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Genetics

# The *rnh* gene is essential for growth of *Escherichia coli*

**(gene replacement/chloramphenicol acetyltransferase/λ prophage curing/ribonuclease H)**

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**ABSTRACT** **We have determined that a functional gene coding for ribonuclease H seems to be essential for cell growth in *Escherichia coli*. A strain was made with two copies of the *rnh* gene by lysogenizing an *E. coli* strain with a λ phage bearing a copy of the *rnh* gene. Inactivation of one of the two copies of the *rnh* gene was accomplished by transformation with a linear DNA molecule that had the gene for chloramphenicol acetyltransferase inserted near the middle of the *rnh* gene. In recombinants that had an inactive gene replacing the normal chromosomal *rnh* gene, the λ*rnh* prophage supplies an intact functional copy of the *rnh* gene. Curing the cells of the λrnh prophage left the cell with an inactive *rnh* gene and resulted in cell death. An intact functional *rnh* gene provided on a plasmid permits normal curing, and cured survivors were readily obtained. The technique described is probably generally applicable for assessing the requirement for other *E. coli* genes.**

RNase H specifically degrades the RNA of DNA·RNA hybrids (1, 2). In eukaryotic cells, two RNase H are present (3), while in *Escherichia coli*, a single RNase H has been identified that exhibits two molecular forms on $NaDodSO_4$/polyacrylamide gel electrophoresis (4). Speculation on a biological function of an RNase H has centered on DNA replication (5), but other functions have been proposed (6). While RNase H is specific for RNA of DNA·RNA hybrids, several other nucleases can also degrade RNA in DNA·RNA hybrids. In *E. coli*, DNA polI (7, 8), exonuclease III (7, 9), and T7 phage gene 6 exonuclease (10) all fall into the category of nucleases that degrade duplex DNA or DNA·RNA hybrids (either strand). The ability of any of these DNases to replace RNase H *in vivo* is only hypothetical.

Previously, we described a mutant of *E. coli* with lower levels of RNase H (4). We have a collection of cells bearing plasmids with either the wild-type or low level *rnh* genes. Until now, variation in RNase H activity over a 200-fold range produced no phenotypic effect. With an *rnh* clone, the enzyme can be overproduced 20-fold without effects on growth rates or on plasmid maintenance. Similarly, a decrease by a factor of 10 in RNase H specific activity in the mutant (FB 2) resulted in no apparent deficiency (4). The failure of changes in RNase H levels to produce an obvious selection scheme for mutants completely defective in RNase H activity led us to consider alternative approaches, especially since there were hypothetical substitutes for RNase H.

Here, we have inactivated the *rnh* gene by insertion of the chloramphenicol acetyltransferase (*cat*) gene, and we recombined the inactive gene in a host cell lysogenic λ*rnh* prophage. In recombinants with an inactive gene replacing the normal chromosomal *rnh* gene, the λ*rnh* prophage supplies an intact functional copy of the *rnh* gene. Curing the cell of the λ*rnh* prophage leaves the cell with an inactive *rnh* gene and results in cell death, indicating that RNase H is essential for cell growth.

## MATERIALS AND METHODS

**Materials.** Restriction endonucleases were obtained from Bethesda Research Laboratories or New England Biolabs. Nuclease S1 and DNA polymerase I (Klenow fragment) were from Bethesda Research Laboratories. T4 DNA ligase and bacteriophage λcI857 were obtained from New England Biolabs. *Sst* I linker DNA was purchased from Collaborative Research (Waltham, MA). [$\alpha$-$^{32}$P]dCTP and [$\alpha$-$^{32}$P]ATP were supplied by Amersham and New England Nuclear, respectively. Antibiotics were purchased from either P-L Biochemicals or Sigma. GeneScreen is a product of New England Nuclear.

**Plasmids.** Plasmid DNA of pBR325 were purchased from Bethesda Research Laboratories. Various plasmids containing the *rnh* gene, pSK750, pSK760, pSK2400, and pFB2400 have been described (11, 12). Constructions of other plasmids are described in this paper.

**Bacteria.** Strains of *E. coli* K-12 used in this study are listed in Table 1. Cells were grown in Luria broth (13). Antibiotics were used at the following concentrations: chloramphenicol (30 μg/ml), ampicillin (100 μg/ml), and tetracycline (15 μg/ml).

**Preparations of Plasmid DNAs and Restriction Endonuclease Fragments.** Large scale purification of plasmid DNA was carried out by a slight modification (11) of the method of Glaser and Cashel (14). Small preparations for screening recombinant plasmid DNAs by restriction endonuclease fragments were made by the alkali $NaDodSO_4$ method of Ish-Horowicz and Burke (15). All DNA fragments, including linear vector DNA, used to construct recombinant plasmids were purified by electrophoresis in agarose gels containing 0.1 M Tris·HCl, pH 8/0.083 M Na borate/0.001 M EDTA (buffer A). The gels were stained with ethidium bromide to locate DNA bands, and then DNA was electroeluted in dialysis tubes submerged in buffer A. The eluant was purified on DE-52 (16).

**Construction of Recombinant Plasmids.** Ligations were done with 0.2–0.5 μg of vector DNA and with a 2- to 5-fold molar excess of DNA fragments. Conditions for ligation were as follows: 14°C for 12 hr in 0.05 M Tris·HCl, pH 7.8/0.01 M $MgCl_2$/0.02 M dithiothreitol/0.001 M ATP/bovine serum albumin (50 μg/ml). The *cat* gene was cloned into the *Bam*HI site of the *rnh* gene of pSK750 (11). Purified 1.33-kilobase-pair (kbp) *Fnu*DII fragment, containing the *cat* gene of pBR325 (17, 18), was ligated to pSK750 DNA partially digested with *Bam*HI and made to have flush ends by nuclease S1 treatment (19). Screening of recombinants for growth in the presence of chloramphenicol or tetracycline, together with analysis of restriction DNA fragments, produced plasmids pCM270 and pCM'270. pCM2401 and pCM2403 were produced as shown in Fig. 1. Fragments A and B of pSK2400 were generated by complete *Sst* II digestion, partial *Pvu* I digestion, and purification by gel electro-

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Abbreviation: kbp, kilobase pair(s).

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Table 1. *E. coli* strains

| Strain | Genotype | Source |
|---|---|---|
| LE392 | *F⁻, supE44, supF58,* Δ(*lacIZY*)*6, metB1, hsdR514* ($r_k^-$ $m_k^+$), λ⁻, *rnh*⁺ *galK2, galT22* | L. Enquist (Molecular Genetics, Minnetonka, MN) |
| FB2 | *HfrH, thi, lac Y482, rha, rnh* | Ref. 4 |
| FB160 | FB2 lysogenic for λ carrying *rnh* of FB2 | This study |
| N3048 | *end, recB21, recC22, sbcB15, leu, ara, his, trp, tsx, thr, thi, lacY, mtl, xyl, galK, proA, argE, str*$^R$, *sup37* | M. Gellert (National Institutes of Health) |
| SK160 | N3048 lysogenic for λ carrying *rnh* of FB2 | This study |
| SK260 | SK160 *cat* interrupting chromosomal *rnh* | This study |
| SK360 | SK160 *cat* interrupting *rnh* at λ locus | This study |
| RW1520 | *HfrH,* Δ *gal-bio,* λ *h80 imm C*⁺ | R. Weisberg (National Institutes of Health) |

phoresis. Fragments A and B of pSK2400 are similar in size and do not resolve well on agarose gel electrophoresis. Therefore, both fragments were present during the construction of pCM2401, using the *Pvu* I/*Sst* II fragment from pCM270. Only clones of the pCM2401 type (20 of 20) were obtained (i.e., there was a selection for cloning into fragment A of pSK2400). Clones, resulting from ligation and transformation of the same mixture of fragments A and B of pSK2400 with the *Pvu* I/*Sst* II fragment of pCM'270, were all (8 of 8) the type shown by pCM2403. Thus, both types of plasmids (pCM2401 and pCM2403) have in common the orientation of the *cat* gene with respect to flanking chromosomal sequences. If we select first for ampicillin resistance and screen those colonies that have inserts in pSK2400 for resistance to chloramphenicol, plasmids might be obtained that carry the *cat* gene but are chloramphenicol sensitive. No evidence for such plasmids could be obtained.

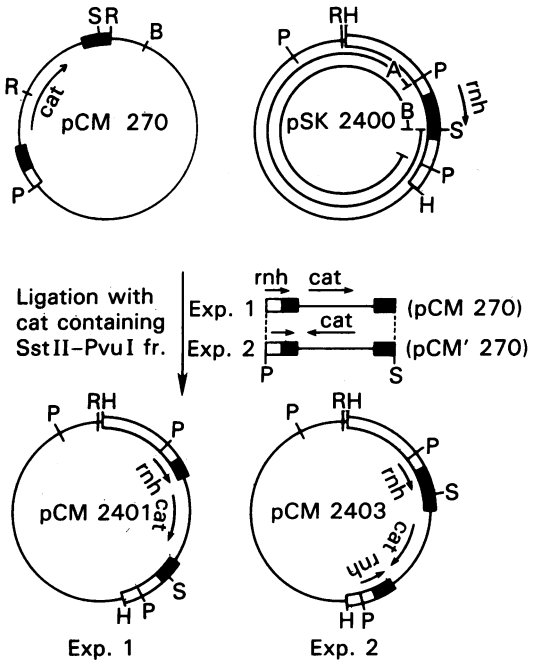


FIG. 1. Construction of plasmids bearing an *rnh* gene interrupted by a *cat* gene. The restriction sites are as follows: R, *Eco*RI; B, *Bam*HI; S, *Sst* II; P, *Pvu* I; F, *Fnu*DII; and H, *Hin*dIII. *Eco*RI sites of pSK2400 and pCM2401 are shown for orientation purposes. Several other *Eco*RI sites are present in the *E. coli* chromosomal portions of these plasmids (11). Arrows denote the direction of transcription of the *rnh* and *cat* genes. The *rnh* gene is shown as solid areas, other *E. coli* DNA is shown as open areas, and the *cat* gene is shown as a single line interrupting the *rnh* gene. The effect of orientation of the *cat* gene on insertion of *Sst* II/*Pvu* I into pSK2400 is presented. A mixture of fragment A (6.16 kbp) and fragment B (6.34 kbp) ligated with either the 2-kbp *Sst* II/*Pvu* I fragment of pCM270 (experiment 1) or of pCM'270 (experiment 2) yields either pCM2401 (experiment 1) or pCM2403 (experiment 2). Fragments A and B of pSK2400 are shown inside the circle representing pSK2400. Fragment A does not contain the 5' portion of the *rnh* gene. Fragment B does not contain the 3' portion of the *rnh* gene.

**Preparation of Recombinant λ DNA [λ(*rnh*$^m$)].** Ends of the 1.6-kbp *Eco*RI fragment of pFB2400 (12) were converted to *Sst* I sites by nuclease S1 digestion (19), *E. coli* DNA polymerase I (20) synthesis, and ligation to *Sst* I linker DNA. After digestion of the modified 1.6-kbp *Eco*RI with *Sst* I and purification of the fragment, ligation to λcI857 cut with *Sst* I produced λ(*rnh*$^m$). Screening for λ(*rnh*$^m$) was by plaque-hybridization (21) of nick-translated pSK760 DNA (22). Nick-translation of DNA was carried out using the kit of Bethesda Research Laboratories as described by the supplier. Plaques were produced by transfection of LE392.

**Transformation of *E. coli* with Linear DNA.** $CaCl_2$-treated *E. coli* were transformed (23) using microgram quantities of DNA. Transformants were selected for chloramphenicol resistance by growth on LB plates containing chloramphenicol and by growth in liquid culture (LB) containing 30 μg of chloramphenicol per ml. All incubations were at 30–32°C to avoid induction of λ.

**Detection of Transformants in Which *cat* Gene Is at the λ Locus.** An overnight culture of *E. coli* RW1520 (grown in Tryptone broth) was diluted into LB broth. After incubation at 37°C, 0.2 ml of *E. coli* RW1520 (midlogarithmic phase) was mixed with 2.5 ml of top agar containing 50 mM $MgSO_4$ and 30 μg of chloramphenicol per ml and poured onto LB plates containing chloramphenicol. After solidification, chloramphenicol-resistant transformants were stabbed onto the plate. Growth of cells at 42°C occurred in all instances. The extent of growth of cells produced from infection of *E. coli* RW1520 by phage carrying the *cat* gene made it easy to distinguish positive from negative results.

**Assay for RNase H.** RNase H activities in extracts were determined by two procedures: (*i*) measurement of acid-soluble radioactivity (24) using [$^{32}$P]poly(rA)·poly(dT) (25). RNase H was partially purified by the polyethylene glycol precipitation method of Carl *et al.* (4). (*ii*) Renaturation of RNase H after electrophoresis in $NaDodSO_4$/polyacrylamide gels (11).

**Southern Analysis.** Chromosomal DNAs (20 μg) isolated by the method of Marmur (26) were digested to completion with either *Hin*dIII or *Sst* I at 37°C for 16 hr using 200 units of enzyme in 200 μl. After extraction of the digest with phenol $CHCl_3$, the DNA was concentrated to 20 μl by ethanol-precipitation and suspension in 10 mM Tris·HCl (pH 8). After electrophoresis in 0.8% agarose gels, the DNA fragments in the gel were treated with alkali, neutralized by sodium phosphate buffer (pH 6.5), and transferred to GeneScreen by electroblotting at 225 mA for 16 hr (27, 28). Hybridization of DNA with nick-translated pSK760 DNA in the presence of dextran sulfate and formamide was carried out as described in the instruction manual of GeneScreen except that the prehybridization and hybridization mixtures did not contain $NaDodSO_4$.

**Curing Cells of Bacteriophage λ.** Ten microliters of exponentially growing cells ($A_{600}$ = 0.2) were diluted into 1 ml of LB broth, which was preheated to 42°C. After various periods of time at 42°C, cells were quickly chilled on ice and diluted to give an appropriate number of cells per plate.

Duplicate plates were prepared, one of which was incubated at 32°C and the second at 42°C. Both plates were initially at room temperature. The percentage of cured cells was calculated from the ratio of the number of cells at 42°C to the number of cells at 32°C. Percent survival was determined by comparing the number of cells growing at 32°C after the heat pulse with a control sample that was not heat-pulsed.

**Transformation of *E. coli* SK260 with pSK760.** $CaCl_2$-treated *E. coli* SK260 was transformed as described by Mandel and Higa (23) except that cells were incubated at 32°C.

## RESULTS

**Cloning the *cat* Gene into the *rnh* Gene.** The *cat* gene was inserted into the *Bam*HI site of the *rnh* gene of pSK750 (11). Two types of plasmids were obtained: (*i*) Those, as shown for pCM270, which are oriented so that transcription of the *cat* and *rnh* genes is from the same strand of DNA and (*ii*) those in which the *cat* gene is transcribed from the opposite strand to that for the *rnh* gene (pCM'270). The *cat* gene splits the chromosomal portion of pSK750 into 270- and 480-base-pair regions. Extension of the chromosomal regions flanking the *rnh* gene was accomplished by inserting the *Sst* II/*Pvu* I fragment of pCM270 into fragment A (an *Sst* II/*Pvu* I fragment of pSK2400). In pCM2401, the *cat* gene is flanked by 1.5- and 0.9-kbp chromosomal DNA sequences.

**Construction of an *E. coli* Strain with Two Chromosomal Copies of the *rnh* Gene.** Replacement of the resident chromosomal *rnh* gene by a double-recombinational event with an *rnh* gene interrupted by the *cat* gene can be accomplished by transformation with linear DNA. Provided inactivation of the *rnh* gene is not a lethal event, chloramphenicol-resistant transformants should be obtained that have lost a functional intact *rnh* gene. If replacement of the *rnh* gene with an inactive *rnh* gene were a lethal event, we would observe transformation of an *E. coli* only if some event produced a functional *cat* gene and restored *rnh* gene or if a compensating mutation arose. Transformation of the insertionally inactivated *rnh* gene into an *E. coli* recipient with two copies of the *rnh* gene, however, would avoid these selection problems, because inactivation of one gene leaves a second *rnh* gene that can function.

We constructed an *E. coli* strain with two copies of the *rnh* gene by lysogenizing *E. coli* N3048 with λ(*rnh*$^m$) carrying a copy of the *rnh* gene. To distinguish between the two functional copies of the *rnh* gene, we chose to clone the mutant *rnh* gene from *E. coli* FB2 (4) into λcI857. The 1.1-kbp *Sst* I fragment of λ was replaced with a 1.6-kbp *Eco*RI fragment of pFB2400. Both of the *Eco*RI ends were converted to *Sst* I sites by introducing *Sst* I linkers after nuclease S1 and DNA polymerase treatment of the purified 1.6-kbp *Eco*RI fragment. Lysogens of N3048 with λ(*rnh*$^m$) are designated SK160. In this strain, inactivation of the normal chromosomal *rnh* gene should produce a cell that has the *E. coli* FB2 mutant level of RNase H activity. Replacement of the *rnh*$^m$ gene at the λ locus can easily be screened as described in *Materials and Methods* by a simple plate test. Moreover, the presence and state of the two *rnh* genes can be assessed by Southern analysis (Fig. 2). The resident chromosomal *rnh* gene (wild type) is present in a 2.4-kbp *Hin*dIII fragment or a large (>10 kbp) *Sst* I fragment. In contrast, the *rnh*$^m$ gene at the λ locus is present in a 5.3-kbp *Hin*dIII fragment or a 1.6-kbp *Sst* I fragment (lanes 5 and 6).

**Transformation of *E. coli* SK160 with a Linear DNA Fragment Containing the *rnh* Gene Interrupted by the *cat* Gene.** Our decision to use N3048 as the parental strain of SK160 was affected by the necessity to transform with linear DNA to replace the *rnh* gene with one interrupted by the *cat* gene. Linear DNA transformation efficiency is higher in *recBC* mutants than in wild-type *E. coli* as a result of the loss of the *recBC* exonuclease (29), a major such enzyme in *E. coli* (30).

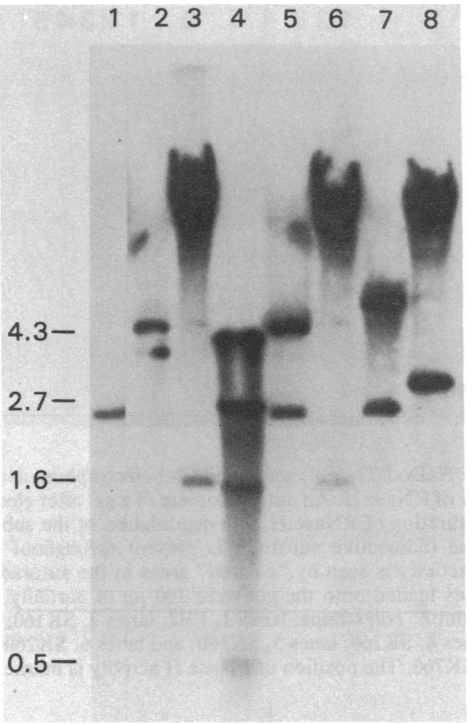


FIG. 2. Probing for the *rnh* genes of chromosomal DNA by Southern blot hybridization. Chromosomal DNAs (20 μg) prepared from *E. coli* strains LE392 (lane 1), SK260 (lanes 2 and 3), SK160 (lanes 5 and 6), and SK360 (lanes 7 and 8) were digested with *Hin*dIII (lanes 1, 2, 5, and 7) or *Sst* I (lanes 3, 6, and 8). Size markers (lanes 1 and 4) were a mixture of digests of pSK760 with either *Eco*RI (8 ng of DNA) or *Acc* I (15 ng of DNA). Size markers are in kbp.

The *sbc* mutation in this strain restores recombination to these cells in spite of the *recB* and *recC* mutations (31). In our case, we observed ≈100 transformants per μg of DNA.

Transformation of *E. coli* SK160 (containing two chromosomal copies of the *rnh* gene) with the 3.7-kbp *Hin*dIII fragment of pCM2401 yielded more than 100 chloramphenicol-resistant colonies. These transformants were examined in some detail. The location of the *cat* gene was determined in three ways.

(*i*) *rnh*$^m$ gene replacement at the λ locus should permit the production of phage that can transduce chloramphenicol resistance to other cells. A simple plate test can be used to screen for cells producing such phage. Fifteen of the 100 colonies that we checked in this manner proved to have the *cat* gene at the λ locus.

(*ii*) Screening of RNase H activity permits the identification of cells in which the chromosomal *rnh* gene has been replaced with one interrupted with the *cat* gene. The level of RNase H changes from wild type to that of the mutant. Seven colonies that exhibited low levels of RNase H activity were examined by the $NaDodSO_4$/polyacrylamide gel renaturation assay (4). All seven had lost wild-type RNase H activity. In Fig. 3, we show results obtained with SK260 as a representative of the seven colonies. The low-level RNase H renatures poorly in these gels (FB2), probably reflecting the low specific activity of the mutant enzyme (32). Other strains (SK160 and SK360) show renaturation of the wild-type activity. SK260-bearing pSK760 demonstrates the high level of renaturation of a wild-type gene on a plasmid.

(*iii*) Southern analysis of DNA from 15 colonies selected by the methods described above confirmed the existence of cells in which the *cat* gene was located at the chromosomal locus (SK260) or in the prophage (SK360). Insertion of the *cat* gene in the chromosomal DNA increases the size of

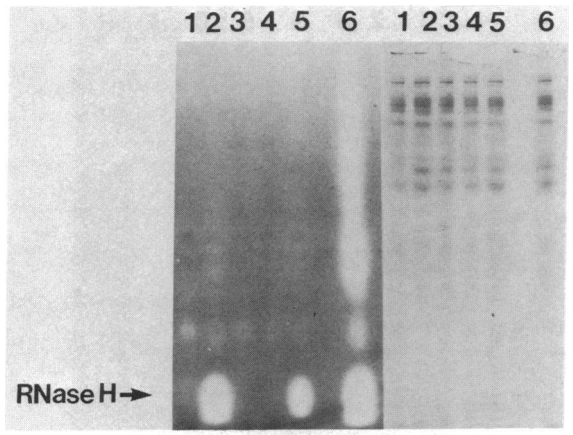


FIG. 3. NaDodSO$_4$/polyacrylamide gel electrophoresis renaturation assay of RNase H. An autoradiogram of a gel after electrophoresis, renaturation of RNase H, and degradation of the substrate is shown. The radioactive substrate is present throughout the gel. RNase H activity is seen by "cleared" areas in the autoradiogram. The samples loaded onto the gel were 100 μg of partially purified extracts from *E. coli* strains: lanes 1, FB2; lanes 2, SK160; lanes 3, FB160; lanes 4, SK260; lanes 5, SK360; and lanes 6, SK260-bearing plasmid pSK760. The position of RNase H activity is marked on the left.

DNA by 1.33 kbp. The 2.4-kbp *Hin*dIII fragment seen in LE392 and SK160 has become 3.7 kbp in SK260. Although replacement of both copies of the *rnh* gene might possibly occur, the 5.3-kbp *Hin*dIII fragment from the λ locus *rnh* is unchanged from SK160. In SK360 the *rnh* gene from the chromosomal locus remains in a 2.4-kbp *Hin*dIII fragment, whereas the λ locus *rnh*$^m$ gene has increased in size. Replacement of the λ locus *rnh*$^m$ gene with one interrupted by the *cat* gene is seen more easily in the lanes containing DNA digested with *Sst* I. The 1.6-kbp *Sst* I fragment of SK160 is 2.9 kbp in SK360. Both sorts of insertions were examined in lysogen-curing experiments.

**Curing Cells of λ Leaves a Single Copy of an *rnh* Gene.** Cells can be cured of λ by a short heat pulse (33) with λcI857 lysogens, because the cI repressor is reversibly thermolabile. The highest percentage of cured cells is obtained after a heat pulse of 3 min at 42°C. SK360 cured of λ should be able to grow at 42°C, because the λ has been removed and it should be sensitive to chloramphenicol. It should, in fact, be identical to N3048. In the case of SK260, survival depends on whether RNase H is essential for cell growth. If RNase H is not essential, survivors should be obtained that grow at 42°C and are chloramphenicol resistant. If RNase H is essential, no survivor should be seen. The results are summarized in Table 2. When the *rnh* gene with the *cat* insertion was present at the prophage *rnh*$^m$ site (SK360), curing occurred giving a high percentage of viable cells that were able to grow at 42°C and were chloramphenicol sensitive. In marked contrast, no viable cured cells were obtained using SK260 in which the chromosomal *rnh* gene was inactive. Other than the location of the *cat* gene, SK260 and SK360 differ in the level of RNase H activity. SK260 has the FB2 mutant level and SK360 has the wild-type level. To eliminate the possibility that curing is defective in cells that have low level RNase H, FB160 was constructed by lysogenizing FB2 with λ carrying the *rnh*$^m$ gene. The results show that the *rnh*$^m$ gene does not alter curing of λ. Thus, the most straightforward interpretation of our results is that a functional *rnh* gene is essential for cell viability.

It is possible that some feature other than RNase H activity *per se* is the reason for the inability to obtain viable cells cured of λ*rnh*$^m$ in SK260. If this were the case, supplying a good copy of the *rnh* gene to SK260 would not aid in obtaining viable cured cells. However, further proof that an active *rnh* gene can complement the defective chromosomal *rnh* gene was obtained by transforming SK260 with the plasmid pSK760 (11). Such a strain has three distinct types of *rnh* gene, the wild-type gene present on pSK760, the mutant (FB2) gene at the λ locus, and the chromosomal *rnh* gene, which is interrupted by the *cat* gene. Of the 760 nucleotide pairs of chromosomal DNA in pSK760, about 575 are required to produce the messenger RNA for RNase H. It is highly unlikely that this plasmid contributed any other *E. coli* function. Cured cells could be obtained from SK260 bearing the plasmid pSK760. These cured cells are chloramphenicol resistant and grow at 42°C (data not shown).

Table 2. Curing of strains

| *E. coli* strain | Genotype | % cure |
|---|---|---|
| SK160 | *Cm*$^s$, *rnh*$^+$, *rnh*$^m$ | 90 |
| FB160 | *Cm*$^s$, *rnh*$^m$ | 76 |
| SK260 | *Cm*$^r$, *rnh*$^m$ | 0 |
| SK360 | *Cm*$^r$, *rnh*$^+$ | 98 |
| SK260-bearing pSK760 | *Cm*$^r$, *rnh*$^+$, *rnh*$^m$ | 84 |

% cure was determined by counting 400–500 colonies. Thus, % cure of 0 means % cure of <0.2%.

**Attempts to Insert an Inactive *rnh* Gene in an *rnh* Haploid.** From evidence presented thus far, an active functional *rnh* gene seems to be required for cell growth. Replacement of the *rnh* (in a strain with only one copy of the gene) by one interrupted with the *cat* gene should be inefficient, at best. Transformation of *E. coli* SK160 (containing two chromosomal copies of the *rnh* gene) with the 5.1-kbp *Sal* I/*Pst* I fragment of pCM2401 yielded >300 chloramphenicol-resistant cells. In contrast, no transformant was observed using N3048 (the parent of SK160 but having a single *rnh* gene). The *Sal* I and *Pst* I sites are the ones found in pBR322 and are not shown in Fig. 1. We also attempted transformation of LE392 with two different restriction fragments of pCM'270. In neither instance did we obtain chloramphenicol-resistant cells. It should be noted that the orientation of the *cat* gene, with respect to the *rnh* gene, differs between pCM2401 and pCM'270.

## DISCUSSION

These results indicate that an intact functional *rnh* gene is required for growth of *E. coli*. Two different experiments support such a conclusion. First, cells survive curing of λ, with concomitant removal of the *rnh* at that locus, only if there is an intact functional *rnh* remaining in the cell, either from the normal chromosomal locus (*E. coli* SK360) or if a plasmid bearing a copy of the *rnh* gene is present (*E. coli* SK260/pSK760). *E. coli* SK260 gave no survivors after being cured of λ (and the functional *rnh* gene). Second, an *rnh* gene with one interrupted by the *cat* gene is readily accomplished in strains containing two copies of the *rnh* gene (*E. coli* SK160), whereas no replacement is detected with the isogenic strain that contains a single copy of the *rnh* gene.

It is reassuring that a large fraction of the cells are cured by the heat pulse (Table 2). Thus, when we determine that RNase H is required for cell growth, the results apply to the vast majority of cells, not just to a special subset. By contrast, the very powerful selective pressures exerted in classical methods sometimes yield strains that are defective in the gene in question but contain a compensating mutation elsewhere in the chromosome [e.g., *E. coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes (34, 35)]. Although we found no chloramphenicol-resistant transformants of haploid *rnh* strains in the experiments reported in this study, it is possible that a more exten-

sive search would yield unusual strains capable of growth in the absence of *rnh* or with very low levels of the enzyme. Having established that *rnh* is essential for normal cell growth, we can begin to search for compensating mutants. Although, the *rnh* gene seems essential for cell growth, the function of RNase H remains unknown.

Duplication of the *rnh* gene permits us to inactivate one copy of the gene. In a sense, this makes strains with such duplications similar to diploid organisms that can carry recessive lethal mutations, because they have a normal copy of the gene. Removal of the functional gene copy by curing the cells of the $\lambda rnh^m$ prophage is somewhat like following the fate of yeast cells by tetrad analysis. In our curing experiments, no viable cured cells could be obtained if the chromosomal gene had been inactivated. In yeast tetrad analysis, only two of the four spores will grow if there is a recessive lethal gene present in the diploid cell. The technique that we used in this study is probably applicable to other genes in *E. coli*. In fact, deletions of a gene can be made if the *cat* gene is cloned in an appropriate manner prior to transformation of a diploid strain.

The orientation of the *cat* gene with respect to flanking regions was unique in the formation of pCM2401 and pCM2403 (Fig. 1). One explanation for the preferential orientation is at the level of transcription (36). In the orientation not obtained, transcription could proceed from the *cat* gene promoter into or through the adjacent chromosomal regions of the plasmid. Overproduction of a gene product from this area could be detrimental. Alternatively, formation of complementary mRNA could result in destruction of mRNA sequences or translational capacity. It is conceivable that situations could arise in which neither orientation of the *cat* gene is permitted. If transcription is the cause of the preference, placing strong transcription termination signals at appropriate sites would be predicted to remove the preference.

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