is a growing recognition that at least some of the catalytic activity associated with the ribosome resides in ligand-induced conformational changes in its RNA component, with the ribosomal proteins serving as modulators of the RNA structure. Translation of mRNA in *E. coli* requires not only ribosomes, consisting of 53 ribosomal proteins and 3 rRNAs, but also tRNAs, aminoacyl-tRNA synthetases, and tRNA-modifying enzymes. In addition, translation requires proteins transiently associated with the ribosome, including initiation factors (IF-1, IF-2, and IF-3), elongation factors (EF-G, EF-Tu, and EF-Ts), and release factors (RF-1, RF-2, and RF-3 [23]). One would assume that the repertoire of proteins necessary for translation in vivo might include the proteins whose function is the unwinding of RNA duplexes. These might facilitate conformational changes in rRNA, tRNA-rRNA interactions, and the melting of mRNA secondary structure. One D-E-A-D box protein in *E. coli*, SrmB, has been implicated in the assembly of ribosomes (41). There are many D-E-A-D box proteins in the yeast *Saccharomyces cerevisiae*, and several are known to be associated directly with ribonucleoprotein particles, such as the ribosome and the spliceosome (13).

Using a strategy designed to find temperature-sensitive mutations affecting amino acid biosynthesis, we have isolated two *E. coli* strains with temperature-sensitive lesions in *rpsB*, the gene encoding ribosomal protein S2. Our aim was to isolate extragenic suppressors of these mutations in anticipation that these might turn up new genes involved in the function or assembly of the translational apparatus. Here we report the isolation of a new gene, called *deaD*, which is located at 68.8 min on the *E. coli* map, in a region that contains genes involved primarily in transcription and translation. This gene, when overexpressed from a high-copy-number plasmid, is able to suppress the phenotype in two *rpsB* mutants. The predicted *deaD* gene product has remarkable similarity to eIF4A and other members of the D-E-A-D family of ATP-dependent RNA helicases. The similarity to a eukaryotic translation factor and its ability to suppress a defect in S2 suggest that the *deaD* gene product may be a hitherto unknown component in the function or assembly of the translational apparatus.

**MATERIALS AND METHODS**

**Bacterial strains and media.** The following bacterial strains were used in this work: JF368 (*relC metF recA ilv rpsL*) [43]; JF6015 (JF368 *rpsB2131*); JF6020 (JF368 *rpsB2132*); CSR603, used for maxicell analysis (*thr-1 leuB6 proA2 phr-1 recA1 argE3 thi-1 uvrA6 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tss-33 supE44*) [50]; JF2419, used to map the temperature-sensitive mutations relative to *dapD2* (*thi-1 relA1 and *dapD2* [10]); JF2420, used to map the temperature-sensitive mutations relative to *dapD4* (*thi-1 relA1 and *dapD4* [10]);
RLA25 (Hfr P4X rpsB [ts25] metB [6]); CAG12105, used to map the temperature-sensitive mutations relative to a Tn10 insertion at 3.5 min (MG1655 zad-3094::Tn10kan [57]); and CAG12204, used for Hfr mapping in the region between 6 and 90 min (KL227 btuB3192::Tn10kan [57]). LB and M9 minimal media were prepared as described previously (39). AT agar was prepared as described (47). When necessary, antibiotics were supplemented to the following concentrations: ampicillin, 100 μg/ml; tetracycline, 20 μg/ml; kanamycin, 100 μg/ml. dapD expression in the dapD host strains was determined by growth on LB agar lacking daminonic acid as described previously (10). Mutagenesis of JF368 was performed by using 50 μg of nitrosoguanidine per ml as described previously (39). Details of plasmids used in this study are given in the text.

Genetic mapping. Recombination-proficient derivatives of E. coli strains JF6015 and JF6020 were constructed by transformation with plasmid pDR1453, which carries a wild-type copy of the recA gene (51). Genetic mapping of the temperature-sensitive mutation in each strain was then carried out by conjugation with Hfr strains and bacteriophage P1 transduction, by using the strategy and strains described previously (57).

Mapping plasmid DNA inserts to the chromosome. Plasmid DNA inserts were mapped to the chromosome by hybridizing a 32P-labeled restriction fragment from the inserts to a DNA blot of an orthogonal field agarose gel electrophoresis (OFAGE) gel, kindly provided by Cassandra Smith (58). The OFAGE DNA blot contained NotI-digested DNA from three strains of E. coli (58). The E. coli DNA NotI restriction fragments have been correlated with the E. coli genetic map (58). This allowed localization of the inserts to a relatively small region of the chromosome. The results showed that the inserts reside on the 1,000-kb NotI A fragment that corresponds to a region between 60 and 80 min on the E. coli map (58). Further mapping was done by scanning the high-resolution restriction map of the E. coli chromosome, in the region indicated (26), comparing the E. coli restriction map with that of the plasmid inserts. A region at coordinate 3150 (26) showed a good match to the restriction map of the inserted pMT1 and pMT2. The hybridization of the insert was confirmed by hybridization of a labeled restriction fragment from the insert to a DNA blot from the phage carrying the susceptor region of the chromosome. Hybridizations to DNA blots were carried out as described previously (38) in the presence of 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 8.25% dextran sulfate at 24°C; the insert-derived restriction fragment was radiolabeled by the random-primer technique (15). The blots were washed twice for 30 min in 0.1× SSC–0.1% sodium dodecyl sulfate (SDS) at 65°C.

Maxicell analysis. Maxicell analyses were performed as described previously (50). E. coli CSR603 (50) was transformed with various plasmids and USG irradiated, and the plasmid-encoded proteins were labeled with [35S]methionine. Samples were analyzed on a sodium dodecyl sulfate–15% polyacrylamide gel.

DNA sequence analysis. Restriction fragments from plasmid pMT1 were cloned into Bluescript vectors pSK and pKS. DNA sequences were determined by the method of Sanger et al. (52). Procedures, including construction of overlapping unidirectional deletions, preparation of single-stranded DNA templates, and dyeodeoxy sequencing, were performed by using reagents and methods provided by the manufacturer (United States Biochemicals). Comparisons with the GenBank data base were performed by using the FASTP program (36) provided in the International Biotechnologies sequence analysis package.

RNA extraction and primer extension. RNA was extracted from various strains of E. coli by using the hot-phenol method (4). The oligonucleotide 5'-CATCTGCAGAAAAG TGGTTTCCA-3' was 5' end labeled with polynucleotide kinase as described previously (38). Primer extension analyses were performed on 10 μg of RNA (as measured by optical density and formaldehyde gel analysis) by using the labeled oligonucleotide. Primer extension was performed as described previously (18).

Recombinant DNA methods. Plasmid DNA preparation, transformation of E. coli, and agarose gel electrophoretic analyses were performed by conventional procedures (38). Restriction endonuclease digestions and ligations were carried out as recommended by the manufacturers (Bethesda Research Laboratories and New England BioLabs).

Nucleotide sequence accession number. The nucleotide sequence presented here has been submitted to the GenBank data base under accession no. M63288.

RESULTS

Strategy and isolation of temperature-sensitive mutants. Histidine biosynthesis in E. coli is regulated at several levels, including an attenuation mechanism that is sensitive to the levels of tRNAHis (25) and a metabolic control mechanism in which ppGpp is a positive effector of transcription (61). Because of the complexity of these mechanisms, one would expect mutational alterations of normal his operon expression to affect a wide variety of cellular processes. Previous studies have already proven this to be the case. Mutations altering histidine biosynthesis have been found in the gyrA and gyrB genes, which encode the subunits of DNA gyrase (48); hisT, which encodes a tRNA-modifying enzyme (33) that affects many species of tRNA; and spoT, which encodes an enzyme that degrades ppGpp (47). All of these mutant genes have pleiotropic effects that extend beyond their His phenotype, yet their effect on his operon expression has helped to elucidate the role of their gene products play in the cell.

We have taken advantage of the histidine biosynthetic system in looking for temperature-sensitive mutations that allow growth on the toxic histidine analog 3-amino-1,2,4-triazole (AT). AT inhibits the imidazolglyceraldehyde-phosphate dehydratase activity, one of the two activities associated with the product of the hisB gene, causing a reduction in synthesis of histidine (64). We have isolated a number of temperature-sensitive mutants, which at the permissive temperature (30°C) were able to grow on AT plates containing 20 mM AT.

The his operon is regulated positively by ppGpp, as are a number of other amino acid biosynthetic operons (61). A strain of E. coli, JF368, was used; this contains a relA mutation, coding for an altered ribosomal protein, L11 (43), which effectively blocks the relA-dependent pathway for the synthesis of ppGpp and lowers the basal level of ppGpp (unpublished observation). The lower basal levels of ppGpp in relaxed (Rel−) mutants renders them more sensitive to inhibition by AT and facilitates selection of AT-resistant mutants (47). The spoT gene product is involved in degradation of ppGpp (2); consequently, mutations in the spoT gene raise the basal level of ppGpp and thereby increase his operon expression. To minimize the probability of isolating spoT mutations, we performed all mutagenesis with strain JF368 that had been transformed with pGA1, a multicopy
plasmid carrying the spoT gene (2). In this strain, SpoT activity does not increase in a gene-dosage-dependent manner (2).

Strain JF368(pGA1) was mutagenized with 50 μg of nitrosoguanidine per ml (39). AT-resistant mutants were selected on M9 minimal medium supplemented with 19 amino acids (minus histidine) and 20 mM AT. The parental strain, JF368(pGA1), grows poorly on 5 mM AT and not at all on 20 mM AT. Colonies that grew on 20 mM AT agar at 30°C were tested for temperature-sensitive growth on AT agar and LB agar at 42°C. AT-resistant colonies arose at a frequency of 10⁻⁶, and approximately 1% of these showed a temperature-sensitive phenotype. Two independently isolated temperature-sensitive mutants, JF6015(pGA1) and JF6020(pGA1), were selected for further analysis. Co-reversion studies for the AT resistance and temperature sensitivity showed that the two phenotypes (in both JF6015 and JF6020) coreverted at a high frequency (30%), indicating that the two phenotypes were probably due to a single mutation. Similar co-reversion frequencies of two phenotypes that are due to a single mutation have been seen in other systems (8) and are much higher than one would expect for mutations in two different genes.

The locus causing the temperature-sensitive and AT resistance phenotypes was mapped genetically. At the same time, a suppressor analysis was undertaken by transforming JF6015 and JF6020 with a pBR322-based wild-type E. coli gene library. For the purposes of further manipulation, both JF6015(pGA1) and JF6020(pGA1) were cured of pGA1 by the method of Maloy and Nunn (37), selecting for loss of tetracycline resistance. Curing the cells of pGA1 had no effect on either the temperature sensitivity or the AT resistance (data not shown).

The temperature-sensitive mutations map to rpsB, the gene encoding ribosomal protein S2. Since both mutant strains were Rec−, all genetic mapping was done in the presence of pDR1453, a pBR322-based plasmid carrying a wild-type copy of recA (51). Transformation of the mutants with this plasmid caused them to become UV resistant, as was expected, but did not affect the AT resistance or temperature-sensitive phenotypes. The approximate map location of the temperature-sensitive mutations was determined by using a set of Hfr strains with kanamycin resistance markers located at known distances from the respective origin of transfer and then screening for linkage of the temperature-sensitive phenotype with the Kanr marker (57). The temperature-sensitive mutations in both JF6015 and JF6020 were mapped to between 90 and 6 min on the E. coli map. A set of isogenic strains of E. coli, each with a single Kanr marker inserted into the chromosome, was used to localize further the temperature-sensitive mutations (57). Bacteriophage P1 transduction showed linkage to a Kanr marker (zad-3094:: Tn10kan) located at 3.5 min. Furthermore, cotransduction of the temperature-sensitive mutation with dapD, an auxotrophic marker for diaminopimelic acid dependence, indicated that in both strains the temperature-sensitive lesion resides very near the rpsB operon (67% cotransduction). The two genes, rpsB and tsf, in the rpsB operon code for ribosomal protein S2 and EF-Ts, respectively. Wild-type clones of these two genes were analyzed for their ability to complement the temperature-sensitive and AT resistance phenotypes. Plasmid pDB9 (1, 5), carrying rpsB and tsf, and plasmid pMT4, carrying only rpsB, were both able to complement the temperature-sensitive and AT resistance phenotypes of strains JF6015 and JF6020 (Fig. 1). Taken together, these data indicate that the mutation responsible for both phenotypes resides in the gene coding for ribosomal protein S2.

Isolation of gene-dosage-dependent suppressors. Both JF6015 and JF6020 were transformed with a wild-type,
pBR322-based *E. coli* library (9) to isolate plasmid-borne genes that could suppress the temperature-sensitive phenotype. We expected to find plasmids that carried a wild-type version of the mutated gene (*rpsB*) and hoped as well to find plasmids that carried genes unlinked to the original temperature-sensitive mutation but that in increased copy could also suppress the temperature-sensitive phenotype. Unexpectedly, the initial pool of clones included only plasmids containing DNA inserts which, when mapped back to the chromosome, were shown to be unlinked to the temperature-sensitive mutation at 4 min. In total, five plasmids carrying DNA inserts from three separate loci, all unlinked to the temperature-sensitive lesion at 4 min, have been characterized. Two clones in particular, pMT1 and pMT2, which share common DNA restriction fragments, completely suppressed the temperature-sensitive phenotype and partially suppressed the AT resistance phenotype (at 30°C, even in the presence of pMT1 or pMT2, the strains are slightly more resistant to AT than is the parent) of JF6015 and JF6020. These clones were selected for further analysis.

**Physical mapping of suppressor plasmids.** The DNA fragments inserted in plasmids pMT1 and pMT2 were localized to a region of the *E. coli* chromosome corresponding to 68.8 min on the *E. coli* map (3). This was done first by hybridizing a 1.4-kb EcoRI probe common to both plasmids to a NotI digest of *E. coli* DNA that had been electrophoresed on an OFAGE gel (58; data not shown). This localized the clones to a 1,000-kb NotI fragment corresponding to between 60 and 80 min on the *E. coli* map (58). Comparison of the restriction maps of the plasmid inserts with the Kohara et al. map of the *E. coli* chromosome (26) showed that the clones reside immediately downstream of the *pnp* gene (46) (Fig. 2). This location was confirmed by hybridization to DNA from Kohara bacteriophages known to map in the 68.8-min region. As illustrated in Fig. 2, a 1.0-kb EcoRI-EcoRV fragment showed hybridization to Kohara phage 13A9 but not to Kohara phage E4G11S (26). Sequence analysis (see below) indicated that part of the insert in pMT1 overlaps with the *pnp* gene, confirming the location of this insert.

**Maxicell and sequence analysis of suppressor plasmids.** Maxicell analysis was carried out with cells carrying plasmids pMT1 and pMT2 in order to identify the plasmid-encoded gene products. Both plasmids were shown to encode a protein of approximately 64 kDa (Fig. 3). The primary structure of this protein was inferred by obtaining the nucleotide sequence of the entire insert of pMT1 (Fig. 4). As shown in Fig. 2, part of this insert overlaps with sequences published previously for the *pnp* gene (46) and then continues counterclockwise on the *E. coli* chromosome for a further 2,705 bp, subsequently overlapping with the *mtr* gene on the 3’ end (22). An open reading frame (ORF) which can encode a protein with a molecular mass of 64 kDa (Fig. 4) was found 1,113 bp downstream from the 3’ end of the *pnp* gene. We have named this new gene *deaD* (see below).

The cluster of genes immediately upstream of *deaD* consists of *nusA*, a postinitiation transcription factor; *infB*, encoding two forms of translation initiation factor IF2B; two ORFs, coding for 15- and 35-kDa proteins; *rpsO*, coding for ribosomal protein S15; and *pnp*, coding for polynucleotide phosphorylase (20). The gene immediately downstream of *deaD* is *mtr*, which encodes a tryptophan-specific permease (22). The intergenic distance between *pnp* and *deaD* is 1,113 bp. Regnier et al. (46) observed the beginning of an ORF, preceding the *deaD* ORF, 108 bp downstream of *pnp* and continuing past the region they sequenced. Our sequencing data indicate that the stop codon (TGA) for the ORF identified by Regnier et al. (46) lies at position ~777 (in Fig. 4). This would create a protein with a predicted molecular mass of approximately 10 kDa. No protein of this molecular mass was visible in our maxicell analysis (Fig. 3). However, it is possible that the ORF requires the *pnp* promoter for expression, and, since neither of the plasmids that we isolated contains a complete copy of the *pnp* gene, we do not
necessarily expect to see expression. The sequence of the region downstream of deaD containing the mtr gene has recently been published (22). The TGA stop codon of deaD lies at position 93 of this sequence. A potential G+C-rich stem-loop structure followed by seven thymidine residues can be seen starting at position 363 in the leader region of mtr (267 nucleotides downstream of the TGA of deaD). This sequence resembles a rho-independent terminator and most probably is the transcriptional terminator for deaD.

Primer extension analysis with an oligonucleotide that hybridizes 62 bp 3' of the putative ATG initiation codon of deaD showed a major reverse transcriptase stop at nucleotide −173 (Fig. 5). A second reverse transcriptase stop was also seen further upstream, near position −650, and may represent a second promoter (data not shown). No primer extension products were seen extending into the pnp cistron, indicating that deaD is probably regulated independently from the genes upstream. Sequence analysis in the region of the reverse transcriptase stop at position −173 revealed a good −10 consensus 5 bp from the putative transcription start and a −35 consensus (21) 17 bp upstream of the −10 (Fig. 4). By comparing the primer extension analysis shown in lane 3 of Fig. 5 with the data shown in either lane 1 or lane 2, it is evident that a strain transformed with pMT1 greatly overproduces the deaD transcript. It is reasonable to assume, although it has not been demonstrated, that this dosage-dependent transcription results in overproduction of the protein.

There is no obvious Shine-Dalgarno (SD) (56) ribosome-binding site upstream of the first ATG of deaD. However, there is a 6-nucleotide sequence, TGATCA, which could hybridize with 16S rRNA immediately 5' to the anti-SD sequence. This site (called a TP site for translation initiation-promoting site) has been shown to be part of the translation initiator region in many E. coli genes (63). The anti-TP site on 16S rRNA is located immediately 5' to the anti-SD and in the case of deaD may be able to circumvent the lack of an SD. There are examples of other genes that lack an obvious SD sequence, such as dnaG, rpsA, rpsK, and malB (63). All contain a TP site within 50 bp 5' to the ATG. In the case of dnaG the sequence AUC (predicted to be part of a TP site) at position −10 may functionally replace the SD sequence. Perhaps the TP site also acts as a ribosome-binding site for deaD. It should also be noted that there is another ATG 18 codons downstream from the proposed start codon of deaD. There is a consensus SD sequence (GAGG) 12 nucleotides upstream of this ATG. At this point we cannot be sure which start codon (or both) is used in the translation of the deaD transcript.

deaD can suppress other temperature-sensitive alleles of rpsB. We tested to see whether deaD could suppress other rpsB(Ts) alleles. The only other rpsB(Ts) allele that has been characterized (both genetically and biochemically) resides in E. coli RL425 (6). Transforming RL425 with either pMT1 or pMT2 (Fig. 2) showed suppression of the temperature-sensitive phenotype on LB agar plates at 42°C (data not shown). Vector plasmid alone (pBR322) or pMT3 (Fig. 2) failed to suppress the temperature-sensitive phenotype. As expected, the temperature-sensitive mutation in RL425 is also complemented by the same rpsB-carrying plasmids (shown in Fig. 1) that complement the temperature-sensitive mutations in JF6015 and JF6020. The observation that deaD, on a high-copy-number plasmid, can suppress a known temperature-sensitive allele of rpsB supports our data mapping the temperature-sensitive mutations in JF6015 and JF6020 to the rpsB gene.

DeaD shares sequence similarity with murine eIF4A. The primary structure of the 64-kDa protein (Fig. 4) was compared with the GenBank database (issue 63) by using the Pustell FastP/FastN programs. This search revealed extensive similarity to the inferred amino acid sequence of the murine translation initiation factor 4A (eIF4A) and to the srmB gene product of E. coli (Fig. 6). Both of these proteins are members of a family of proteins known as the D-E-A-D family (named after an Asp-Glu-Ala-Asp motif that is conserved in all members of the family [34]). eIF4A, an ATP-dependent RNA helicase, is part of the mRNA cap-binding structure (eIF4F) and probably functions by denaturing RNA secondary structure, thereby eliminating it as an impediment to translation (45, 59). By association, all members of the D-E-A-D family are thought to be ATP-dependent RNA helicases (34).

The region over which the D-E-A-D family shows similarity encompasses 388 of the entire 408 amino acids of the eIF4A protein (40). Among the D-E-A-D box proteins listed in Fig. 6, DeaD shares the greatest similarity with eIF4A (51%; 36% identity) and the least with MSS116 (41%, using the conservative substitutions listed in the legend to Fig. 6; 25% identity). Protein secondary-structure predictions for DeaD and other D-E-A-D box proteins (32) predict a pre-
FIG. 4. Nucleotide and predicted amino acid sequence of the deaD gene. The putative ATG is labeled +1, and the sequence beginning at -882 starts where that described by Regnier et al. (46) ended. The stop codon for an ORF starting downstream of pnp is boxed. The -10 and -35 sequences for an E. coli promoter are underlined. The translation initiator region (with homology to a TP site (63)) 11 bp upstream of the first ATG is also underlined.

ponderance of α-helical structure throughout the protein, with a consensus helix-turn-helix motif, normally characteristic of DNA-binding proteins (42), found in the conserved a-TDV-ARGLDa-V region (a stands for aliphatic). This motif has been observed previously in a number of D-E-A-D box proteins (32), and with the addition of new proteins it appears now to be conserved in all members of this family. As noted by Leroy et al. (32), this observation indicates that the helix-turn-helix motif may act as a general nucleic acid-binding structure that is important for RNA-binding proteins as well as DNA-binding proteins. Most of the D-E-A-D box proteins have domains that extend beyond the central region of similarity with eRF4A. DeaD, as well as SrmB, P68, and PRP5, has highly charged carboxy-terminal domains. These charged regions are similar to domains found in other proteins that are implicated in RNA binding, such as the 70K U1 small nuclear ribonucleoprotein particle protein, and may be important for either protein-protein or protein-nucleic acid recognition (44). Another motif embedded in the charged carboxy-terminal domain of DeaD is an arginine-rich consensus (RPRRERRD), which is found in a number of proteins thought to bind to regions of RNA.

FIG. 5. Primer extension analysis of the deaD transcript. The major transcription start site was mapped by extension of a 20-nucleotide primer, complementary to the underlined sequence, with reverse transcriptase (18) and analyzed on a 5% polyacrylamide-urea sequencing gel. The deoxy sequencing ladder was generated by using the same oligonucleotide. The sequence of the actual 5' end of the mRNAs that is complementary to the extended products is shown on the right. Lanes: 1, primer extension analysis with RNA isolated from the parent strain, JF368; 2, primer extension analysis with RNA isolated from one of the mutants, JF6015; 3, primer extension analysis with RNA isolated from strain JF6015 transformed with pMT1.
**FIG. 6.** Alignment of DEAD and seven other members of the helicase family, eIF4A, PL10, VASA, P68, TIF1/2, MSS116, and SRMB. Pairwise computer alignments (using the amino acid single-letter code) were altered slightly to give optimal matches for the eight sequences. Identical residues and conservative substitutions in six of the eight proteins are listed in boldface under each row, as are the branched-chain aliphatic amino acids, I, L, and V (a), and the basic (b) and acidic (c) amino acids. Conservative substitutions used to arrive at percent similarities are grouped as follows: IL, DE, KE, and TB.

DEAD E. coli NH2- 28aa... LGLKAPIEALNLKYGKPSQAPGECPPHLL---NQRGVDLGMAATG
EIF-4A mouse NH2- 36aa... MNSLESLLGLFYGASTPAIQARAILPC---KQDGVIASAQSG
PL10 mouse NH2- 183aa... VEGEERNMGETRYTRPRFVQKHAPIIIK---EKRMLDMACQST
VASA fruit fly NH2- 249aa... ALDRIIIDNNVNSKGSFEKPDIAQREACLPI---SGRDMACQST
P68 human NH2- 78aa... ANFRPVANIDYRQNPQETQAQGQPWSP---SGLSMDVGQAAG
TIF1/2 yeast NH2- 26aa... MeldenNRVQFVQPFEFAQPKPAQI---HEGDLVAQAAG
MSS116 yeast NH2- 110aa... CVSLEDFKKEHAPAPFQPSQPKPKEQIKLPISESDHVIDVIATKRG
SRMB E. coli NH2- 86aa... LEIDRSEISLQKPTPTAQAQAIAPFAL---DGRDLVGASAP

DEAD SGKTAAPFLPLQLN---DPLEKA---PQIVLAPRELAVQ---EEMDTSFSKFRGV
EIF-4A TGKTAAPFLSLQIQ---ELDKA---TQLAVLAPRELALQ---KVVALMDYGCA
PL10 SGKTAAPFLPISQYDTPGEAAMRNKGRAYGKRQYISPLAVLAPQAV---EAFRKSRSVR
VASA SGKTAAPFLPLLKLAPHELELR---PQVIVSPRELAIQIF---NEARKAFESYLK
P68 TGKTAAPFLPADRLPG---CQVRVRPVQDPRELQG---EAMGVRKPRAVR
TIF1/2 TGKTSFISAAQR---QVAAAYCRCRLK
MSS116 TGKTSFIFIPFQH---INTKFDQSGYM---VKAIVAPDRDLQTEAEEVKEHHYDK
SRMB TGKTAAYALLPHL-LDFPRKKSFG---SPPLILRTPLEAQLVS---DHAELAKYHLD

DEAD TNIQIA---PESQHTALISATMP---IRRTRMFKEPQ---QVRIQVSQTVRTDPISQGYTVMWG
EIF4A DIFQKL---SNQTOVYFSA---VRTKVFQMPRDP---IRRIVKKEETLSDGQFYINER
PL10 RVEQGD---TPMFKVRRHTMSATPKREIQMLAFLDBEY---VFILVUGRSTENIQTQKVWVEEA
VASA RINTIV---TMPKHQTMQVTP---SIPKPVEQVTVGR---VFPAVIDGQGRSCTQVEYNN-Y
P68 KIVQDI---BPDQTLMWATSRPQVRLEAFDLY---THINIGALELSANNRILQIVVDYC-D
TIF1/2 QIPTFQ---PPTQTVVQSLTNP---VRTKVFQMPRDP---VRLIVKKEETLSDGQFYINER
MSS116 TISIGILNEKNSKASDINLTLFLSATDKVQLKLANMMKNKCEFELTDVKEQPAERHIDQSVSISEKF
SRMB IATIF-GGVAY---MHNAYFVESE-QNDTVATTGGLVLYTQIEEKFDCARVETLIDEARLDMFGAQDIE

DEAD RNKEAVRFL---EAAEFDAAAIFVVTRTKNATLVEAER---GYN-SALSDKDMQARLEQTLELDKR
EIF4A EWDLTCRL---ETLTTTQAVQFPLTRKVDWLETMK-HAR---DFTVSMHGDMQKDERVIFREFRSKS
PL10 DKSIPDLLL---ATKGDSLIVLFYETKNGASLDFLEDY---YGAFTCSTHGRSDQDRDEEAHFQGRSK
VASA RINTIV---TMPKHQTMQVTP---SIPKPVEQVTVGR---VFPAVIDGQGRSCTQVEYNN-Y
P68 VEKDEKIRLMEEMIENKSENKFEVPKRDCTRLEKRM---GPWAMNGKDQKEQDERWLMFNEKF
TIF1/2 EYKYETCLY---DISSQTVATVIFCTRKEVETKL-RND---KPTSVAISLDPQEQRDTMIFK
MSS116 SIFAAVEHIKQIKKERSDKNOFIAFIMPSTFILSCKNREFGKFPIDLEDGKIF
SRMB EHHTALVL---KQPEATRISFVYVRKERVHLANV---REAG-GINNCLRGEMVGRKNEAIIKLE

DEAD LLIDIALTDAVARGLDVERISLVLNVDYIDMPSVESYRIGTRGACRAVALLFENERRELLRRIMTML
EIF-4A SVRLITDFTLARIDQVQSVLNYIDFTRTNYIHRGTRGGRFGRVKAINTMVETDEKLDLDEITYNS
PL10 SVLPATVAARLIDQVQSVLNYIDFTRTNYIHRGTRGGRFGRVKAINTMVETDEKLDLDEITYNS
VASA MKVLITASVARGSLIDQVQSVLNYIDFTRTNYIHRGTRGGRFGRVKAINTMVETDEKLDLDEITYNS
P68 APILITDTSVARGLIDQVQSVLNYIDFTRTNYIHRGTRGGRFGRVKAINTMVETDEKLDLDEITYNS
TIF1/2 SRLITDFTLARIDQVQSVLNYIDFTRTNYIHRGTRGGRFGRVKAINTMVETDEKLDLDEITYNS
MSS116 SGILVCTDVGANGDMDFTVHQLVISQVPEPALNYIHRGTRGGRFGRVKAINTMVETDEKLDLDEITYNS
SRMB VNVNLIDDFTLARIDQVQSVLNYIDFTRTNYIHRGTRGGRFGRVKAINTMVETDEKLDLDEITYNS

DEAD IPEVEPLNAEL 175aa- COOH
EIF4A IEPMLNADVL 38aa- COOH
PL10 KQVPSMLNHKA 38aa- COOH
VASA SGQPVDPLRST 38aa- COOH
P68 NQANIPKQLVL 139aa- COOH
TIF1/2 IEILPSDIATLL 1aa- COOH
MSS116 IAKQEKYKTFSE 153aa- COOH
SRMB IKARIVDELPRK 62aa- COOH

FIG. 6. Alignment of DEAD and seven other members of the helicase family, eIF4A, PL10, VASA, P68, TIF1/2, MSS116, and SRMB.
secondary structure, including the \( \lambda N \) protein and the HIV-Tat protein (30).

**DISCUSSION**

Isolation of mutants with an altered ribosomal protein. We have cloned a gene from *Escherichia coli* which encodes a protein that shares significant similarity with a group of proteins called the D-E-A-D family. This is a growing family of proteins whose founding member is the murine protein translation initiation factor eIF4A. *deaD* was cloned on the basis of its ability, when present on a high-copy-number plasmid, to suppress temperature-sensitive mutations in *rpsB*, the gene encoding ribosomal protein S2.

Ribosomal protein S2 binds late in the assembly process of the 30S subunit, and its binding is stimulated by S3 as well as other ribosomal proteins. S2 is among the group of proteins, which also includes S9, S10, and S14, that are necessary for efficient incorporation of ribosomal protein S1 into the ribosome (29). Previous studies have shown that a temperature-sensitive *rpsB* mutant (RL425) has not only reduced levels of S2 but also reduced levels of ribosomal protein S1 (6). S1 has an RNA helix-unwinding activity, is important for the binding of mRNA to the ribosome, and influences the affinity of ribosomes for the different mRNA initiation sequences (60, 62). Therefore, the phenotypes associated with our temperature-sensitive mutants may be caused either directly by a mutated S2 or indirectly by the inability of an altered S2 to cooperate in the incorporation of another protein, such as S1, into the 30S subunit.

Although some ribosomal proteins appear to be dispensable under some conditions, most are essential for ribosomal assembly and protein synthesis; thus, it is not surprising that mutations in *rpsB* give rise to a temperature-sensitive growth phenotype. However, how a mutation in *rpsB* causes the cell to become resistant to the histidine analog AT is still a matter of conjecture. One possibility, which is consistent with results obtained for all other AT-resistant mutants, is that the *rpsB* mutations enhance histidine biosynthesis, thereby rendering the cell resistant to AT. Since the mechanism of *his* operon attenuation involves the stalling of ribosomes at “hungry” histidine codons in the *his* leader region (33), it is conceivable that a defect in the translation machinery could cause contextual stalling of ribosomes at these sites, thus mimicking a decrease in concentration of tRNA\(^{\text{His}}\). Alternatively, the mutation in *rpsB* may slow the growth of cells to a point that results in increased ppGpp, causing an increase in *his* operon expression. The strains used in this study carry a *relC* mutation that effectively blocks the *relA*-dependent pathway for ppGpp synthesis. Any increase in ppGpp levels would have to come from the *relA*-independent pathway (12).

A second possibility is that a mutant *rpsB*, even at the permissive temperature, causes a decrease in synthesis of a subset of proteins that may be particularly sensitive to alterations in the translational apparatus. Perhaps one of these is a membrane protein necessary for transport of AT into the cell. The situation could be reminiscent of a search for extragenic suppressors of conditional-lethal mutations that affect the protein export pathway in *E. coli*. Many extragenic suppressors of both *secY*(Ts) and *secA*(Ts) protein secretion mutants map to genes whose products are part of the translational apparatus (31). These include a number of ribosomal protein genes, tRNA genes, and the gene for initiation factor 2 (31, 55). One mutation, which was isolated as an extragenic suppressor of *secA*(Ts) and which maps to *rpsO* (secC), the gene encoding ribosomal protein S15, itself blocks the synthesis of transported proteins (16). The exact mechanisms underlying these observations are not clear; however, they suggest an interplay between the protein export system and the protein synthesis system. Therefore, a defect in a ribosomal protein could specifically affect the synthesis of membrane proteins, which in turn may cause AT resistance; this could also be responsible for the conditional-lethal phenotype seen in our strains. Germane to this hypothesis is the fact that we have characterized two other gene-dosage-dependent suppressors of the *rpsB*(Ts) mutants, one of which maps very near a gene whose product has been implicated in regulation of membrane protein synthesis (unpublished observations).

A gene-dosage-dependent suppressor of temperature-sensitive *rpsB* mutants encodes a putative RNA helicase. We have isolated a number of plasmid clones that can suppress the temperature-sensitive phenotype of *rpsB*. Two of these plasmids carry a gene-dosage-dependent suppressor, called *deaD*. The *deaD* gene product has extensive similarity to a family of proteins called the D-E-A-D family (34). The D-E-A-D family is a subset of a larger superfamily of proteins that consists of ATP-dependent proteins involved in nucleic acid recombination and/or replication (24, 54). To date, only P68 and eIF4A have been shown biochemically to have RNA unwinding activity; however, the high degree of similarity among these proteins suggests that RNA binding and unwinding is a characteristic function of the entire family. The ability of *deaD* to suppress a defect in a ribosomal protein suggests that it has a role in translation. Other D-E-A-D family members known to play a role in translation are the mouse translation initiation factor 4A, its yeast analogs TIF1/2 (35), and SPB4 (44) and *E. coli* SrmB (41) proteins, both of which are thought to function in ribosome assembly.

In *E. coli* an *srmB* clone suppresses a temperature-sensitive mutation in *rplX*, the gene encoding ribosomal protein L24 (41). This mutation causes a defect in ribosome assembly that can be overcome by overproducing *srmB*. Nishi et al. (41) suggest that *srmB*, when overexpressed, may stabilize an unstable ribosome assembly product. *DeaD* shows no more similarity to SrmB (48%) than to some of the other eukaryotic proteins in this family (Fig. 6); therefore, it is unlikely that the two proteins share the same function in the cell. In fact, we have shown that *deaD*, when overexpressed, cannot suppress the *rplX* temperature-sensitive mutation and that *srmB*, when overexpressed, cannot suppress the *rpsB* temperature-sensitive mutation (data not shown).

**Models for DeaD function.** What is the normal role of DeaD in *E. coli*? Given its similarity to eIF4A and its ability to suppress a mutation in a ribosomal protein, it is possible that DeaD has a role in translation. Overproduction of *deaD* on a high-copy-number plasmid was shown to overproduce the *deaD* transcript (Fig. 5) and presumably leads to an increase in the amount of DeaD protein. Increasing the cellular concentration of DeaD may either enhance its natural role or allow it to take on secondary functions which it might not normally perform.

Figure 7 illustrates two possible models for the natural role of DeaD and the way in which increasing the amount of DeaD allows suppression of the temperature-sensitive mutations. As is shown in Fig. 7A, DeaD may be part of the normal translation machinery, but it may be rate limiting. Simply increasing the concentration of the protein may enhance the efficiency of translation in the mutants, coun-

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terbalancing the detrimental effect of a mutant ribosomal protein S2. For example, the mutant S2 causes a decrease in the amount of ribosome-associated S1 (whose association with the ribosome is partially dependent on S2 [29]); perhaps increasing the concentration of a second ribosome-associated RNA helicase could compensate for this deficiency. It is of interest that Ganoza et al. (19) have isolated a ribosome-associated factor, called W, which has an approximate molecular mass of 62 kDa and is necessary for efficient translation of MS2 phage RNA in vitro. W is thought to act after translation initiation by stimulating protein chain elongation, perhaps by removing noncognate, deacylated tRNA from the ribosome (19).

In the second model (Fig. 7B), DeaD could be a translation accessory factor that activates or enhances the translation of specific genes, perhaps by denaturing RNA secondary structure. Its effect on rpsB may then be direct or indirect. An RNA helicase may be necessary for efficient translation of the rpsB mRNA. Increasing the amount of DeaD may allow more S2 to be made, effectively compensating for a defect in the S2 protein. Indirectly, overproducing DeaD may enhance the expression of proteins that are undersynthesized (which may cause or contribute to the temperature-sensitive phenotype as well as the AT resistance phenotype) in the rpsB mutants, thereby correcting the temperature-sensitive phenotype. This is somewhat similar to the role that eIF4A is proposed to play in the internal initiation of translation and in the translation of naturally uncapped mRNAs, such as poliovirus and encephalomyocarditis virus. According to this model, proposed by Sonenberg (59), free eIF4A (not complexed with eIF4F) and eIF4B (which shows affinity for the AUG) bind to single-stranded mRNA, allowing ribosome binding and internal initiation of translation.

As an alternative to the above models, DeaD may act on the ribosome at an earlier stage in a manner similar to that postulated for SrmB, stabilizing an unstable intermediate in the assembly of ribosomes. The existence of at least two D-E-A-D proteins, facilitating similar processes, may
indicate that there are at least two stages in ribosome assembly that require enzymatically modified conformational changes in rRNA.

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