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Functional interaction of heat shock protein GroEL with an RNase E-like activity in *Escherichia coli*

(endoribonuclease/mRNA stability/RNA processing)

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ABSTRACT **The highly specific endoribonuclease activities of RNase E (which processes ribosomal 9S RNA into p5S RNA) and RNase K (which initiates decay of the ompA mRNA) are inferred to play a central role in RNA processing and mRNA decay in *Escherichia coli*. *In vivo* both activities are affected by a conditional mutation of the *ams/rne* gene that seems to be complemented at nonpermissive temperatures by a fragment of the *groEL* gene. Analysis of the relationship between the two nucleases and the heat shock protein revealed that GroEL interacts functionally with an RNase E-like activity but not with an RNase K activity, a *groEL* mutation affected 9S RNA processing but not *ompA* mRNA cleavage, RNase E activity could be precipitated with an antibody against GroEL, and a highly purified GroEL preparation contained RNase E activity but not RNase K activity. When purifying RNase E activity, we obtained a preparation containing two major proteins of 60 and 17 kDa. The size and the N-terminal sequence identified the 60-kDa protein as GroEL.**

Processing and degradation of RNA in *Escherichia coli* involves the action of highly specific endoribonuclease activities, such as RNase K and RNase E (1, 2). RNase E activity has been originally defined by the cleavages that process the 9S rRNA precursor into the p5S product (3), but the number of RNA species where RNase E has been suggested to control processing and/or degradation is growing (for review, see ref. 4). Cleavages in the transcript encoding the outer membrane protein A (OmpA) have been attributed to RNase K, which appears to regulate the degradation of the *ompA* mRNA as a function of the bacterial growth rate (1, 5, 6). RNase E and RNase K have been partially purified, but the catalytic polypeptides have not yet been identified (1, 2, 7). Results obtained *in vivo* with the cleavages in *ompA* mRNA (RNase K activity) and with the processing of 9S RNA (RNase E activity) suggest that the two activities are regulated inversely by growth rate changes (8). *In vivo*, cleavages in both 9S RNA (RNase E activity) and in the *ompA* 5′ untranslated region (RNase K activity) are dependent on the *E. coli ams/rne* gene (9). However, *in vitro*, inhibition of cleavages appears restricted to RNase E activity when extracts from *ams/rne* mutants are tested at a nonpermissive temperature (10). Mutations in this gene have been found to repress degradation of bulk mRNA and anabolic and catabolic cleavages in a variety of RNA species (for review, see ref. 4). The *ams/rne* gene, also designated *hmpl* gene, has been cloned and characterized by several groups and its gene product has been identified (9, 11–15). The gene encodes a 114-kDa protein that migrates as a 180-kDa band on a denaturing gel (15). The *ams/rne* (*hmpl*) gene product has been suggested to be synonymous with RNase E, though no evidence has been reported that the *ams/rne* (*hmpl*) gene protein has nuclease activity (9, 11–15). Interestingly, in an earlier attempt to clone the gene, a DNA segment containing a fragment of the *groEL* gene was reported to complement the lethal phenotype of a conditional mutation in the originally designated *ams* gene (16).

The *groEL* gene codes for GroEL, which belongs to the 60-kDa heat shock protein (Hsp60) family (the so-called chaperonins) (17). As a chaperonin, GroEL is assembled in a high molecular weight structure that is composed of two stacked seven-subunit rings (18, 19). Members of the Hsp60 family have been shown to mediate the folding of proteins into their native form (18). The GroEL oligomer interacts specifically with another heat shock protein, GroES (20). Except for defects in phage assembly, only a few phenotypic changes have been identified in cells with conditional lethal mutations in the *groEL* and *groES* genes (21). For example, RNA synthesis is reduced >4-fold in these mutant strains when they are shifted from a permissive to nonpermissive temperature (22). Further analysis of this phenotype suggested that the activity of RNA polymerase is impaired in the absence of a functional GroEL–GroES complex (23).

The reported complementation of the *ams* mutant by a *groEL* fragment (16) suggests functional interactions exist between GroEL and the *ams/rne*-dependent nuclease activities. We report here that the processing of 9S RNA is impaired in mutant cells with a deficient GroEL protein and that a protein identical to GroEL is present in RNase E preparations purified on the basis of 9S rRNA cleavage activity. Moreover, experiments with antibodies raised against GroEL and purified GroEL protein suggested that GroEL interacts functionally with an RNase E-like activity but not with RNase K activity.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *E. coli* CAN 20-12E/18-11 (RNase I⁻, II⁻, D⁻, BN⁻, T⁻ (24) used for purifying RNase E was kindly provided by M. Deutscher (University of Connecticut). *E. coli* MC4100 and the derivative strain NRK117 (*groEL44*, *zje*::Tn*10*) (25) were kindly provided by T. Yura (Kyoto University). The plasmid pTH90 (Fig. 1) containing a section of 9S RNA, was constructed by inserting the *Eco*RI–*Xba* I fragment from M13mp19 derivative 224, kindly provided by J. Christiansen (University of Copenhagen), into the respective site of pUC19. Derivative 224 is similar to construction 18 described in ref. 26 except that the *Alu* I fragment is in the opposite direction. The plasmid pUH101, carrying the complete *ompA* gene, was constructed by digesting pTac-*ompA* (6) with *Pst* I and *Nru* I and inserting the *Pst* I–*Nru* I fragment into the *Pst* I–*Nru* I site of pUH100 (1).

***In Vitro* Transcription and RNase Activity Assay.** RNA substrates were generated as run-off transcripts from plas-

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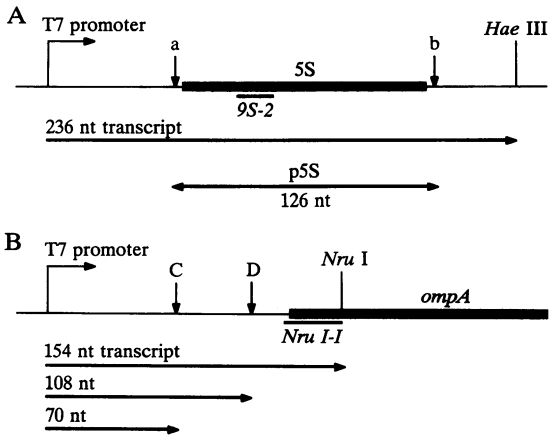

FIG. 1. Maps of the 9S and *ompA in vitro* transcripts. The template sections of plasmids pTH90 (*A*) and pUH101 (*B*) relevant for the *in vitro* transcriptions are shown. (*A*) The 9S template. (*B*) The *ompA* template. Positions of the T7 RNA polymerase initiation sites and the restriction sites (*Hae* III and *Nru* I) terminating the *in vitro* run off transcripts are depicted. Vertical arrows a and b mark RNase E (26) and C and D mark RNase K (1) cleavage sites relative to the templates. The transcripts, their major cleavage products, and their lengths in nucleotides are indicated. Part of the coding region of the *ompA* gene and the region defining the mature 5S RNA are defined by solid bars. *9S-2* and *Nru I-I* show the position of the 9S RNA-specific primer and *ompA* mRNA-specific primer used for primer extensions, respectively. nt, Nucleotide(s).

mids pTH90 and pUH101, linearized with *Hae* III and *Nru* I, respectively. Linearized DNA (2 μg) was transcribed in 50-μl reaction mixtures as described (10). RNase K and RNase E activity were assayed as described (1).

Partial Purification of RNase E. Cells were grown to late-logarithmic phase, washed, and centrifuged. All subsequent steps were performed at 4°C. Frozen cells [50 g (wet weight)] were suspended in 50 ml of buffer A [20 mM Tris·HCl, pH 7.8/5 mM $MgCl_2$/0.1 mM dithiothreitol/0.1 mM EDTA/5% (wt/vol) glycerol] supplemented with 500 μl of 100 mM phenylmethylsulfonyl fluoride and 40 mg of lysozyme. The cells were lyzed by freezing and thawing. The lysate was centrifuged (30,000 × *g* for 30 min) and the supernatant was centrifuged again (100,000 × *g* for 2 h). The pellet was dissolved in 4 ml of buffer A/0.65 M KCl and centrifuged (100,000 × *g* for 2 h). The supernatant (P-100) was treated with 0.2 mg of DNase I (Worthington) for 30 min and fractionated by ammonium sulfate (32.6 g/100 ml). The precipitate was collected (12,000 × *g* for 15 min), and the pellet was suspended in 3 ml of buffer A and dialyzed against three 1-liter changes of buffer B (20 mM sodium/potassium phosphate, pH 6.5/5 mM $MgCl_2$/0.1 mM dithiothreitol/0.1 mM EDTA/5% glycerol). The dialyzed fraction was loaded onto a CM-Sepharose CL-6B column (Pharmacia, 2.5 × 11 cm) equilibrated with buffer B (flow rate, 32 ml/h), the samples were eluted with a linear gradient (600 ml; 0–0.5 M KCl in buffer B), and 10-ml fractions were collected. The active fractions were concentrated (PEG, M_r 20,000), dialyzed against buffer A, and then loaded onto a DEAE-Sepharose CL-6B column (Pharmacia, 2.5 × 10 cm) equilibrated with buffer A. Proteins were eluted with a linear gradient (600 ml) of 0–0.35 M KCl. The active fractions from the second peak were pooled and dialyzed as described before and loaded onto a Sephadex G-100 column (Pharmacia, 1.5 × 73 cm) equilibrated with buffer A. The proteins were eluted with buffer A and 2.5-ml fractions were collected. The active fractions were pooled and concentrated in a Microsep microconcentrator (Filtron, Bjärret, Sweden) with a molecular mass cut-off of 10 kDa. The proteins were fractionated by SDS/PAGE on a 10% gel (27) and electroblotted onto a poly(vinylidene difluoride) membrane (Applied Biosystems). The protein bands were excised from the membrane and sequenced on an Applied Biosystem 477A sequencer for 20 cycles. In some cases RNase K and RNase E activities were separated with FPLC. The P-100 extract (12 mg) was loaded onto a 1-ml Mono Q Sepharose column (Pharmacia) equilibrated with buffer A, and the proteins were eluted with a 0–0.65 M KCl gradient (see Fig. 3).

Purification of GroEL. GroEL protein was purified as described (28). The purity was ≈95% as judged by SDS/PAGE and silver staining (29).

Temperature-Shift Experiments and Primer Extension. Cells were grown in 100 ml of Luria broth (LB) at 30°C to midlogarithmic stage. Ten milliliters of the culture was immediately put on ice and 10 ml of the culture was shifted to 45°C for 20 min before being put on ice. Cells were harvested and RNA was extracted with the hot phenol method as described (30). Primers and primer extensions were as described (8). After the primer-extension reaction with reverse transcriptase, the samples were analyzed by PAGE (6% gel/8.3 M urea). The gel was exposed to Fuji RX x-ray film and the signals were quantified as described (8).

Immunoprecipitation. Antibodies against GroEL were a kind gift from D. Nelson (University of Rhode Island). GroEL was purified and separated by SDS/PAGE, and the excised band material was injected into a rabbit. Antiserum (3 μl) was incubated overnight at 4°C with 50 μl of protein A-Sepharose CL-4B (Pharmacia) in 500 μl of 50 mM Tris·HCl, pH 7.5/2% Triton X-100/150 mM NaCl/1 mM EDTA. The beads were washed twice in 1 ml of Nonidet P-40 buffer (10 mM Tris·HCl, pH 7.5/1% Nonidet P-40/150 mM NaCl/2 mM EDTA), twice in 1 ml of Nonidet P-40 buffer/0.5 M NaCl, and once with 1 ml of buffer A and, finally, suspended in 100 μl of buffer A. A concentrated protein sample (5 μg) from Mono Q-separated fractions was added to the suspension and incubated overnight at 4°C. The supernatant was collected and the pellet was washed as described above.

RESULTS

Previous results have suggested that there is an interaction between the *groEL* gene and the *ams/rne* gene that affects RNase E and RNase K activities (16). Cells carrying a conditional lethal mutation in the *groEL* gene were grown to logarithmic phase at the permissive temperature (30°C) and then shifted to nonpermissive temperature (45°C). Total RNA was extracted from mutant and control cell lines, before and after the temperature shift, and the amount of 9S RNA per total RNA was determined by primer-extension reactions (8). After the temperature shift, the amount of 9S RNA showed an increase by a factor of three in the mutant cells and a decrease to less than one-third of the initial concentration was noted in the control cells (Fig. 2). The reduction of 9S RNA in the control cells is in agreement with the reported diminution of rRNA synthesis during a temperature shift (22, 31). In contrast, an accumulation of 9S RNA in the *groEL* mutant at the nonpermissive temperature is unexpected, as a dramatic drop in RNA synthesis is the phenotypic deficiency observed for *groEL* and *groES* mutations (21, 22). The magnitude of the accumulation of 9S RNA in the mutant cells, despite the drop in RNA synthesis, suggests that processing of 9S into p5S RNA is decreased by at least one order of magnitude in the absence of a functional GroEL protein. We also observed that at the permissive temperature the level of 9S RNA in mutant cells is lower than in control cells (Fig. 2). This finding may represent an aberrant 9S RNA metabolism in *groEL*^{ts} mutations even at the permissive temperature. In parallel primer-extension reactions, the same RNA samples were analyzed for the amount of *ompA* mRNA and RNase

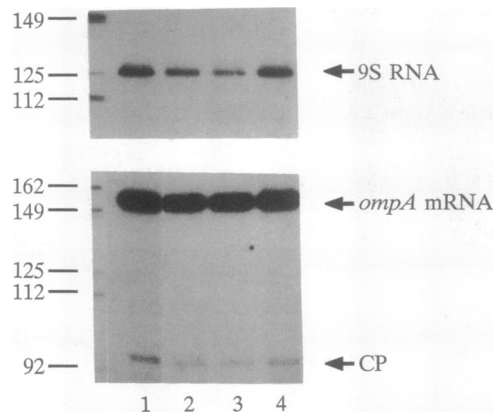

FIG. 2. *In vivo* analysis of 9S RNA and *ompA* mRNA by primer-extension assays. *E. coli* with a thermolabile GroEL protein and an isogenic wild-type strain were grown to midexponential phase at 30°C and then a part of both cultures was shifted to 45°C. Total RNA was extracted from all four cultures. The four RNA samples were incubated in parallel assays with reverse transcriptase and an excess of a radiolabeled DNA primer specific for 9S RNA (*Upper*) or ompA mRNA (*Lower*). The primer-extension products were analyzed on denaturing polyacrylamide gels (6%) next to a size standard and the signals were analyzed as described in ref. 8. The lengths of the size standards (in nucleotides) are indicated on the left and the arrows on the right indicate the positions of 9S RNA, *ompA* mRNA, and RNase K cleavage products (CP). Lanes: 1, wild type at 30°C; 2, wild type at 45°C; 3, GroEL mutation at 30°C; 4, GroEL mutation at 45°C. The ratio of *ompA* mRNA to cleavage products did not vary by >10%.

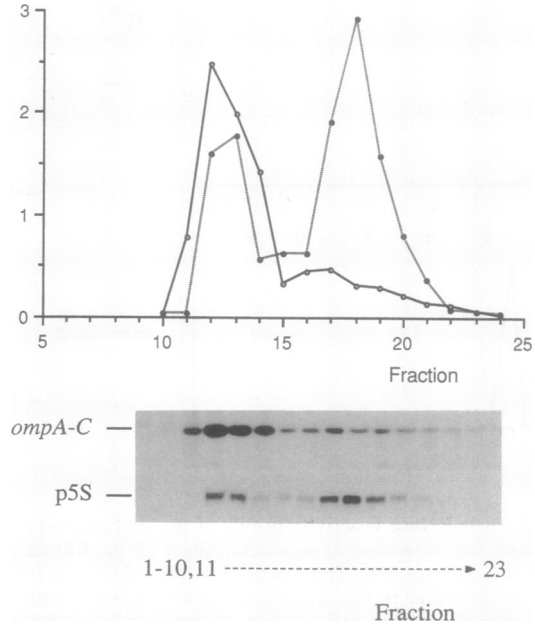

FIG. 3. RNase K and RNase E activities of the fractions from the Mono Q column. Single fractions and pooled fractions of the void fractions (1–6 and 7–10) from the Mono Q column were assayed for RNase K and RNase E activities. A 5-μl aliquot from each fraction and the two void fractions was incubated with the same stoichiometric amounts of radiolabeled *ompA* RNA or 9S RNA (Fig. 1) in separate assays for 30 min. RNA substrates were in excess; only a fraction of the RNA in the assays was converted into cleavage products during incubation. After the reactions, the *ompA* RNA and the 9S RNA were analyzed (in the order of the fractions derived from the column) on separate denaturing 6% polyacrylamide gels. RNA cleavage products ompA-C and p5S were identified by size standards in the gels, which were exposed to x-ray films for the same time period. (*Lower*) The autoradiogram section showing the ompA-C and p5S bands. The fractions are indicated below the autoradiogram. (*Upper*) Signal strength (determined by densitometric evaluation) of the *ompA*-C and p5S bands is plotted against fraction numbers. Dotted line, RNase E activity; solid line, RNase K activity.

K-specific cleavage products (Fig. 2). In comparison with 9S RNA, the cellular concentration of *ompA* mRNA remained almost unchanged (<20% alteration) upon temperature shift of the mutant cells, a result supporting the proposal that the metabolism of the two RNA species is different in the two strains. Most indicative for the activity of RNase K is, however, the ratio of cleavage products to uncleaved *ompA* mRNA (1, 6), which is not affected by the *groEL* mutation (the intensities of *ompA* mRNA and cleavage products were evaluated by densitometry). Thus, the results suggest that a deficient GroEL protein impairs the cleavages in 9S RNA but not those in *ompA* mRNA.

This result, and other features that seem to distinguish RNase E and RNase K activities (8), led us to comparatively follow their separate activities (*ompA* mRNA cleavage and 9S RNA cleavage; Fig. 1) during purification. When cell lysates were made in low salt buffer, we observed that both RNase E and RNase K activities remained in the ribosome pellet after a 100,000 × *g* centrifugation and that the activities could be released from the pellet after washing in high salt buffer. The activities obtained from the ribosome wash were either fractionated using CM-Sepharose followed by DEAE-Sepharose or by a Mono Q column. In all three ion-exchange columns, RNase E and RNase K activities were partially separated from each other (data not shown). In the Mono Q column, RNase E and RNase K activities occur in two peaks of the collected fractions; the first peak contains both RNase E and RNase K activities and the second peak consists mostly of RNase E activity (Fig. 3). Although a complete separation of RNase E and RNase K activities was not achieved, these data further suggest that the two enzymes are not identical.

The fractions containing predominantly RNase E activity were size-fractionated on a Sephadex G-100 column. RNase E activity was eluted with the void fractions, indicating that the enzyme is present in a complex >150 kDa (Fig. 4*A*). Analysis of the void fractions by SDS/PAGE and silver staining showed two prominent protein bands at ≈60 and ≈17 kDa (Fig. 4*B*). The N-terminal sequence of the larger protein (AAKDVKFGNDARVKMLRGVN) and its size identified it as GroEL (32). The N-terminal sequence obtained from the 17-kDa band did not show any significant homology with the predicted sequence of the *ams/rne* gene product (15) or with any other protein sequence in the data base. It is worth noting that there were fainter bands detected on the silver-stained gel migrating above the GroEL position (Fig. 4*B*); their sizes, however, do not match the predicted position of the *ams/rne* (*hmp1*) gene product (180 kDa) (15). We consider the possibility that the RNase E-like activity in the void of the gel filtration was associated with the oligomeric configuration of the ≈800-kDa GroEL complex (18, 19).

If it is correct that GroEL functionally interacts with RNase E, then it should be possible to immunoprecipitate the nuclease activity from *Escherichia coli* extracts with antibodies raised against purified GroEL. Anti-GroEL antibodies were attached to protein A-Sepharose. The Mono Q fractions containing RNase E activity were allowed to bind to the antibodies and the complexes formed were recovered by centrifugation. The supernatant and the rinsed pellet were assayed for RNase E activity with 9S RNA substrate. In Fig. 5*A*, it can be seen that anti-GroEL antibodies are able to precipitate RNase E activity almost completely from the supernatant and that the activity can be recovered in the rinsed and resuspended pellets. It is noteworthy that nonnative proteins that interact with GroEL/Hsp60 have been found to coprecipitate with the chaperonin less efficiently

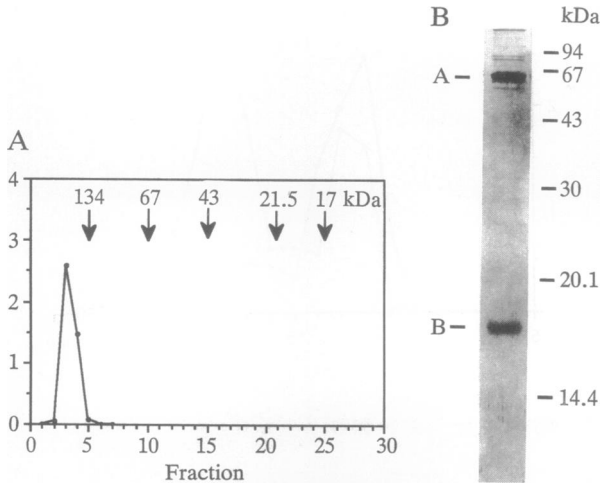

FIG. 4. (*A*) RNase E activity profile of the gel-filtration column. The native size of RNase E was determined by gel filtration on a Sephadex G-100 column. The fractions were assayed for RNase E activity as described in Fig. 2 (processing of 9S RNA into p5S). The relative intensity of the p5S product, determined by densitometric evaluation, is plotted against the fraction number. The size of the protein complex in the active fractions was determined by relating it to standard proteins fractionated in parallel. Arrows at the top indicate positions of the molecular mass standards: dimeric bovine serum albumin (134 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), trypsin inhibitor (21.5 kDa), and myoglobin (17 kDa). (*B*) SDS/PAGE of RNase E preparation. RNase E-containing fractions from the Sephadex G-100 column were separated on a SDS/15% polyacrylamide gel followed by silver staining. Fraction 3 is shown. Molecular mass standards (Pharmacia) are indicated on the right. Bands: A, 60-kDa band identified as GroEL; B, other major band at 17 kDa.

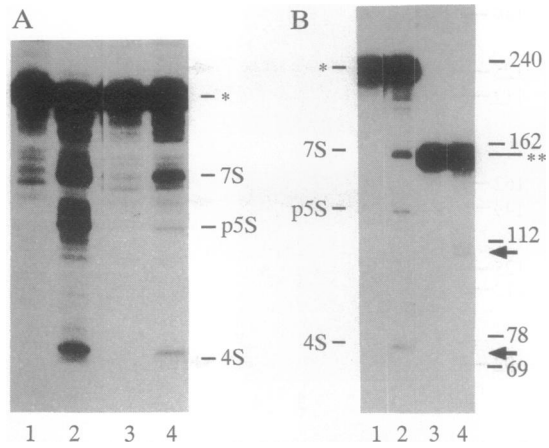

FIG. 5. (*A*) Precipitation of RNase E with anti-GroEL antibodies. Mono Q fractions were incubated with purified anti-GroEL antibodies attached to protein A-Sepharose beads, which were recovered by centrifugation. Aliquots of the supernatant and the resuspended beads were incubated with radiolabeled 9S RNA in RNase E assay buffer and analyzed on a denaturing 6% polyacrylamide gel with material from the control assays. Lanes: 1, 9S RNA incubated without addition of protein (control); 2, 9S RNA incubated with RNase E (peak fraction from Mono Q column shown in Fig. 2); 3, 9S RNA incubated with supernatant obtained from the antibody precipitation; 4, 9S RNA incubated with the resuspended precipitates obtained from the antibody precipitation. The 9S RNA transcript (*) and the RNase E-specific cleavage products are indicated on the right. 4S and 7S, cleavage products obtained when 9S is cleaved only at the a site (Fig. 1); 4S, product derived from the transcription start to the a site; 7S, product derived from the a site to the end of the transcript. (*B*) 9S RNA and *ompA* mRNA cleavage with a GroEL preparation. GroEL (5 μg) was incubated with an equal stoichiometric amount of radiolabeled *ompA* RNA or 9S RNA (Fig. 1) in parallel assays for 60 min. RNA substrates were in excess, as described in Fig. 2. The RNA samples were analyzed on a denaturing 6% polyacrylamide gel and exposed to x-ray film. Sections from the autoradiograph are shown. Lanes: 1, 9S RNA incubated without added protein; 2, 9S RNA incubated with 5 μg of GroEL protein; 3, *ompA* RNA incubated without added protein; 4, *ompA* mRNA incubated with 5 μg of GroEL protein. On the left, the 9S transcript (*) and the RNase E-specific cleavage products are indicated. On the right, the positions (and size in nucleotides) of the radioactive size standards, electrophoresed on the same gel are depicted. The *ompA* mRNA (**) and the two degradation products (arrows) are shown. The two degradation products did not comigrate with the *ompA* cleavage products (Fig. 1, arrows C and D) obtained from a RNase K digestion (data not shown).

than RNase E activity (33, 34). This indicates a comparatively strong attachment of the nuclease activity to GroEL. We also analyzed the amount of GroEL in the two peak fractions of the Mono Q column (Fig. 3) and detected GroEL only in the second peak where RNase E activity was dominant (data not shown). It is possible that the antiserum contains antibodies that bind directly to the putative RNase E. We consider such an interpretation as unlikely, since the antibodies were raised against a purified GroEL protein excised from a denaturing SDS gel. However, even such an interpretation is in favor of a strong association between RNase E and GroEL, as it suggests that RNase E copurifies with GroEL throughout the antigen preparation and is trapped underneath the GroEL band on the denaturing gel.

To further support the data suggesting a strong functional interaction between RNase E activity and GroEL, we probed for RNase E activity in a relatively pure GroEL preparation with <5% contaminants, which was made for chaperonin studies (29). The GroEL preparation indeed contained RNase E activity (Fig. 5*B*), although less than the purest fraction of the RNase E preparation, when the amount of GroEL protein added to each assay is taken in consideration (conversion of 9S RNA into p5S RNA per unit time per mg of protein). This can be explained, assuming that relatively few oligomers in the GroEL preparation contained RNase E activity, since the preparation was enriched for chaperonin activity.

The same stoichiometric amounts of 9S RNA and *ompA* mRNA were incubated with equal protein amounts of the GroEL preparation (Fig. 5*B*). No detectable level of RNase K activity (cleavage in *ompA* mRNA) was found in the GroEL preparation, and the comparatively faint degradation products from *ompA* mRNA seen on the gel after incubation with the GroEL preparation do not comigrate with the RNase K cleavage products, denoted by arrows C and D in Fig. 1 (data not shown). Such degradation products were found in a previous study (35) and may be the result of contaminating exonucleolytic activities in the GroEL preparation. Thus, we conclude that an RNase E activity but no RNase K activity is found in the GroEL preparation.

DISCUSSION

Four lines of evidence support the conclusion that GroEL functionally interacts with an RNase E-like activity; a mutation in the *groEL* gene impeded 9S RNA processing *in vivo*, RNase E activity was precipitated from partially purified extracts with antibodies raised against highly purified GroEL protein, a GroEL preparation purified for chaperonin activity contained substantial amounts of an RNase E-like activity, and GroEL copurified with RNase E activity. The results of previous studies are also compatible with our conclusion that an RNase E-like activity is bound to GroEL. An activity attributed to RNase E was found to fractionate with an apparent molecular mass >1500 kDa in a gel-filtration column (36) and a protein in the range of 60 kDa, as judged from a denaturating gel (7), was identified as a major component in

an RNase E preparation. Although the present study shows that GroEL functionally interacts with an RNase E-like activity, our data do not positively identify the catalytically active components that define RNase E activity. We consider it unlikely that GroEL *per se* is the nuclease, as the specific activity of the GroEL preparation purified for chaperonin studies was lower than that of the purest RNase E fractions. However, such an interpretation needs to be verified by showing that no RNase E activity is found in a GroEL preparation that is free of contaminating proteins.

RNase E is attributed to the *ams/rne* (*hmp1*) gene, which encodes a gene product resembling a filamentous transmembrane protein with multiple functional domains (9, 11–15). We did not find in our RNase E preparation (Fig. 4*B*) components that seem to be identical with the *ams/rne* (*hmp1*) gene product. The apparent absence of the putative RNase E protein may be explained as the result of proteolytic cleavage; RNase E activity may reside in smaller proteolytic fragments derived from the intact protein. Such smaller "RNase E" entities may be obscured by the predominant 60- and 17-kDa bands in the preparation or be identical with the two faint bands with sizes of 70 and 75 kDa, respectively (Fig. 4*B*). Another explanation is that the RNase E-like activity that copurifies with the GroEL protein is different from the RNase E activity attributed to the *ams/rne* (*hmp1*) gene product. The 17-kDa band found in our preparation would then be a strong candidate for the catalytic entity of the RNase E-like activity that functionally interacts with the GroEL protein. Finally, the possibility should not be excluded that GroEL itself, perhaps by the virtue of an as yet undiscovered RNA binding property, may take part in the RNase E-like activity.

The activities of both RNase E (9S RNA processing) and RNase K (*ompA* mRNA cleavage) are reduced in intact cells with mutations in the *ams/rne* gene (9), which is often taken as an argument that the two nucleases are identical. Clearly, however, the motivation to isolate an activity that is specific for cleavage in *ompA* mRNA (RNase K) was based on the finding that, unlike RNase E activity, RNase K activity is not temperature-sensitive *in vitro* when crude extracts from *ams/rne* mutant cells were compared with their congenic counterparts (10). Furthermore, fractionation of extracts from *E. coli* with a mutation in the *ams/rne* gene on a Mono Q column showed three peaks, two unresolved peaks containing RNase E and RNase K activities and a third peak with RNase E activity only. At the nonpermissive temperature, only this third RNase E activity was affected (ref. 4). This result supports our former observation (10) that RNase K activity can be distinguished from a thermosensitive RNase E activity in extracts from an *ams/rne* mutant. In agreement with these studies, we were able to fractionate extracts from cells with the wild-type *ams/rne* gene into two major peaks, the first peak containing RNase E and RNase K activities and the second peak containing only RNase E activity (Fig. 3). In this study we present further evidence suggesting a difference between RNase E and RNase K activities; 9S RNA processing and not *ompA* mRNA cleavage appears to be affected *in vivo* in the *groEL* mutant, and RNase K activity is not detectable *in vitro* in the GroEL preparation that contains an RNase E-like activity. Thus, if RNase E and RNase K are to share the same catalytic component(s), one has to postulate that this component(s) interacts with different auxiliary factors that in turn modulate the cleavage specificity. It is worth remembering in this context that RNase E activity and RNase K activity are inversely affected by shifts in growth rates (8).

As heat shock proteins have been reported to regulate gene expression in response to various forms of cellular stress (37) and RNase E activity shows an immediate response to growth rate changes (8), we speculate that 9S RNA processing is regulated by the interactions between GroEL and the identified RNase E activity.

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