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Isolation and Mapping of a Mutation in *Escherichia coli* with Altered Levels of Ribonuclease H

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A mutant of *Escherichia coli* with altered levels of ribonuclease (RNase) H was isolated after mutagenesis with ethyl methane sulfonate. A procedure for assaying RNase H in partially purified extracts was used to screen approximately 1,500 colonies for variations in RNase H activity. Confirmation of a lower level of RNase H in the mutant was accomplished by analysis of RNase H in sodium dodecyl sulfate-polyacrylamide gels. By Hfr, F', and P1 transduction mapping, the genetic locus responsible for the lower levels of RNase H was located at 5.1 min on the *E. coli* chromosome. This mutation (*rnh*) represents a new locus on the *E. coli* chromosome. The only phenotypic characteristic of this mutation which has been observed to date is the lower level of RNase H (30% of parental values).

Shortly after the discovery of RNase H (an enzyme that specifically degrades RNA that is hydrogen bonded to a complementary DNA strand) in calf thymus (29), a number of investigators suggested that such an enzyme might be responsible for removing RNA which acts as a primer for DNA synthesis (18, 33). Since the discovery of RNase H in calf thymus, RNase H has been found in a wide variety of organisms (8, 9, 12, 14, 19, 22, 25, 26, 28, 37). In fact, more than one RNase H has been described in several organisms (5, 7, 38). RNase H's with either exonucleolytic activity (e.g., retrovirus reverse transcriptase RNase H [19, 22] which produces mainly dinucleotides) or endonuclease activity (e.g., cellular enzymes which produce a rather unique distribution of oligonucleotides as degradation products [19; R. Crouch, unpublished data]) have been observed.

In *Escherichia coli* at least three different enzymes are known to degrade RNA in a DNA-RNA hybrid. DNA polymerase I (19, 22) and exonuclease III (19, 35) can degrade either the DNA or the RNA of DNA-RNA hybrids. In contrast, RNase H degrades only the RNA component of the hybrid and thus is the only one of these enzymes that is a ribonuclease. Analysis of the participation of each of these enzymes in various cellular processes is possible through the study of mutations in each of these enzymes. Exonuclease III is a dispensable gene under normal laboratory conditions (36). Mutants of DNA polymerase I are of two types: (i) those that are defective in polymerization (10) yet retain the 5'→3' exonuclease activity (21), and (ii) those that alter the 5'→3' exonuclease (20, 27). Mutants defective in polymerase activity were used to indicate that DNA polymerase I is not responsible for normal replication (10). On the other hand, mutations in the 5'→3' exonuclease activity suggest that this activity is necessary for cellular replication (20, 27). Although polymerization by DNA polymerase I is not required for normal DNA replication, strains lacking the polymerase activity of DNA polymerase I are unable to replicate ColE1. Recently, Itoh and Tomizawa (17) reported that RNase H stimulates initiation of ColE1 DNA synthesis in vitro even though the polymerization in their system requires DNA polymerase I, one of the enzymes with the potential to supplant RNase H. Itoh and Tomizawa suggest that the more efficient initiation results from the cleavage of the RNA to generate a specific, more efficient end for priming. Such a selective degradation of RNA in DNA-RNA hybrids has been observed (M. Dirksen and R. Crouch, unpublished data). Furthermore, RNase H has been reported to be a factor involved in discrimination of replication of single-stranded DNA viruses (31, 32).

No mutation that is known to alter the activity of RNase H is yet available to ascertain the relationship of RNase H to a possible function in DNA replication. As a first step toward this goal, we have isolated a mutant with altered levels of RNase H and have mapped the genetic locus responsible for the modified levels of RNase H.

MATERIALS AND METHODS

Bacterial and phage strains. Strains of *E. coli* K-12 used in this study are listed in Table 1. P1 C/R 100 Km was obtained from Ed Hafener. A ColE1 derivative carrying ampicillin resistance and λ *int* was a generous gift of Aki Kikuchi.

Reagents and materials. [α-^{32}P]ATP was purchased from New England Nuclear Corp. Hexokinase was obtained from Sigma Chemical Co., and *Micrococcus luteus* polynucleotide phosphorylase was a generous gift of O. Uhlenbeck. Lysozyme, bovine serum albumin (fraction V), calf thymus DNA, deoxycholate, ethyl methane sulfonate, and dithiothreitol were from Sigma Chemical Co. Polydeoxyribosylthymine [poly(dT)] was from Miles Laboratories, Inc. Charcoal-impregnated paper was a product of Schleicher & Schuell Co. Plastic microtiter dishes and covers were obtained from Cook Engineering Co. (Dynatech Corp.).

Preparation of RNase H substrate. [^{32}P]φX DNA-RNA and ^{32}P-labeled polyriboadenylate·polydeoxyribosylthymine {[^{32}P]poly(rA)·poly(dT)} were prepared as described previously (8). For some preparations of [^{32}P]poly(rA)·poly(dT), [α-^{32}P]ADP was polymerized with *M. luteus* polynucleotide phosphorylase in the following reaction: a total of 5 ml contained 40 mM glycylglycine, pH 9, 1 mM [α-^{32}P]ATP (53 μCi/μmol), 4 mM dithiothreitol, 4 mM $MgCl_2$, and 8 mM glucose. The reaction was initiated by the addition of 88 μg of hexokinase (495 U/mg), which converts ATP to ADP. After 30 min at 37°C, polymerization was begun by the addition of 75 μl of *M. luteus* polynucleotide phosphorylase, and incubation was continued for an additional 60 min. A 0.25-ml amount of 10% sodium dodecyl sulfate (SDS) terminated the reaction. Precipitated proteins were removed by centrifugation at 4°C for 10 min at 5,000 rpm in the SS-34 rotor of the Sorvall centrifuge. The resulting [^{32}P]poly(A) was separated from unincorporated precursor by passage over a G-50 Sephadex column (1 by 60 cm) which had been previously equilibrated with 0.1 M NaCl plus 0.01 M Tris-HCl, pH 7.9. Peak fractions were combined, yeast tRNA was added (10 μg/ml), and 2 volumes of ethanol was used to precipitate the poly(A). After 2 h at −20°C, the poly(A) was collected by centrifugation at 0°C for 30 min at 10,000 rpm.

Media. Luria broth (L broth) and M940 medium are described in Miller (24). Zubay Super Broth is as defined by Zubay (40).

Mutagenesis. To 5 ml of a stationary-phase culture of KS351 grown in L broth, 0.15 ml of ethyl methane sulfonate was added. After 10 min, cells were collected by centrifugation at 5,000 × *g* for 5 min and washed with 5 ml of phosphate-buffered saline (7 g of Na_2HPO_4, 3 g of KH_2PO_4, 4 g of NaCl, and 1 ml of 1 M $MgSO_4$ in 1 liter of water). The cells were suspended in 5 ml of phosphate-buffered saline, diluted 20-fold in L broth, and grown to a concentration of 6 × 10^8 to 7 × 10^8 cells per ml (approximately 10 h). Aliquots (0.1 ml) were diluted into 0.9 ml of L broth, mixed 1:1 with 20% glycerol, and frozen at −70°C. About 15% of the colonies that would grow on L agar failed to grow on minimal plates after mutagenesis.

Preparation of cell extracts. The general procedures of growth, storage, and preparation of extracts for use in mass screening techniques have been given in detail by Milcarek and Weiss (23). Individual colonies from mutagenized cells were grown in microtiter tubes to stationary phase in 50 μl of Zubay super broth. Pellets were collected by centrifugation, the supernatant was removed, and lysis was accomplished by the addition to each well of 25 μl of a 4:1 mixture of solution A plus solution B (see below for the contents of solutions A through E). The cells were suspended by blending in a Vortex mixer and left on ice for 15 min. To each well of the tray 25 μl of solution C was

TABLE 1. *E. coli strains*

Strain	Genotype/phenotype	Source
KS351	Hfr H *thi lacY482 rha*	Konrad (20)
PC238	*argH pyrF lac gal trp his ilv purC* thi *mal xyl mtl* Str^r *ton thy drm*	S36, S. Brenner
PC430	PC238 Spc^r	Spontaneous mutation from this laboratory resistant to 100 μg of spectinomycin per ml
FB2	KS351 *rnh*	Ethyl methane sulfonate mutagenesis of KS351
KLF1/AB2463	F101/*thr-1 leuB6 thi-1 argE3 his-4 proA2 recA13 lacY1 galK2 mtl-1 xyl-5 ara-14 rpsL31 tsx-33* λ$^-$ *supE44*	CGSC[a] (4250)
KLF4/AB2463	Same as KLF1/AB2463 but F104 in place of F101	CGSC (4251)
E126	F+ *nadC13 mel-1 supE57 tyrT58*	CGSC (5080)
CD4	HfrC *metB1 proA3 relA1 metD88 lac-3 malA36 tsx-76* λ^r λ$^-$	CGSC (5096)
E293	*thr leu thi lac thy* SuII$^+$ *tonA* Str^r *dnaE293 met*	Wechsler and Gross (34)
χ354	Δ(*proA,B-lac*)*111*	R. Weisberg (from R. Curtiss III)
LE30	*mutD5* Str^r Azi^r *galU95*	L. Enquist (E. Cox)

[a] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

then added, and incubation was continued for 10 min. To freeze-thaw the extracts, the trays were placed in a slurry of dry ice-ethanol and then placed in a water bath at room temperature. When thawing was complete, the trays were returned to an ice-water bath. DNA and RNase H were precipitated by the addition of 100 μl of solution D per well and incubated on ice for 10 min. The trays were then centrifuged at 3,000 rpm for 10 min, and the supernatant was removed. Elution of RNase H was accomplished by the addition of 50 μl of solution E to each of the trays. After the contents were blended in a Vortex mixer, the trays were centrifuged for 10 min at 3,000 rpm. At this point, the supernatants can be frozen at −20°C until assayed. Screening could be accomplished on 400 colonies per day by this procedure. For mapping experiments, the procedure for preparation of cell extracts was essentially as described for mass screening. In the case of mapping experiments, 5-ml cultures (in L broth) were centrifuged for 10 min at 3,000 rpm, 0.5 ml of solution A was added, and the cells were suspended by blending in a Vortex mixer. After 15 min at 0°C, 0.125 ml of solution B was added, and incubation was continued for an additional 5 min. Solution C (0.625 ml) was added, and the tubes were transferred to room temperature until a clear, viscous lysate was obtained (3 to 5 min). A 1.75-ml amount of solution D was then mixed into the lysate. After 10 min, the lysate was centrifuged for 10 min at 10,000 rpm. RNase H was eluted from the pellet by the addition of 0.2 ml of solution E, and the solution was freed of precipitant by centrifugation in a Beckman microfuge for 5 min. In addition to assaying for RNase H, the protein concentration of each extract was determined by using the Bio-Rad protein assay of Bradford (4).

Assay of RNase H. Three assays for RNase H were used. Type A was used for mass screening. An amount of extract from wild-type cells sufficient to degrade 60% of the poly(rA)·poly(dT) substrate in 30 min at 42°C was used (about 4 μl for extracts prepared as described). Each well of a microtiter tray (on ice) received 50 μl of 40 mM Tris-HCl, pH 7.6, 4 mM $MgCl_2$, 1 mM dithiothreitol, 30 μg of bovine serum albumin per ml, 27 μM [^{32}P]poly(rA), 34 μM poly(dT), and 4% glycerol {[^{32}P]poly (rA) and [^{32}P]poly(dT) had been previously incubated at 37°C to form the hybrid}. Trays were incubated for 30 min at 42°C and placed on ice. Then 25 μl of denatured calf thymus DNA (1.5 mg/ml) and 25 μl of 30% Cl_3CCOOH were added. The trays were centrifuged for 30 min at 0°C. Cl_3CCOOH-soluble products from 25-μl aliquots were bound to charcoal-impregnated paper (Schleicher & Schuell Co.). The paper was processed for autoradiography as described by Milcarek and Weiss (23). Colonies with low apparent activity of RNase H were rechecked in a similar assay but with a scintillation counter to quantitate the extent of digestion.

Type B employed [^{32}P]φX DNA-RNA as substrate. Reaction mixtures of 25 μl contained 10 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 50 mM NaCl, and 3.7 pmol (ribonucleotides) of [^{32}P]φX DNA-RNA (3,500 cpm/pmol). For mapping experiments, the media varied so that the final protein concentration in the extracts was very different from one extraction to another. Consequently, protein determinations were used to adjust the size of aliquot to be assayed (0.2 μg of protein per 25-μl assay). Acid solubility was determined as described previously (13).

Type C was based on the reconstitution of RNase H in SDS-polyacrylamide gels containing [^{32}P]poly(rA)·poly(dT). The procedure is briefly outlined in Fig. 1 and will be described in detail elsewhere (manuscript in preparation). Reconstitution was done at 42°C.

Solutions. Reagent A was 10 mM Tris-HCl, pH 7.9, 0.1 M NaCl, and 25% (wt/vol) sucrose. Reagent B was 0.3 M Tris-HCl, pH 7.9, 0.1 M disodium EDTA, and lysozyme (4 mg/ml). Reagent C was 1 M NaCl, 0.02 M EDTA, pH 7.0, and 0.08% deoxycholate. Reagent D was 0.157 M NaCl, 0.01 M dithiothreitol and 17% (wt/vol) polyethylene glycol 6000. Reagent E was 2 M NaCl, 0.01 M Tris-HCl, pH 7.9, 0.01 M dithiothreitol and 5% (wt/vol) polyethylene glycol 6000.

RESULTS

Detection of mutation. After mutagenesis of *E. coli* KS351 with ethyl methane sulfonate, colonies were chosen at random and assayed by procedure A (see Materials and Methods) for RNase H. Approximately 1,500 colonies were picked, and after a more sensitive assay, three were chosen for further study. The SDS-polyacrylamide gel reconstitution assay (similar to that shown in Fig. 1) indicated that of these three only one seemed to have altered levels of RNase H. Assays of extracts from FB2 showed consistently lower levels of RNase H (Table 2) than the parental strain or other strains tested and much lower rates of degradation of poly(rA)·poly(dT) in SDS-polyacrylamide gel assays (Fig. 1).

Genetic mapping. (i) Hfr mapping. Since the RNase H mutation was initially isolated in an Hfr strain, it was necessary to transfer this mutation to a multiply auxotrophic female strain to facilitate genetic mapping of the locus. Accordingly, a spontaneous spectinomycin-resistant mutant (PC430) of a multiply auxotrophic female strain (PC238) was used in a cross with FB2. Ara$^+$ Str^r and Arg$^+$ Str^r recombinants were tested for inheritance of low levels of RNase H by procedure B in Materials and Methods. Of the 23 Arg$^+$ recombinants 4 were low in RNase H activity, whereas 16 of 20 Ara$^+$ recombinants exhibited low levels of RNase H. These results suggested that the *rnh* locus is relatively closer to the *ara* locus than to the *argH* locus.

(ii) F′ mapping. To map the locus more closely, an Arg$^+$ Ara$^-$ RNase H$^-$ recombinant (auxotrophic for a variety of markers) was crossed with a variety of F′ donor strains by using spectinomycin to select against the streptomycin-resistant F′ donors. This approach assumed that the RNase H mutation in FB2 was recessive to the wild-type allele and that mero-

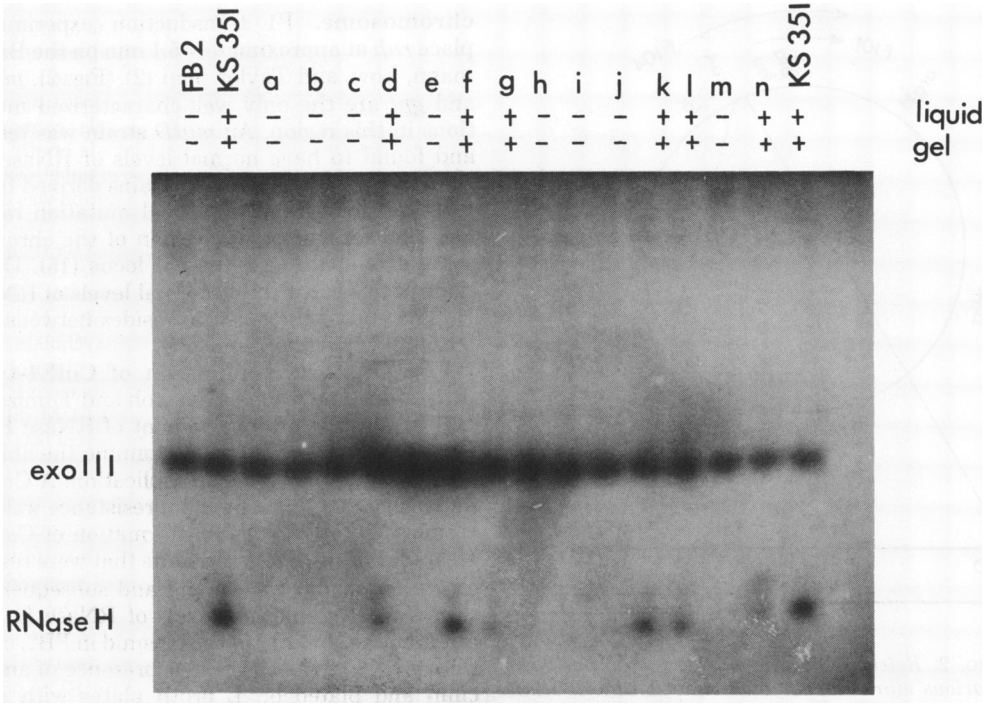

FIG. 1. *SDS-polyacrylamide gel assay of RNase H. Lysozyme-EDTA extracts of 14 metD$^+$ transductants and KS351 (parent) and FB2 (mutant) strains were electrophoresed in a 15% SDS-polyacrylamide gel which contained [^{32}P]poly(rA)·poly(dT). After electrophoresis, gels were soaked in several changes of 0.05 M NaCl–0.01 M Tris, pH 7.9–10 mM $MgCl_2$–1 mM 2-mercaptoethanol at 42°C. Autoradiography results in a film exposed uniformly by the [^{32}P]poly(rA)·poly(dT), except in those areas in which the substrate has been degraded and the radioactivity has leached from the gel. Such degradation appears as a light area in a dark background. For ease of visualization, a contact print is shown here; positive degradation appears as a dark area on a light background. ExoIII marks the location in the gel to which exoIII would migrate. We have not shown that the band of activity seen in these gels is exoIII [see text for documentation that exoIII degrades poly(rA)·poly(dT)]. RNase H marks the position seen for highly purified RNase H in these gels. Protein concentrations of the crude extracts were measured, and volumes of extracts that were electrophoresed were adjusted to ensure constant protein concentrations. Correspondence between liquid and gel assays of RNase H is shown by the plus and minus signs.*

diploid strains carrying an F′ factor with the RNase H$^+$ allele would show at least wild-type levels of RNase H. It was found that F′ 104 restored wild-type activity whereas F′ 101 did not (Fig. 2). Both F′ 101 and F′ 104 were maintained in an RNase H$^-$ Ara$^-$ Arg$^+$ strain by growth in media with arabinose as the carbon source. Six colonies were isolated from arabinose-MacConkey plates that were unable to utilize arabinose as a carbon source. Presumably, these colonies arose from the spontaneous loss of the F′ 104. Cultures from each of these colonies were tested for RNase H by procedure B in Materials and Methods. Each of the six isolates exhibited the mutant level of RNase H, whereas the control merodiploid showed a high (wild-type) level of RNase H. The F′ mapping, thus, located *rnh* between the termini of F′ 101 and F′ 104 (i.e., between *leu* and *lac*).

TABLE 2. *Comparison of RNase H activity in partially purified extracts*[a]

Strain	Substrate degraded (pmol)	Protein (μg/assay)	pmol of φX DNA-RNA/μg of protein
FB2	0.15	0.12	1.25
KS351	0.53	0.13	4.1

[a] Assays were performed on partially purified extracts by method B (see text); incubations were for 30 min at 37°C.

P1 transduction. To confirm the provisional assignment of the *rnh* locus, P1-mediated crosses were carried out with a variety of markers in this region. The results are shown in Table 3. The *rnh* locus shows a 36% linkage to the *metD* locus and a 40% linkage to the *proA* locus. No linkage was detected to *dnaE* or *nadC*. Our decision to concentrate on the mutation in

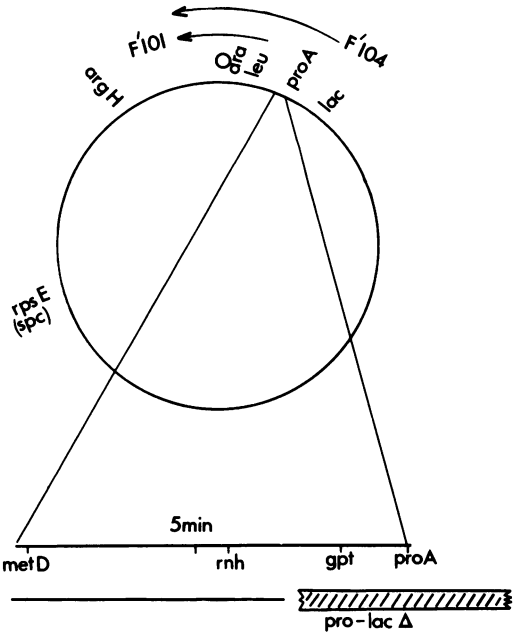

FIG. 2. *Relevant genetic markers. Map positions of various markers are noted either on the 100-min circle or on the exploded view around the 5-min region of the E. coli map.*

TABLE 3. *Transduction analysis of rnh relative to nadC, dnaE, metD, and proA*[a]

Selected marker	Linkage (*rnh*/selected marker)
nadC	0/23
dnaE	0/10
metD	9/25
proA	23/47

[a] Transductants were grown in small cultures, extracts were made, and RNase H was assayed by method B (see text). Protein assays were performed on each extract, and specific activities of RNase H for each transductant were compared with corresponding extracts from KS351 (parent) and FB2 (mutant).

FB2 was based on assays for RNase H of two types (procedures B and C in Materials and Methods). Hfr, F', and P1 mapping experiments utilized procedure B exclusively. For confirmation that the low levels of RNase H detected by procedure B were indeed due to a deficiency in the activity of RNase H, colonies of *rnh*$^+$ *metD*$^+$ and *rnh*$^-$ *metD*$^+$ P1 recombinants were picked at random and assayed by the SDS-polyacrylamide gel reconstitution assay. The results (Fig. 1) show a complete correspondence between low levels of RNase H activity as detected by procedure B and low levels of the bands corresponding to the RNase H activity on the gels.

***rnh* represents a new locus on the *E. coli* chromosome.** P1 transduction experiments place *rnh* at approximately 5.1 min on the Bachmann, Low, and Taylor map (2) (Fig. 2). *mutD* and *gpt* are the only well-characterized mutations in this region. An *mutD* strain was tested and found to have normal levels of RNase H. Conversely, FB2 and other strains derived from FB2 did not exhibit abnormal mutation rates. Deletions of the *pro-lac* region of the chromosome extend through the *gpt* locus (15). Deletions of this sort exhibit normal levels of RNase H. The end of the deletion resides between 5.1 (*rnh*) and 5.45 (*gpt*) min.

FB2 supports replication of ColE1-type plasmids. The results from Itoh and Tomizawa (17), suggesting an involvement of RNase H in ColE1 replication, led us to examine the ability of FB2 to support plasmid replication. A ColE1 derivative carrying ampicillin resistance was introduced into FB2 by transformation of $CaCl_2$-treated cells (6). Transformants that were resistant to ampicillin were found and subsequently shown to have mutant levels of RNase H. To examine the stability of the plasmid in FB2, cells were grown in the absence or presence of ampicillin and plated on L broth plates with and without ampicillin. No difference in plating efficiencies was observed. In a second experiment, individual colonies, grown on L broth plates without ampicillin, were tested for growth on L broth plates with ampicillin. Again, no loss of ampicillin resistance was observed (0 of 200 colonies). Therefore, the mutation in FB2 does not contribute to a remarkable instability of ColE1 plasmids.

DISCUSSION

Since there seemed to be no obvious method for selecting RNase H mutants without presupposing a function for the enzyme, a mass screening technique was developed which was used to assay relatively crude cell extracts for RNase H activity. The straightforward procedure of assaying crude lysates for enzymatic activity as described by Milcarek and Weiss (23) was not applicable for RNase H. RNase H activity cannot be accurately measured in initial lysates (3), due in part to interference by endogenous DNA (Carl, personal observation), the multitude of enzymes that will degrade the substrate, and, for poly(rA)·poly(dT), an endogenous inhibitor of RNase H (Dirksen and Crouch, unpublished data). A solution to this problem was suggested by the work of Gross et al. (11), who devised a technique for screening for RNA polymerase mutants. In this procedure RNA polymerase was first precipitated with the endogenous DNA of the extract in the presence of low salt and

polyethylene glycol. Subsequently, RNA polymerase was eluted from the precipitate with high salt, permitting accurate determinations of the RNA polymerase activity.

The observation that RNase H binds strongly to DNA (9) suggested that a similar procedure might be applicable to RNase H. We found that this was the case. Extracts prepared as described in this paper, a method similar to that of Gross et al. (11), can be used to assay RNase H. The degradation of DNA-RNA hybrids is proportional to the amount of extract used and to the time of incubation, thus providing the possibility of assaying the enzyme in relatively crude extracts. Furthermore, after coprecipitation with DNA by polyethylene glycol and elution of the RNase H activity, the activity is inhibited more than 90% by *N*-ethylmaleimide, which supports the idea that the assay measures RNase H (3). The ultimate validation of the assay depends, of course, on the isolation of the mutant FB2 and the results shown in Fig. 1 demonstrating agreement between the SDS-polyacrylamide gel reconstitution assay and the assay of the polyethylene glycol-treated extracts. The procedure described in this paper has permitted us to detect a mutation that results in a lower level of RNase H. Our estimate of the residual RNase H activity in FB2 in these partially purified extracts indicates that about 30% of the wild-type activity is present in FB2 (Table 2).

Utilization of the mass screening technique has also allowed us to map the locus responsible for the altered level of RNase H. A new locus (*rnh*) which maps at approximately 5.1 min on the *E. coli* chromosomal map (2) has been defined as a result of our mapping. *rnh* has no properties to suggest that it is related to any known gene that maps in this region. Recently a series of mutations that map in or near *mutD* have been shown to be slightly abnormal in DNA synthesis (16). *dnaQ* mutations map closer to *mutD* than to *proA*, suggesting that *dnaQ* and *rnh* are nonallelic.

Historically, the copurification of RNase III and RNase H through several steps (8, 28) has been noted. Both of these enzymes attack similar, yet different, substrates. Such results suggest a close relationship between these enzymes. It is interesting that *rnc*, the locus for RNase III (1, 30), and *rnh* map 180° apart on the *E. coli* map. It has been suggested that this relationship of map positions reflects an evolutionary relationship between the genes involved (39).

FB2 and strains derived from FB2 exhibit no unusual features as a result of having lowered levels of RNase H activity. The strongest suggestion of involvement of RNase H in DNA replication comes from the results of Itoh and Tomizawa (17). Our results with a ColE1-type plasmid in FB2 indicate that if ColE1 plasmids require RNase H for replication, the residual activity in FB2 is sufficient for such a purpose.

Evidence that the mutation in FB2 exerts its influence on RNase H and not on exonuclease III or DNA polymerase I comes from a variety of experiments. First, the assay of RNase H (type A) used for screening for the mutant is sensitive to *N*-ethylmaleimide, ruling out DNA polymerase I (insensitive to *N*-ethylmaleimide) as a contributor in this assay. Second, the reconstitution assay of RNase H (Fig. 1) demonstrates the alteration of RNase H activity in the region of the gel in which RNase H is found without significant alterations in exonuclease III activity. Finally, the map position of the mutation in FB2 (5 min) is unrelated to that of DNA polymerase I (85 min) or exonuclease III (38 min).

The mutation in FB2 does not allow us to decide whether the alteration is in the structural gene for RNase H or whether it represents some regulatory factor resulting in lower levels of RNase H. The enzyme from FB2 is not obviously temperature sensitive, but such determinations are subject to a variety of problems. The differences observed between RNase H obtained from the FB2 mutation and wild-type RNase H as shown in the SDS-polyacrylamide gel reconstitution assay (Fig. 1) do permit us to make some important conclusions concerning the problem itself. From other observations (Crouch, unpublished data), it was suggested that the two rapidly migrating bands of RNase H are related by a simple cleavage of the larger protein to generate a smaller protein that retains the ability to be both reconstituted and enzymatically active. Both bands of RNase H disappear (or are diminished) when the RNase H level as measured by procedure B is low (Fig. 1). If the two bands of RNase H activity were the result of two separate genes for distinct RNase H's, the mutation in FB2 would be required to diminish both of these. Although such a situation is possible, the simplest explanation is that each of the bands of RNase H is derived from the same gene and that the mutation in FB2 affects this single gene.

The mutation in FB2 is probably too leaky to produce any noticeable phenotypic alteration in this strain other than the low levels of RNase H activity detected in extracts. However, now that we know the location of the *rnh* gene, localized mutagenesis should allow us to isolate other alleles at this locus which have greater effects on the level of RNase H. Some of these mutants will hopefully provide clues to the in vivo function of the enzyme.

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