Structural analysis of the rhlE gene of Escherichia coli

Haruo Ohmori

Institute For Virus Research, Kyoto University
53 Shougoin, Kawahara-machi, Sakyo-ku, Kyoto 601-01, Japan
(Received 10 October 1993)

ABSTRACT

The E. coli chromosome is known to carry at least five genes, each of which codes for a “D-E-A-D” box protein that is presumed to possess an ATP-dependent RNA helicase activity. Four of such genes (srmB, deaD, dhpA and rhlB) were already mapped on the E. coli chromosome and their DNA sequences determined. We here report the complete nucleotide sequence of the remaining rhlE gene located at about 17.8 min on the E. coli genetic map. RhlE protein possesses all of the motifs (I to VI) conserved among prokaryotic and eukaryotic “D-E-A-D” proteins and has an arginine-rich carboxyl-terminal region. A null mutant of the rhlE gene was constructed by a new method with a CoIE1 plasmid mutant that replicates in RNase HI-deficient bacterial strains, but not in the wild-type strains. The ΔrhlE mutant can grow normally, implying that the rhlE gene product is nonessential for bacterial cell growth.

1. INTRODUCTION

The “D-E-A-D” box proteins are a family of proteins found in a wide variety of organisms from bacteria to humans, which have a core region homologous to eIF-4A, a murine translation initiation factor (Schmid and Linder, 1992). This family of proteins share G-K-T (motif I) and D-E-A-D (motif II) sequences known to be a common A-motif and a special version of B-motif, respectively, for ATP binding sites (Walker et al., 1982). They also share other sequences such as P-T-R-E-L-A (motif IA) and H-R-I-G-R-T-G-R (motif VI) which are unique to this family (Gorbalenya et al., 1989; Linder et al., 1989). The “D-E-A-D” box proteins are assumed to have ATP-dependent RNA helicase or RNA-dependent ATPase activity, while such an enzyme activity has been demonstrated only with a few members of them, for example, the prototype eIF-4A (Ray et al., 1985) and the human p68 protein (Hirling et al., 1989; Iggo and Lane, 1989). Individual members of the family possess specific amino- and carboxyl-terminal regions of variable lengths for respective functions, which are in most cases related to rearrangement of RNA structures during translation, ribosomal biogenesis, or RNA processing.

* The sequence of rhlE and its flanking regions has been submitted to GenBank and given the accession number L02123.
At least five D-E-A-D box genes have been found in *E. coli* (Kalman et al., 1991). First, the *srmB* gene was identified as a multiple copy suppressor for a temperature-sensitive (ts) mutation in the ribosomal protein L24 (Nishi et al., 1989). Purified SrmB protein was shown to have RNA-dependent ATPase activity. Another gene, *deaD*, was also identified as a multiple copy suppressor of the ts mutations in the ribosomal protein S2 (Toone et al., 1991). SrmB and DeaD proteins appear to have different functions or specificities since overexpression of DeaD protein does not suppress the ts mutation in L24 and overexpression of SrmB protein does not suppress the ts mutations in S2 (Toone et al., 1991). The third gene, designated *dbpA* for D-E-A-D box protein, was found by low stringency screening with a cDNA probe from the *S. pombe* homologue of the human p68 protein (Iggo et al., 1990). The function of this gene is unknown. Furthermore, PCR screening with oligonucleotides corresponding to the common motifs (I, IA, II, and VI) identified two more genes, named *rhlB* and *rhlE* after RNA-helicase like protein (Kalman et al., 1991). The *rhlB* gene is necessary for viability only in some genetic backgrounds, and the conditional lethality is not complemented by the presence of a multiple copy plasmid carrying the *srmB* gene (Kalman et al., 1991). There are least information with regard to the *rhlE* gene; its partial sequence determined with the PCR fragment (from motif I to motif VI) and a rough mapping by hybridization of the fragment with the Kohara phage clone λ # 204 have been reported (Kalman et al., 1991). During the course of studying a gene, designated *rarB*, which is presumed to participate in the alternative replication mechanism of the *E. coli* chromosome independent of oriC and dnaA (to be published elsewhere), we noticed that the *rhlE* gene is located very closely to the *rarB* gene. This paper describes mapping on the *E. coli* chromosome, sequencing, and isolation of a null mutant, of the *rhlE* gene.

2. MATERIALS AND METHODS

*Media and strains*

LB [1% Bactotryptone (Difco), 0.5% yeast extracts (Difco), and 0.5% NaCl, pH 7.4] was used for cultivation of bacteria, and NZCYM (Sambrook et al., 1989) was used to grow lambda phages. L-agar contained 1.6% agar in LB. If necessary, ampicillin and kanamycin were added at a final concentration of 50 and 20 μg/ml, respectively.

The *E. coli* K-12 strains used are JM101 [supE, thi, Δ(lac-proAB)/F', traD36, proA⁺B⁺, lacI⁰ΔM15] (Yanish-Perron et al., 1985), its isogenic strains AK101 (rnhA::cat) (Kanaya et al., 1990) and MV1190 [Δ(srl-recA)306::Tn10] (Vieira and Messing, 1987). The phage λ #204 of the Kohara library (Kohara et al., 1987) was provided by Dr. Kohara (National Institute of Genetics, Japan). The λgt-λC phage (Thomas et al., 1974) was used for gene disruption experiment and provided by Dr. Inokuehi (Kyoto University, Japan). The phagemid vectors carrying an
ampicillin (Ap)-resistance gene, pTZ18U and pTZ19U were obtained from Bio-
Rad (Richmond, CA, USA) and used for subcloning and sequencing. pUC4K
(Pharmacia-LKB, Uppsala, Sweden) was used to prepare the DNA fragment
containing the kanamycin (Km)-resistance gene.

DNA manipulations

Most procedures for DNA manipulation and bacterial transformation followed
the standard protocols (Sambrook et al., 1989). E. coli chromosomal DNAs were
prepared and subjected to Southern blotting analysis as described (Silhavy et al.,
1984), using a nonradioactive DNA labeling and detection kit from Boehringer
Mannheim (Mannheim, Germany).

Construction of pTZ19Urhh and other plasmids

An RNase H-sensitive replication mutant, pTZ19Urhh, was constructed from
pTZ19U by sequence-directed mutagenesis of the Kunkel method (Kunkel, 1985).
pTZ19Urhh has deleted the two bases at the unique HaeII site (from 5'-AGCGCC-
3' to 5'-AGCC-3') in the region encoding the primer-precursor (RNA II) for
initiation of the plasmid replication, and consequently its replication becomes
sensitive to RNase H (Masukata and Tomizawa, 1984; Naito et al., 1984; Ohmori
et al., 1987); that is, the plasmid can replicate in RNase HI-deficient (rnhA^{-})
strains of E. coli, but not in the wild-type strains containing the active enzyme.
DNAs of pTZ19Urhh and its derivatives were prepared from the strain AK101
carrying such plasmids. Other plasmids were constructed in this experiment.

DNA sequence analysis

DNA sequencing by the dideoxy chain terminator method (Sanger et al., 1977)
was done with single-stranded DNAs prepared by infection of the helper phage
M13K07 (Vieira and Messing, 1987), using a Sequenase kit from US Biochemicals
(Cleveland, OH, USA). Computer analyses of the nucleotide and amino-acid
sequences were done with the SDC-GENETYX programs (Software Develop-
ment, Tokyo, Japan).

3. RESULTS

The nucleotide sequence of the rhIE gene

The PCR-amplified DNA fragment of the RhIE core region was found to
hybridize to the Kohara phage λ #204 (1B4) DNA (Kalman et al., 1991). The E.
coli chromosomal DNA fragment carried by the phage was prepared after EcoRI
digestion of the phage DNA and treated with SmaI and other restriction enzymes
to locate their cleavage sites within the fragment. The fragment (Fig. 1) was
found to contain some additional sites for EcoRV, BglI, and PvuII to those shown
in the original map (Kohara et al., 1987). The 858 base-pairs (bp) sequence of the
PCR-amplified *rhlE* fragment (GenBank database Accession Number X56037), contained single sites for *EcoRV* and *SmaI*, respectively, within a very short (109 bp) distance. This information allowed us to locate the *rhlE* fragment at around 2.0 kilo-bases (kb) away from the left end of the *E. coli* chromosomal segment. Figure 2 shows the 2600 bp sequence determined, which corresponds to the 842–845 kb region of the Kohara map and also to the 17.8 min region of the genetic map between *chlAM* and *glnQPH* (Bachmann, 1990). This location of the *rhlE* gene differs from the assignment by Rudd (1992), who mapped the gene on the adjacent *BamHI* segment (the B1-B2 segment in Fig. 1), relying on the 858 bp sequence of the PCR-amplified *rhlE* fragment. Our sequence differs at 12 sites from the above-mentioned sequence of the PCR fragment, and generates three amino acid changes from the published RhlE core sequence (Kalman et al., 1991), all at the nonconserved residues. The coding region probably starts with ATG at 540–542, since it is preceded by a good candidate (GGAG at 531–534) for the Shine-Dalgarno sequence. This open reading frame (ORF) terminates with TGA at 2004–2006, which is followed by a repetitive extragenic palindromic element (REP) (Higgins et al., 1987). A short ORF in an opposite direction (f160, Fig. 1) terminates at 2138–2136, although there is no evidence that it is indeed translated. The REP element is also found in the 5.2 kb downstream, repeated twice, near the ends of two oppositely directed ORFs (Fig. 1). Another putative ORF

---

**Fig. 1.** Physical map of the *E. coli* chromosomal region carried by λ +204. The *E. coli* chromosomal region is flanked by an *EcoRI* site at each end, derived from the cloning vector λEMBL4. The second horizontal line from the top indicates the physical map position of the *E. coli* chromosome (Kohara et al., 1987). The open square indicates a *SmaI* site (another *SmaI* site present in this region is not precisely localized), and the filled squares indicate *BglII* sites. The symbols B, H, E, R, G, K, S, and V represent *BamHI*, *HindIII*, *EcoRI*, *EcoRV*, *BglII*, *KpnI*, *PstI*, and *PvuII*, respectively. The vertical lines with an open circle denote sites not present in the original Kohara map, but found from the DNA sequence. The rightmost *BamHI* site (B4) was regenerated during cloning of the *Sau3A* partial digest of *E. coli* DNA into the *BamHI* site on λEMBL4. The two vertical arrows indicate the sites of mini-Tn10 (Km) insertion in the *rarB11* and *rarB19* mutants (our unpublished data). Four rightward (clockwise direction in the genetic map) and two leftward ORFs as indicated were found in the determined DNA sequence from the left *PstI* site to the second *BamHI* (B2) site (8356 bp in total, our unpublished data). The thin horizontal arrows indicate the direction of transcription. The thick horizontal bar represents REP sequences and the vertical bar represents a putative Rho-independent transcription termination signal.
preceding *rhlE* terminates at 327–329, which is followed by a palindromic sequence that might function as a transcription terminator signal. These surrounding sequences suggest that the *rhlE* transcription is monocistronic.

```
PstI
1  CTGCCAGCGGAGAGATCTGATTTCTGAGCGCCGATCCGCGTGCGCGGAC
61  AAGGCGTGAGAGAGAGTGATACAAATTCTCTCGAAGGGATGATGTTGAGTTGG
121  CGGCCCAGGCCGCGTTCTGAGGAGAGAGGATCTGATTTCTGAGCGCCGATCC
181  GCTTGGCTGGGCGGCTGAGGAGAGAGGATCTGATTTCTGAGCGCCGATCC
241  TGGCCAGCGGAGAGATCTGATTTCTGAGCGCCGATCCGCGTGCGCGGAC
-35  GGAATATCCATATGCGACATACATCTCTGATATTTGATATATGACTCACATCG
-10  GGGGAGTTGTCCAGAGATGGCTAAAGAGTTTTTACGACAACGATCAGCAGAAGTTATCC
SD
481  CCTTCTACGATATCTGCGATACACCTACCGGTAAGGGGCGGCGTCCGAGAGAGTTG
541  TGGCTTTGATGCTTCTTTGGGTATAGCCGCCGATATCCGCGCGGCTGCGCGGAC
2  SFDLSGLSPDLIRAVAEQGGTT
601  ACCCGGACCCCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
22  REPITPIQQQAIPAVLGERDL**Q**P**L**G**D**
661  TGGATGGCTAGCGCGCGGAGCGGCCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
42  MASAQGTGTGKTAGFTLPLQLAGTK**GKT**
motif I
721  AACAACCCTGATCAGCTAGGCTGCACCGGGAAATGGCGTTGGATCGCTGCGCTGATTC
62  HLIIRQPHAKGRVRAPLILL**L**
781  TTACCGGCAACCGCTGAGCTGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
82  TPTRELAAQIGENVRYDSKYPCTRLEAQ*
motif IA
841  ACCTGAAACATCTGTCGTGCTGTGTTCTGTGTGAGATTAACGGGACATGATTG
102  LNIIRSLVVVFVGSINPQQMKGG
SnaI
901  AACTGCGTGGCCGGCCCTGATGCTTGGCGGATCCGGCAGGTTGCTGCTCCGGGAC
122  LRGGGVDLPFTGRRLDLHEL**T**GRL
961  ATCAGAACTGCGTGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
142  QNAVKLDQVEILVLDEADRM**DEAD**M
motif II
1021  TGCTCGACATGCGGTTTATCCGAGTATCGGCTGGCGTTGATAAACAAAATACCTCGGCAAGC
162  LDMGFIHDIRVLYLTLKLPAKR**G**F
1081  GCCAAGAATGTTATATCTCCTGCCAGTACCTCTGAGATTTAAAGCCCTGCGGAAAAAC
182  QNLFLSACPSTFSSDIDIKALAEKQLFSAT
motif III
1141  TGTTGCACATCCCGTCGGGAAACTGAAATGCGACCCGGCAATCCGGCTGGATCAGGTGA
202  LHNFEIEIEVARRRNTSDQVT
1201  CCCAGACGTCTACATTGGCTGATAGAAGAACGGGAAACGCGGATTTGCTGCTGCAGCAACTGATTG
222  QHVHFVFDKRKRKRELLSHMIGL
```
Fig. 2. The nucleotide sequence around the *rho*E gene. The 2600 bp sequence from the *PstI* site to the first *BamHI* (B1) site is shown. The initiation and termination codons of the *rho*E gene and the preceding ORF, and the sequence complementary to the termination codon of *f160* are indicated in boldface. The putative -35 and -10 sequences for the *rho*E transcription and the Shine-Dalgarno sequence are also shown in boldface. The palindromic sequences probably acting as a transcriptional terminator and some restriction sites are underlined. Amino-acids are denoted by single letters. The identical residues among all of the five *E. coli* “D-E-A-D” box proteins are indicated below the *RhoE* sequence, and the conserved (not identical but similar) one are indicated by asterisks.
Expected properties of RhlE protein

From the deduced amino acid sequence, molecular weight of RhlE protein (488 amino-acid residues) is estimated to be 54 kilodalton (KD), similar to those of the four other E. coli D-E-A-D box proteins (50 KD of SrnB, 64 KD of DeaD, 46 KD of DbpA, and 47 KD of RhlB). RhlE protein has a core region with all of the well-conserved motifs and a relatively long carboxyl-terminal region, which is rich in basic amino acids, especially arginine (17 Arg and 10 Lys vs 5 Asp and 10 Glu residues among the total 150 residues in the carboxyl-terminal region beyond motif VI). The RRRPRK sequence at 443–449 may correspond to the arginine-rich RNA binding motif as found in λN and HIV Tat proteins (Lazinski et al., 1989; Calnan et al., 1991). Another characteristic sequence in the RhlE carboxyl-terminal region is GRQQRGGGRGQGGQQPPRRREGG at 390–416. Similar glycine-rich sequences containing arginines were also found in the carboxyl-terminal region of the other D-E-A-D box proteins, the yeast P 68 homologues DBP2 of S. cerevisiae and dbp2 of S. pombe (Iggo et al., 1991). The Drosophila vasa protein, which also belongs to the D-E-A-D family, contains a glycine-rich heptad repeat, F/SRGGGE/QGG, five times in the amino-terminal region (Lasko and Ashburner, 1988; Hay et al., 1988). These glycine-rich sequences are believed to contribute for RNA binding since such sequences are found in some other RNA binding proteins (Jong et al., 1987). The presence of the above two characteristic sequences in the carboxyl-terminal region of RhlE supports that the region functions as a domain for RNA binding.

Disruption of the rhlE gene

To examine the rhlE gene function, isolation of a null mutant of the gene was undertaken. The method adopted here was to use a mutant of ColE1-type plasmids that can replicate in RNase HI-deficient (rnhA−) strains of E. coli, but not in the wild-type strains (Ohmori, 1988). Derivatives of such a mutant plasmid carrying an E. coli chromosomal segment can be maintained as a high-copy-number plasmid in rnhA− hosts, but in the wild-type strains they can be maintained only in a state integrated into the host chromosome by homologous recombination. For such a purpose, we constructed a mutant of pTZ19U (pTZ19Urrh) by introducing a 2 bp deletion in a region essential for replication in the wild-type cells, as described in MATERIALS AND METHODS. This plasmid and its derivatives were propagated in an rnhA− strain, AK101. The 5 kb EcoRI-BglII DNA fragment containing the rhlE coding region (see Fig. 1) was inserted between the EcoRI and BamHI sites of pTZ19Urrh to construct pKH 6001. Subsequently, about two-thirds of the rhlE gene, flanked by the SmaI site at the position 934 and the StuI site at the position 1978, was replaced by the 1.2 kb HincII fragment carrying the kanamycin-resistance gene of pUC4K. The resultant ampicillin-resistant (ApR) and kanamycin-resistant (KmR) plasmid (designated pKH6002, Fig. 3) generated KmR transformants with JM101 (the rnhA+
Fig. 3. A schematic representation of the events during transformation. One homologous recombination event at the right (R) side of the rhlE gene generates a Ap$^S$ Km$^R$ transformant shown in the lower right, and two such events, once at each side (L and R), generates a Ap$^S$ Km$^R$ transformant. The thick horizontal bar with the letter a or b indicates the DNA region that hybridizes with the probe used for Southern blotting analysis (see Fig. 4).

parental strain of AK101) at the frequency of about $10^{-4}$ of AK101, but it generated no Km$^R$ transformants with MV1190 (a ΔrecA derivative of JM101) implying that recA-dependent homologous recombination underlay establishment of the transformants in RNase HI-containing cells. Most of the 38 Km$^R$ transformants of JM101 thus obtained were resistant to ampicillin, but four of them were sensitive to the drug. It suggested that the entire sequence of the pKH 6002 genome was integrated in the chromosome in the major Ap$^R$ Km$^R$ transformants and the intact rhlE gene was replaced by the Km$^R$-inserted gene in the minor four Ap$^S$ Km$^R$ transformants. This result was as expected, since the replacement requires two homologous recombination events once at each side of the kanamycin-resistance gene, while the integration requires such an event just once at either side (Fig. 3). However, the appearance of the disruptants at about only one order less than that of the integrants suggested that the rhlE gene could be disrupted without an accompanied suppressor mutation (see below).

To confirm the DNA rearrangement occurred in the Ap$^R$ Km$^R$ and Ap$^S$ Km$^R$ transformants, the chromosomal DNAs were isolated from such transformants and also from the parental strain JM101, and subjected to Southern blotting analysis with a probe carrying a part of the rhlE gene (474 bp EcoRV fragment from 573 to 1046). The DNA sample from JM101 or the Ap$^S$ Km$^R$ transformants generated a single band, which had the size expected from the intact sequence or the Km-inserted one, respectively, (Fig. 3 and Fig. 4, lanes 1, 3, and 4). The DNA sample from the Ap$^R$ Km$^R$ transformant generated both of the two bands (Fig. 4, lane 2). This result is consistent with the conclusion that the rhlE gene is replaced by the corresponding region with Km$^R$-insertion in the Ap$^S$ Km$^R$
Fig. 4. Southern blotting analysis of two types of transformants. Chromosomal DNAs were prepared as described (Silhavy et al., 1984). Their PstI digests were electrophoresed in an 1% agarose gel, transferred to a Zeta-Proxy™ blotting membrane (Bio-Rad), and hybridized with digoxigenin-labeled EcoRV fragment. The hybridized probes were detected with antidigoxigenin:alkaline phosphatase conjugate. Lane 1, JM101; lane 2, ApKmR transformant; lanes 3 and 4, two independent ApKmR transformants; lane 5, length markers (1145 and 664 bp fragment). The band a corresponds to the 1809 bp fragment from PstI (at position 1) to PstI (1810) and b corresponds to the 940 bp fragment from PstI (1) to PstI (the end of the kanamycin-resistance gene).

transformants, and the whole sequence of pKH6002 is integrated in the ApKmR transformant.

Since the above results do not necessarily exclude the possibility that the ApKmR transformants might carry a secondary mutation allowing the disruptants to survive, the rhlE gene disruption was examined by another method using a lambda phage vector with the temperature-sensitive repressor mutation c1857 (Silhavy et al., 1984). The whole sequence of pKH6002 linearized by EcoRI was inserted into λgt-λC (Thomas et al., 1974) to construct λΔrhlE::Km. JM101 strain lysogenized with this hybrid phage could be identified by the ApKmR and temperature-sensitive phenotype. Curing of the lysogenized phage by a short (1 min) heat pulse at 42°C generated temperature-resistant clones at approx. 10^{-2}. Nearly half of them were resistant to kanamycin and sensitive to ampicillin, implying that the rhlE gene can be disrupted without loss of viability, not accompanied by any suppressor mutation. Since the disruptants showed no difference from the original strain with regard to growth rate or colony size, the rhlE gene appears to be dispensable for cell growth under the normal conditions.

4. DISCUSSION

In this paper we have described mapping of the rhlE gene on the E. coli chromosome and determination of its complete sequence. We developed a new
method for gene disruption using a mutant of ColE1-type plasmid. The conventional methods for gene disruption in *E. coli* using a ColE1-type plasmid can be applied to only in special bacterial strains, because they take advantage of the fact that the vectors cannot be replicated in *polA* strains (Gutterson and Koshland, 1983), or cannot be stably maintained in *recB* *recC* *sbcB* (Winans et al., 1985) or in *recD* strains (Russell et al., 1989). Contrarily, the plasmid constructed in this work cannot be replicated in the wild-type strains, although it can be maintained as a high-copy plasmid in *rnhA* strains. The gene disruption method with this plasmid can be, therefore, applied to most of *E. coli* strains, even without separating the vector portion from the chromosomal region. If the gene in question were dispensable, transformation with the circular DNAs generates integration of the whole plasmid sequence into the chromosome predominantly and disruption/replacement less frequently. The relative frequency of the disruptants against the integrants can be increased by using the linearized DNAs for transformation, because integration becomes unfavorable unless the cleaved DNAs were religated within the cells. With this newly developed method, we were able to isolate a null mutant of the *rhlE* gene with no discernible growth defects. The *rhlE* gene might be just a remnant of an old gene which has lost its role during evolution, or its function might be exerted by one of the other D-E-A-D box genes in *E. coli*.

The *E. coli* chromosome carries many helicase genes. Most of the DNA helicase genes were first identified through isolating mutants defective in DNA replication, repair or recombination, and then their gene products were purified and shown to possess an activity to unwind duplex regions of DNA-DNA or DNA-RNA (Matson, 1991; and references therein). In contrast, studies on the putative RNA helicases encoded by the D-E-A-D box genes in *E. coli* are at present limited to isolating and sequencing of the genes. The *E. coli* strains in which *rhlB* or *rhlE* was disrupted were isolated, but they show no definite phenotype (Kalman et al., 1991; this work). The *srmB* and *deaD* genes, when overexpressed, suppress the temperature-sensitive mutation in the ribosomal protein L24 and S2, respectively, but their normal functions at a single copy level on the chromosome are obscure (Nishi et al., 1988; Toone et al., 1991). Since no functional complementation is observed between *srmB* and *deaD* or between *srmB* and *rhlB* (Kalman et al., 1991; Toone et al., 1991), these putative RNA helicases probably have specificity for RNA to act on, recognizing a primary sequence or secondary structure of substrate RNAs. Such specific interactions with RNA might be due to the carboxyl-terminal regions of the D-E-A-D box proteins, which differ in length and sequence but contain more basic amino-acids than acidic ones in general. Identification of such an RNA substrate and biochemical studies on the products of the D-E-A-D box genes will facilitate further understanding the functions of the *E. coli* RNA helicases.
I thank Dr. M. Cashel for informing me the DNA sequence of the rhlE PCR fragment, Drs. S. Kanaya, H. Inokuchi, and Y. Kohara for providing bacterial and phage strains, and also Dr. T. Nagata for improving the manuscript. This work was supported in part by Grants for the Priority Research Field ‘Comprehensive Analysis of the E. coli Genome’ from the Ministry of Education, Science and Culture, Japan.

REFERENCES


Linder, P., Lasko, P. F., Ashburner, M., Leroy, P., Neilson, P. J., Nishi, K., Schnier, J. and
Masukata, H. and Tomizawa, J. (1984). Effects of point mutations on formation and structure of
Biol. 40, 289–326.
suppressing replication-defective mutations of the ColE1 plasmid. Proc. Natl. Acad. Sci. USA
81, 550–554.
protein is a suppressor of an Escherichia coli mutant defective in 50S ribosomal subunit assembly.
plasmid to replicate in Escherichia coli cells with or without RNase H. J. Mol. Biol. 198, 223–
234.
Ray, B. K., Lawson, T. G., Kramer, J. C., Cladaras, M. H., Grifo, J. A., Abramson, R. D., Merrick,
Rudd., K. E. (1992). Alignment of E. coli DNA sequences to a revised, integrated genomic
restriction map. In: A Short Course in Bacterial Genetics (ed.: J. H. Miller), Section 2A. Cold
Microbiol. 6, 283–292.
presumed ATP-dependent RNA helicase, can suppress a mutation in rpsB, the gene encoding
Vieira, J. and Messing, J. (1987). Production of single-stranded plasmid DNA. Methods in En-
zymol. 153, 3–11.
α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a
common nucleotide binding fold. EMBO J. 8, 945–951.
Yanish-Perron, C., Vieira, J. and Messing, J. (1985). Improved M13 phage vectors and host strains:
nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33, 103–119.