Identification of the dnaQ gene product and location of the structural gene for RNase H of Escherichia coli by cloning of the genes

(mutator/maxicell/insertion mutant)

TAKASHI HORIUCHI, HISAJI MAKI, MASAO MARUYAMA, AND MUTSUO SEKIGUCHI

Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812, Japan

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ABSTRACT  By in vitro recombination we have constructed hybrid plasmids capable of complementing a conditional lethal mutator mutation, dnaQ49, in Escherichia coli K-12. The dnaQ" plasmids consist of a full-length pBR322 DNA and a 1.5-kilobase DNA fragment derived from the E. coli chromosome. Specific labeling of plasmid-encoded proteins by the maxicell method revealed that the 1.5-kilobase insert codes for two proteins, one of whose molecular weight is 25,000 [the 25-kilodalton (kDal) protein] and the other whose molecular weight is 21,000 (the 21-kDal protein). Because insertion of y6 sequence into the dnaQ gene of the plasmid resulted in disappearance of the 25-kDal protein, it was concluded that the 25-kDal protein is the dnaQ gene product. The 21-kDal protein was identified as RNase H on the basis of the following evidence. (i) Cells harboring the dnaQ" plasmids, with or without the y6 insertion in the dnaQ gene, had a 5- to 7-fold higher level of RNase H activity than cells harboring pBR322. (ii) After induction of cells that are lysogenized with dnaQ"-transducing λ phages, RNase H activity increased considerably. A similar high level of RNase H activity was observed with transducing phages whose dnaQ function was inactivated by insertion of a transposon, Tn3, into the gene. (iii) The plasmid-encoded RNase H, labeled with [35S]methionine, was purified in a manner essentially similar to that of the chromosome-encoded enzyme. These results suggest that the dnaQ gene and the structural gene for RNase H, termed gene rnh, are closely linked and located at 5 min on the linkage map.

Mutators are a special class of mutations that render many other genes unstable (1). In Escherichia coli, many types of mutators have been isolated and characterized, mostly by genetic means (2-4). Although speculative interpretations for mechanisms leading to increment mutation frequency by the mutator mutation have been proposed, little is known about the nature of products encoded by mutator genes and their functions.

Recently, we discovered a strong mutator mutation that also caused temperature-sensitive growth and defective DNA synthesis in cells carrying the mutation (5). It was found that the mutation was in a new gene, designated dnaQ, which was located at 5 min on the E. coli genetic map.

By using in vitro recombination techniques we have constructed plasmids carrying the dnaQ gene. The hybrid plasmids contain a 1.5-kilobase (kb) insert of E. coli DNA that codes for two proteins whose molecular weights are 25,000 and 21,000. By biochemical and genetic analyses, the 25-kilodalton (kDal) protein was identified as the dnaQ gene product and the 21-kDal protein, as RNase H. It was suggested that the dnaQ gene and the structural gene for RNase H are adjacent on the E. coli chromosome.

MATERIALS AND METHODS

Bacteria, Plasmids, and Phages. All bacterial strains used in these experiments are derivatives of E. coli K-12. Strains KH1171 (F-, recA1 dnaQ49 strA) and KH1188 (Hfr, dnaQ49) were isolated in this laboratory. Strain C5R603 (F-, utrA6 recA1 phr-1) is a gift of D. Rupp and was used in maxicell experiments (6). Strain KP370 (F-, recA1), provided by T. Miki, was used in y6 insertion experiments (7). The vector plasmid pBR322 (8) and the vector phage λgtAC (9) were furnished by Y. Sakaki and Y. Nakamura, respectively.

Construction of Hybrid Plasmids. F- strains harboring the ColE1 hybrid plasmids in the Clarke and Carbon colony bank (10), provided by K. Ueda, were screened by conjugation. Cells harboring pLC28-22 and pLC34-20 were found to complement the dnaQ and dnaE defective mutants, respectively. The plasmids and their derivatives were isolated (10) and digested with EcoRI. The DNA fragments were inserted into the EcoRI site of pBR322. Details of the construction and properties of the hybrid plasmids will be published elsewhere. Transformation and transfection were performed as described by Mandel and Higa (11).

Transposition of the y6 Sequence to Plasmids. The y6 sequence was inserted into plasmids according to the method of Guyer (7). Strain KP370 (F-, recA1) was transformed with the dnaQ" plasmids, into which the y6 was to be inserted, and the transformants were subsequently conjugated with strain KH1171 (F-, recA1 dnaQ49 strA). Because only the plasmids that received the y6 from F factor can be transferred to the recipient cells (7), the recombinants showing ampicillin- and tetracycline-resistance characters were selected and their plasmids were analyzed. The existence of the y6 sequence (5.7 kb) in the plasmids was confirmed by electrophoresis of the DNA after EcoRI digestion. Some of the plasmids with the y6 sequence exhibited dnaQ- character, probably due to insertion of the y6 inside the dnaQ gene.

Construction of Hybrid Phages. The dnaQ"-transducing λ phage, designated AdnaQ+, was constructed by recloning the 1.5-kb DNA fragment derived from pMM5, which carries the dnaQ gene (see Table 1), into a vector phage, λgtAC (9). To transpose Tn3 to the AdnaQ+, the phage was propagated in cells that harbor pKY2533 (ColE1: Tn3). Progeny phages that had acquired ampicillin resistance, conferred by Tn3 (12), were isolated and their DNAs were analyzed.

Labeling of Plasmid-Encoded Proteins. The maxicell method of Sancar et al. (6) with a minor modification (13) was used. Bacteria were grown at 37°C in M9 medium supplemented with 1% Casamino acids and 0.2% glucose. When OD at 660 nm reached about 0.4 (3x10^8 cells per ml), the culture was irra-

Abbreviations: kb, kilobase pair(s); kDal, kilodalton(s).
diated with ultraviolet light (60 J/m²). After the culture was shaken for 1 hr, D-cycloserine was added to give a final concentration of 100 μg/ml and the culture was shaken overnight at 37°C. One milliliter of the culture was centrifuged: the cells were washed and resuspended in 1 ml of M9 supplemented with 25 μg each of 18 amino acids other than methionine and cysteine. [35S]Methionine (1000 Ci/mmol; 1 Ci = 3.7 × 10¹² becquerels) was added to give 82 μCi/ml, and the proteins synthesized under these conditions were labeled for 1 hr at 37°C. The labeled cells were centrifuged, washed, resuspended in 0.1 ml of a buffer, and heated for 2 min. The sample (25 μl) was applied to 12.5% NaDodSO₄/polyacrylamide gel.

Assay of RNase H Activity. The procedure for preparation of cell extract was essentially the same as described by Wickner et al. (14). RNase H activity was assayed according to Berkoener et al. (15). The reaction mixture contained 3 μM poly(A) (483 cpm/μmol; 40–140 nucleotide residues) hybridized with 10 μM poly(dt), 40 mM Tris-HCl (pH 7.7), 4 mM MgCl₂, 1 mM dithioerythritol, bovine serum albumin at 30 μg/ml, 4% (vol/vol) glycerol, and an extract. The reaction ran for 20 min at 30°C, and then the radioactivity converted into acid-soluble form was determined. One unit of activity was defined as the amount of enzyme producing 1 nmol of acid-soluble material in 20 min at 30°C.

Analysis of RNase H. E. coli CSR603 harboring pMM5 or pBR322 (60 ml each) was labeled with [35S]methionine (1000 Ci/mmol; 40 μCi/ml) by the maxicell method. The procedure and the conditions were as described in a preceding section except that the dose of ultraviolet irradiation was 30 J/m². The labeled cells were collected by centrifugation, washed, and frozen in liquid nitrogen. The cells were thawed, resuspended in 2 ml of 2.5 M NaCl/1 mM EDTA/1 mM 2-mercaptoethanol/20 mM Tris-HCl, pH 7.4, and disrupted by sonication. The crude extract (1.5 ml), polyethylene glycol 6000 was added to a final concentration of 10%, and the mixture was centrifuged. The supernatant fluid was dialyzed against buffer C (16) and 1 ml of the dialysate was applied to a column of phosphocellulose (2 ml). The column was washed with 4 ml of buffer C and eluted with a linear gradient of KCl (0–0.8 M) in buffer C. Fractions (20 drops each) were collected and assayed for RNase H activity. Fractions at or around a peak of the enzyme activity were concentrated and subjected to NaDodSO₄/polyacrylamide gel electrophoresis followed by staining and fluorography.

Other Methods. Polyacrylamide gel electrophoresis in the presence of NaDodSO₄ was essentially the procedure of Laemmli (17). Fluorography was done by soaking the stained gel for 1 hr in ENHANCE (New England Nuclear) and for 1 hr in water, drying, and exposing to x-ray film (Kodak XR-1) at −70°C. Protein was determined by the method of Lowry et al. (18).

RESULTS

Construction of Plasmids Carrying the dnaQ gene. Hybrid plasmids capable of complementing dnaO49 mutation were made by in vitro recombination using a multicopy plasmid, pBR322, as vector. Both high mutability and conditional lethality of the dnaQ mutant were almost completely suppressed by introduction of the dnaQ+ plasmids into the cells. Similarly, a plasmid carrying the dnaE gene was constructed, which served as a control.

One of the dnaQ+ plasmids, pMM5, carried a 1.5-kb EcoRI fragment derived from E. coli chromosome; the other, pMM4, had a 4.5-kb EcoRI fragment (Table 1). The 4.5-kb fragment of pMM4 was further divided into a 1.5-kb fragment and a 3-kb fragment by EcoRI treatment. Thus, the dnaQ gene seems to reside on the 1.5-kb insert of the hybrid plasmids. On the other hand, pMM1, which complements the dnaE but not the dnaQ defect of recipient cells, had a 9-kb EcoRI fragment.

Identification of the dnaQ Gene Product. To identify the dnaQ gene product, proteins encoded by the dnaQ+ plasmids were specifically labeled by the maxicell method developed by Sancar et al. (6) and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. Besides two known plasmids encoded proteins (25 and 37 kDal), pMM5 produced two specific proteins, a 25-kDal protein that comigrated with 25-kDal chymotrypsinogen A and a 21-kDal protein that ran slightly faster than 21.5-kDal soybean trypsin inhibitor (Fig. 1). The corresponding bands were found also in pMM4 (data not shown).

To determine which protein is specified by the dnaQ gene, we introduced the y8 sequence into pMM5. Two types of plasmids with y8 insertion were selected: pMM5::y8(dnaQ+), which still complements the dnaQ mutation, and pMM5::y8(dnaQ−), which does not complement the mutation (Table 1). Then, proteins produced by these plasmids were analyzed by gel electrophoresis. pMM5::y8(dnaQ−) produced the two proteins originally found in pMM5 but pMM5::y8(dnaQ−) lacked the 25-kDal protein (Fig. 1, lanes D and E). It is likely,
therefore, that the dnaQ gene codes for a protein whose size is approximately 25 kDal.

Increased Level of RNase H Activity in Cells Harboring dnaQ* Plasmids. In a search for an enzyme activity related to the dnaQ phenotype we noticed that an extract of cells that harbor the dnaQ* plasmids contained a higher level of RNase H activity than that of normal cells. RNase H activity of cells harboring pMM4 or pMM5 was 5- to 7-fold that of cells harboring pBR322 (Table 2). RNase H activity in cells harboring pMM1, which carries the dnaE gene but not the dnaQ gene, was normal.

In these experiments, 3H-labeled poly(A) hybridized with unlabeled poly(T) was used as substrate to assay the enzyme activity. When [3H]poly(A) alone was used no difference among the extracts was observed. Moreover, RNase H activity in cells harboring dnaQ* plasmids was sensitive to N-ethylmaleimide, as was observed with the enzyme in normal cells (15). From these results it was suggested that a gene that controls RNase H activity is present in the dnaQ* plasmids.

To confirm this, we recloned the 1.5-kb DNA into λ phage vector. Fig. 2 shows one of the representative results. After induction of cells that are lysogenized with dnaQ*-transducing phage (AdnaQ*), RNase H activity increased almost linearly. The level at 3 hr of incubation was about 8 times the initial level.

![Graph showing increase in RNase H activity](image)

**Table 2. RNase H activity in cells harboring various plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>E. coli gene on plasmid</th>
<th>Specific activity, units/mg protein</th>
<th>Ratio to pBR322</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBR322</td>
<td>—</td>
<td>17.9</td>
<td>1.0</td>
</tr>
<tr>
<td>pMM4</td>
<td>dnaQ</td>
<td>102.6</td>
<td>5.7</td>
</tr>
<tr>
<td>pMM5</td>
<td>dnaQ</td>
<td>120.1</td>
<td>6.7</td>
</tr>
<tr>
<td>pMM1</td>
<td>dnaE</td>
<td>16.3</td>
<td>0.9</td>
</tr>
<tr>
<td>None</td>
<td>—</td>
<td>4.4</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* Strain CSR603 was used as host for these plasmids.

In the case of control λ phage, no such increase was observed.

**Table 3. RNase H activity in cells harboring plasmids with y8 insertion**

<table>
<thead>
<tr>
<th>Plasmid*</th>
<th>dnaQ genotype of plasmid</th>
<th>Specific activity, units/mg protein</th>
<th>Ratio to pBR322 : y8</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBR322 : y8</td>
<td>None</td>
<td>21.2</td>
<td>1.0</td>
</tr>
<tr>
<td>pMM4</td>
<td>dnaQ*</td>
<td>134.9</td>
<td>6.4</td>
</tr>
<tr>
<td>pMM4 : y8 (dnaQ*)</td>
<td>dnaQ*: y8</td>
<td>182.0</td>
<td>8.6</td>
</tr>
<tr>
<td>pMM5 : y8 (dnaQ*)</td>
<td>dnaQ*: y8</td>
<td>167.1</td>
<td>7.9</td>
</tr>
<tr>
<td>pMM5 : y8 (dnaQ*)</td>
<td>dnaQ*: y8</td>
<td>154.0</td>
<td>7.3</td>
</tr>
</tbody>
</table>

* The host for the plasmids was strain CSR603.

In the case of control λ phage, no such increase was observed.

**Table 4. RNase H activity in induced AdnaQ* lysogens**

<table>
<thead>
<tr>
<th>Phage*</th>
<th>dnaQ genotype on phage</th>
<th>Specific activity, units/mg protein</th>
<th>Ratio to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>10.6</td>
<td>1.0</td>
</tr>
<tr>
<td>AdnaQ*</td>
<td>dnaQ*</td>
<td>47.0</td>
<td>4.4</td>
</tr>
<tr>
<td>AdnaQ(Tn3)-6</td>
<td>dnaQ*: Tn3</td>
<td>88.5</td>
<td>8.3</td>
</tr>
<tr>
<td>AdnaQ(Tn3)-3</td>
<td>dnaQ*: Tn3</td>
<td>86.0</td>
<td>8.1</td>
</tr>
</tbody>
</table>

KH1188(ACI857 Qam) was used as host for phages. All phages carried CI857 and Qam mutations in addition to the dnaQ allele. Extracts were prepared from cells incubated for 3 hr after heat induction.
Fig. 3. Resolution of RNase H by phosphocellulose chromatography. Extracts of cells, whose plasmid-encoded proteins were labeled with [35S]methionine, were applied to a phosphocellulose column, and the proteins were eluted by a linear gradient of KCl. RNase H activity was assayed and the fractions at or around a peak, indicated by a bracket, were used for further analyses, as shown in Fig. 4. (A) CSR603(pBR322); (B) CSR603(pMM5).

DISCUSSION

We have isolated and characterized a conditional lethal mutator (dnaQ49) mutant in E. coli K-12 (5). All the dnaQ-defective mutants thus far examined show the following pleiotropic phenotype (refs. 5 and 20; unpublished results): (a) strong mutator activity; (b) inability to produce colonies at 44.5°C in NaCl-free amide gel electrophoresis. Fig. 4 A and B present patterns of proteins present in each of the enzyme fractions from the two types of cells. In the peak fractions (fractions 15 and 16) of CSR603 (pMM5) there was a distinct band which corresponds to the 21-kDal protein, whereas only a faint band was observed at the corresponding position of the CSR603(pBR322) sample.

Clearer resolution of the protein was obtained with fluorography (Fig. 4 C and D). A band for the 21-kDal protein was found in fractions 15 and 16 of CSR603(pMM5) but not in the corresponding fractions of CSR603(pBR322). These results indicate that the 21-kDal protein encoded by the dnaQ+ plasmid is indeed RNase H.

Fig. 4. Analysis of RNase H fractions by NaDodSO4/polyacrylamide gel electrophoresis. Enzyme fractions indicated by brackets in Fig. 3 were concentrated by lyophilization and applied to 12.5% NaDodSO4/polyacrylamide gels. After electrophoresis, the gels were stained with dye and dried (A and B). The gels were then exposed to x-ray films for 16 days for fluorography (C and D). The numbers above the gels correspond to fraction numbers in Fig. 3: C, sample of whole cells of CSR603(pMM5); M, molecular weight markers; arrows, pMM5-specific 21-kDal protein. (A and C) Proteins of CSR603(pBR322); (B and D) proteins of CSR603(pMM5).
L-broth plates; (c) decreased level of DNA synthesis under the restrictive conditions; (d) increased sensitivity to DNA-intercalating reagents or inhibitors of DNA gyrase, such as 5-aminooacridine and novobiocin; and (e) enhancement of thermosensitivity of dnaE mutants, having mutations in the gene for DNA polymerase III. These properties of the mutants suggested that the dnaQ product might be a component of the cellular DNA synthesizing machinery or a factor stabilizing the chromosome structure and that its defectiveness may cause a decrease in the fidelity of DNA replication.

It seemed to be important to identify the dnaQ gene product and to elucidate its function. As a first step in this direction we constructed plasmids carrying the dnaQ gene by in vitro recombination. The hybrid plasmids contain a 1.5-kb insert of E. coli DNA that codes for two proteins; one of the proteins, with a molecular weight of 25,000, was identified as the dnaQ gene product.

Among many proteins known to be involved in DNA replication and related processes, there are some whose molecular weights are around 25,000. These include subunit ε of the DNA polymerase III core enzyme (21) and discriminatory factor β (22). There is a possibility that the dnaQ gene may encode one of these proteins. Availability of the dnaQ+ plasmids makes it possible to examine such a possibility.

In the course of analysis of the dnaQ+ plasmids, we found that a protein with a molecular weight of 21,000 also was encoded by the 1.5-kb DNA insert. The protein was identified as RNase H on the basis of the following criteria (15, 16, 19); (a) the molecular weight is identical to that RNase H of E. coli; (b) the enzyme specifically hydrolyzes RNA in a DNA-RNA hybrid, and the activity is inhibited more than 90% by N-ethylmaleimide; and (c) the enzyme is purified in a manner similar to that of E. coli RNase H. Thus, it is evident that the gene for RNase H resides on the DNA fragment. Because the radioactive 21-kDal protein was produced under conditions such that only plasmid-encoded proteins are labeled, it was concluded that the gene is the structural gene rather than the regulatory gene. According to the current convention for nomenclature of genes (23), the gene is to be named rnh.

The sum of the molecular weights of the two proteins, the dnaQ gene product and RNase H, is approximately 46,000, which is just within the coding capacity of the cloned DNA fragment. From the space limitation it seems that there is no other gene in intact form in the fragment, implying that dnaQ and rnh are adjacent. The dnaQ gene has been mapped at 5 min on the E. coli chromosome (5, 23). The rnh gene must lie within 0.1 min from the dnaQ locus.

Recently, Carl et al. (24) isolated an E. coli mutant with a moderately low level of RNase H activity (30% of parental level). The mutation (rnh) has been mapped at 5.1 min on the E. coli chromosome. Because this is very close to the rnh locus determined in the present study, it seems that the mutation arose in the structural gene for RNase H.

From the mode of action of RNase H it has been supposed that the enzyme might be responsible for removing RNA that acts as a primer for DNA synthesis (25, 26). Recently, Ito and Tomizawa (27) reported that RNase H has a specific role in the process of initiation of CoeI DNA synthesis in vitro. However, definite evidence that RNase H is involved in cellular DNA replication has not been available. The plasmids carrying the rnh gene should be useful in solving this problem.

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