

The *ams-1* and *mre-3071* Temperature-Sensitive Mutations in the *ams* Gene Are in Close Proximity to Each Other and Cause Substitutions within a Domain That Resembles a Product of the *Escherichia coli mre* Locus

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Two temperature-sensitive mutations, *ams-1* and *mre-3071*, in the *ams* (altered mRNA stability) gene have been used extensively to investigate the processing and decay of RNA in *Escherichia coli*. We have sequenced these temperature-sensitive alleles and found that the mutations are separated by only 6 nucleotides and cause conservative amino acid substitutions next to a possible nucleotide-binding site within the N-terminal domain of the Ams protein. Computer analysis revealed that the region altered by the mutations has extensive sequence similarity to a predicted gene product from the *mre* (murein pathway cluster e) locus of *E. coli*, which has been implicated previously in determining bacterial cell shape.

The *ams-1* and *mre-3071* temperature-sensitive mutations of *Escherichia coli* were initially described as increasing the chemical half-life of total pulse-labeled RNA (13) and causing the accumulation of 9S rRNA (1) at the nonpermissive temperature, respectively. These mutations, both of which are conditionally lethal, have been mapped to a region near the 5' end of the *ams* gene (2), and both have been reported to have pleiotropic effects on the activities of a number of ribonucleases, for example, RNases E (1), K (15), F (11), and III (23). While there is no direct evidence that the *ams* gene encodes any ribonucleolytic activity, the temperature sensitivity of RNase E extracts isolated from the temperature-sensitive strains (6, 16, 17) and the increase in RNase E activity in cells containing additional copies of the *ams* gene (2) have led to the prevailing view that *ams* encodes RNase E (6) or a product required for RNase E activity (2). It has been argued that the *ams* dependency of some non-RNase E cleavages (11, 15, 23) in vivo is due to an aberrant action of the temperature-sensitive Ams protein at the nonpermissive temperature (6).

Recently, the sequence of the entire *ams* gene has been published (4), extending and correcting previously reported sequences (7, 9). Computer analysis has revealed limited homology between the C terminus of the Ams protein and several RNA-binding proteins, including a mitochondrial ribosomal protein from *Neurospora crassa*, the S1 ribosomal protein of *E. coli*, and the 70-kDa human small nuclear ribonucleoprotein (9). In addition, similarity between regions of Ams and two eukaryotic structural proteins, H1 neurofilament and dynamin, a protein implicated in the movement of macromolecules in eukaryotes, has led to the suggestion that the *ams* gene may have a role in the overall biogenesis of RNA by mediating, for example, the translocation of RNA from the interior of the nucleoid to sites of RNA decay (4). While the Ams protein has been shown to

cross-react with monoclonal antibodies raised against myosin heavy chain from *Saccharomyces cerevisiae* and Ams antibodies have been shown to identify nonmuscle myosins (5), comparisons of amino acid sequences have not identified regions in Ams that resemble myosin (4).

***ams-1* and *mre-3071* mutations.** As a step toward elucidating the biological role of the *ams* gene, we have determined the nucleotide sequences of the *ams-1* and *mre-3071* temperature-sensitive mutations. Previously, these mutations had been localized to a region near the 5' end of the *ams* gene (2) in *E. coli* HAK117 and N3431, respectively. In order to sequence the chromosomal regions containing these temperature-sensitive mutations, two convergent oligonucleotides were first employed to amplify, by the polymerase chain reaction (20), an 861-bp region that begins at the second codon of the *ams* gene (Fig. 1A). Sequencing the resulting DNA by the chain-terminating method (21) revealed that these two independently isolated mutations (1, 13) are located very close to each other: the *ams-1* mutation is a G→A transition at position 736 in the nucleotide sequence reported by Casaregola and coworkers (4), and the *mre-3071* mutation is a C→T transition at position 742 (Fig. 1B). We speculate, on the basis of their close proximity to each other, that the temperature-sensitive mutations may affect the same structure or function in the Ams protein.

The *ams-1* mutation causes the substitution of glycine, a small amino acid, for serine, a small polar amino acid, at position 66 in Ams. Similarly, the *mre-3071* mutation also causes a conservative change: phenylalanine, a hydrophobic aromatic amino acid, is substituted for leucine, a hydrophobic aliphatic amino acid, at position 68.

Further analysis of the *ams* gene. Small segments of 20 to 50 amino acids that encompass the conservative substitutions were compared with entries in the data bases (Swiss-Prot 23, PIR 34, HIV-AA 4, A-GeneSeq 2.0, GenPept, and NRL3D 9.1), using the FASTDB program (3) to identify similarity to proteins with known function or structure. As shown in Fig. 2, a segment of the Ams protein extending

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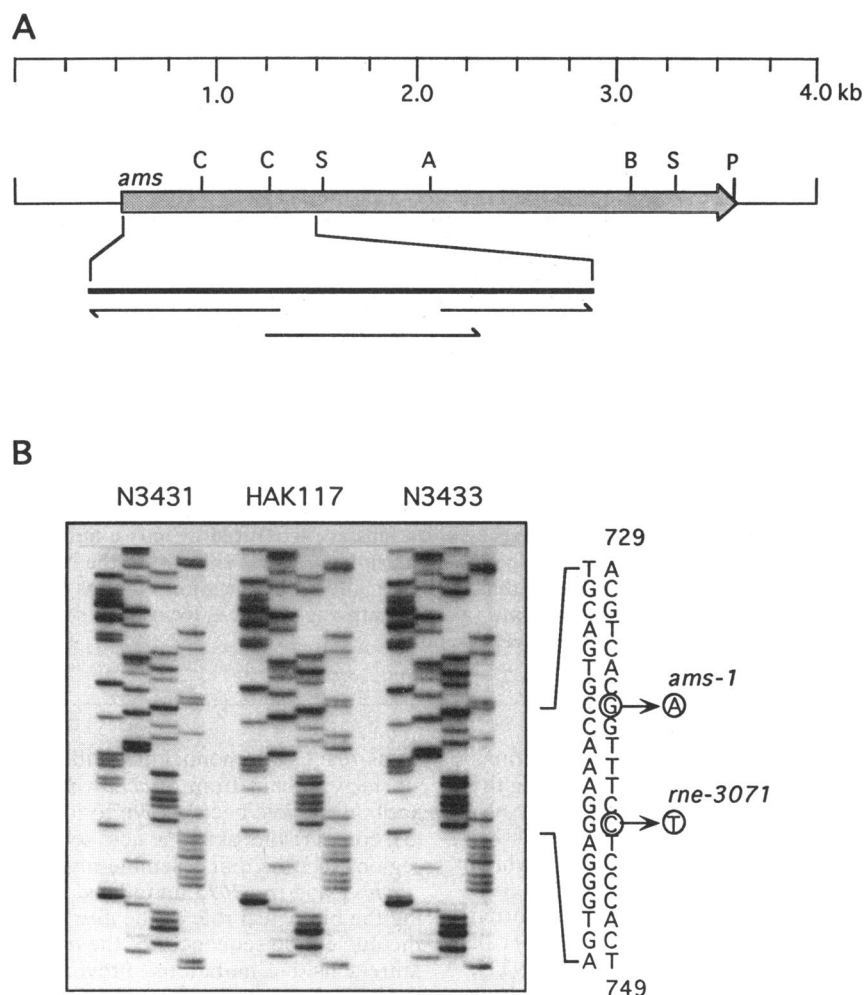


FIG. 1. Location and sequence of the temperature-sensitive mutations. (A) Strategy for amplifying the region at the 5' end of the *ams* gene by the polymerase chain reaction and sequencing the resulting DNA by using *ams*-specific oligonucleotides as primers. The thick black bar delineates the amplified region, and the arrows indicate the direction and extent of overlapping sequence. Abbreviations: C, *Cla*I; S, *Sac*II; A, *Afl*II; B, *Bam*HI; P, *Pst*I. (B) Locations of the *ams-1* and *mre-3071* mutations from *E. coli* HAK117 and N3431, respectively. The sequence of the wild-type allele from N3433 is also shown. The sequence ladders are from the leftward extension, and the numbering of the nucleotides is as published by Casaregola et al. (4).

from amino acids 39 to 61 had 78.3% sequence similarity (59.1% sequence identity) to the corresponding region in the predicted product of *orfF* from the *mre* locus of *E. coli* (26): we have designated this segment HSR (high similarity region) 1.

Using GENALIGN (Intelligenetics 5.4), which aligns sequences to create the optimal number of matches, we also found extensive similarity between the N terminus of the Ams protein (amino acids 1 to 489) and the entire *orfF* gene product, 49.5% sequence similarity (34.1% identity) in an overlap of 464 amino acids (Fig. 2). In addition, we note that particularly high sequence similarity exists between amino acids 316 to 350 of Ams and amino acids 317 to 351 of the *orfF* gene product, 80.0% similarity (71.4% identity) in an overlap of 38 amino acids. This segment has been designated HSR 2.

The high degree of homology between the N-terminal half of the Ams protein and the entire *orfF* gene product supports the notion that the N terminus may be a functionally independent domain of Ams (4) and, furthermore, suggests

that these proteins may share similar function(s) and/or structure(s). However, the role of *orfF* in shaping the cellular form of *E. coli* is unclear, as is the precise role of the *ams* gene in RNA decay. Although *orfF* is deleted along with *orfE*, *mreD*, *mreC*, and *mreB* in the $\Delta mre-678$ strain, which is characterized by a round cell shape (25), only *mreB*, *mreC* and *mreD* are required to restore *E. coli* to its normal rod shape (24).

Additional computer analysis using QUEST (Intelligenetics 5.4) was undertaken to identify sequences within the Ams and *orfF* gene product that were similar to protein consensus patterns within the PROSITE 9.1 and KeyBank 10 databases. As shown in Fig. 3, HSR 1 matches both the segment A and segment B sequence components of a modified version (8) of the ATP-binding consensus sequence proposed by Walker and coworkers (27). The HSR 1 sequence matches and aligns with the consensus sequence at all of the conserved positions without introducing additional gaps (Fig. 3). The corresponding sequence in the putative *orfF* gene product also matches the consensus sequence. Given

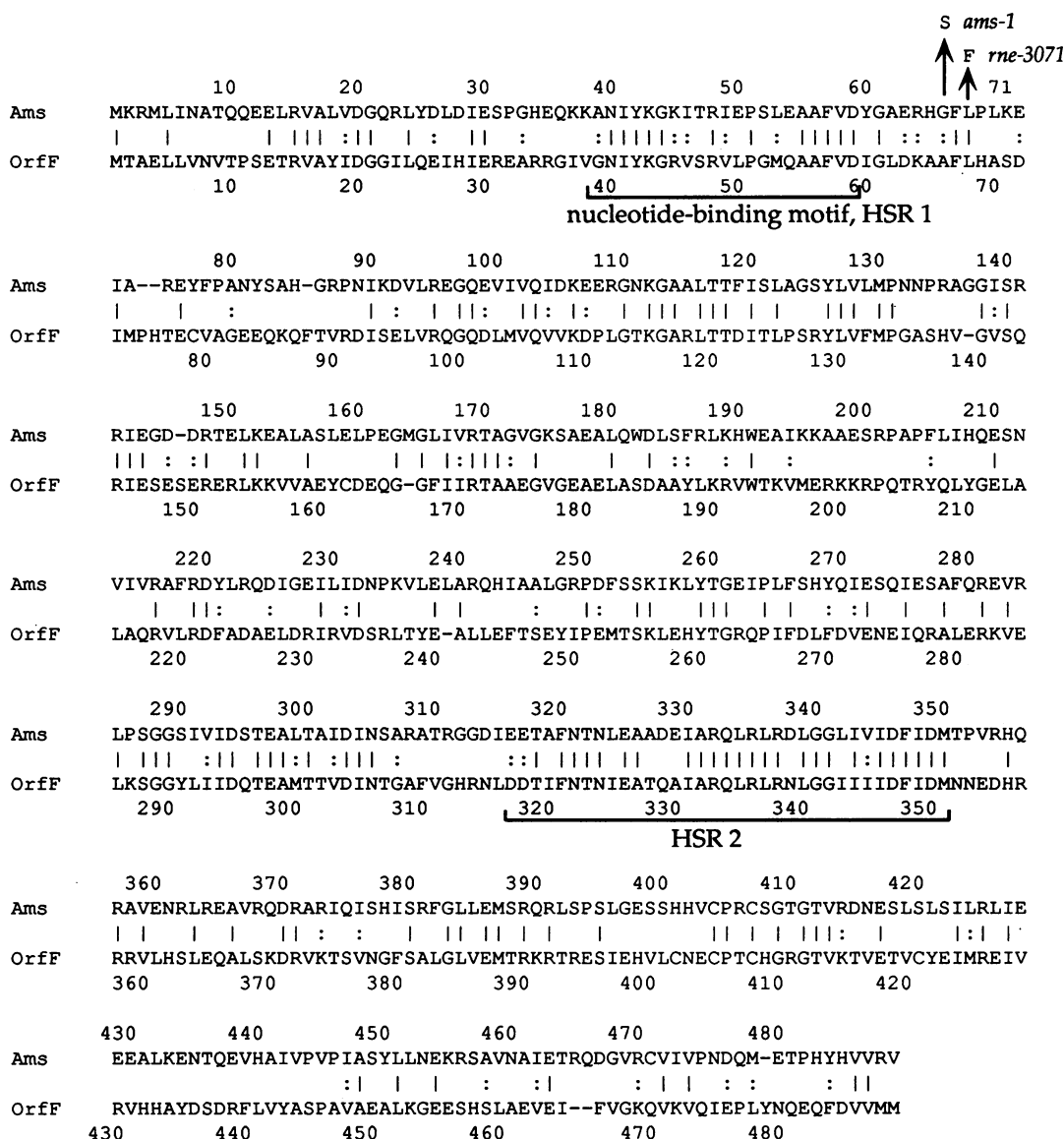


FIG. 2. Optimal alignment of the Ams protein and the predicted *orfF* gene product from the *mre* locus using GENALIGN. The conservative amino acid substitutions caused by the temperature-sensitive mutations are also shown. Regions of particularly high sequence similarity (HSR) and the proposed nucleotide-binding site are underlined. Identical and similar amino acid substitutions are indicated by vertical lines and colons, respectively.

the independent matching of the *orfF* gene product and the Ams protein with both the segment A and segment B sequences, we speculate that this region may be involved in nucleotide binding in both proteins, although not necessarily ATP binding, since related sequences have also been found in proteins that bind other nucleotides such as flavin adenine dinucleotide, GTP, and NAD (18, 22). Furthermore, because of the close proximity of this region to the amino acids substituted by the *ams-1* and *rne-3071* mutations, it is possible that an Ams-nucleotide interaction is blocked at the nonpermissive temperature in the mutants.

Previously, another potential nucleotide-binding site was suggested for the Ams protein (4) between amino acids 169 and 177 (RTAGVGKS). This suggestion was based on sequence similarity (boldface type) to a consensus sequence derived for the segment A component (also called P-loop

motif) of ATP- and GTP-binding sites (GXXGXGKT/S) (22). No similarity was found to the segment B component, but this sequence is not conserved in all nucleotide-binding domains (22). Although the *orfF* gene product also contains two glycine (G) residues between amino acids 176 to 178, this region does not contain the highly conserved lysine (K) residue nor the conserved threonine (T) and serine (S) residues characteristic of ATP- and GTP-binding sites (22). QUEST did not identify any consensus patterns within HSR 2 and FASTDB comparisons using HSR 2 did not identify any significant similarity in the data bases other than the *orfF* gene product entry.

Using the FASTDB program, the entire N-terminal sequence (amino acids 1 to 489) of the Ams protein was analyzed for similarity to other entries in the recent versions of the data bases. Other than the observed homology be-

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