Gene, 85 (1989) 25–33
Elsevier

GENE 03284

The P2 phage *old* gene: sequence, transcription and translational control

(Bacteriophage; codon usage; plasmid; promoter; recombinant DNA; tRNA)

Elisabeth Haggård-Ljungquist [a], Virginia Barreiro [a], Richard Calendar [b], David M. Kurnit [c] and Hans Cheng [b]

[a] Department of Microbial Genetics, Karolinska Institutet, S-10401 Stockholm 60 (Sweden); [b] Department of Molecular and Cell Biology, Wendell M. Stanley Hall, University of California, Berkeley, CA 94720 (U.S.A.) Tel. (415)642-5951 and [c] Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109 (U.S.A.) Tel. (313)747-4747

Received by Y. Sakaki: 18 April 1989
Revised: 15 June 1989
Accepted: 17 July 1989

SUMMARY

The *old* (*o*vercoming *l*ysogenization *d*efect) gene product of bacteriophage P2 kills *Escherichia coli recB* and *recC* mutants and interferes with phage λ growth [Sironi et al., Virology 46 (1971) 387–396; Lindahl et al., Proc. Natl. Acad. Sci. USA 66 (1970) 587–594]. Specialized transducing λ phages, which lack the recombination region, can be selected by plating λ stocks on *E. coli* that carry the *old* gene on a prophage or plasmid [Finkel et al., Gene 46 (1986) 65–69]. Deletion and sequence analyses indicate that the *old*-encoded protein has an M_r of 65 373 and that its transcription is leftward. Primer extension analyses locate the transcription start point near the right end of the virion DNA. A bacterial mutant, named *pin*3 and able to suppress the effects of the *old* gene, has been isolated [Ghisotti et al., J. Virol. 48 (1983) 616–626]. In a *pin*3 mutant strain, carrying the *old* gene on a prophage or plasmid, the amount of *old* transcript is greatly reduced. The effect of the *pin*3 mutation is abolished by the wild-type allele of *argU*, an arginine tRNA that reads the rare Arg codons AGA and AGG, which are used for eight of the 14 Arg codons in the *old* gene. Thus the *pin*3 allele probably stalls translation of the *old* mRNA, causing this mRNA to be degraded. Isoelectric focusing and electrophoretic analysis identify the *old* gene product as a basic protein of approx. 65 kDa.

INTRODUCTION

Temperate coliphage P2 encodes a gene called *old* that causes killing of *E. coli recB* and *recC* mutants (Sironi, 1969) and interferes with the growth of phage λ (Lindahl et al., 1970). Interference with λ growth involves the inhibition of λ late gene expression and the inhibition of *E. coli* RNA and protein

Correspondence to: Dr. E. Haggård-Ljungquist, Department of Microbial Genetics, Karolinska Institutet, Box 60400, S-10401 Stockholm (Sweden) Tel. (46-8)7287338; Fax (08)339380; *and to:* Dr. R. Calendar, Department of Molecular and Cell Biology, Wendell M. Stanley Hall, University of California, Berkeley, CA 94720 (U.S.A.) Tel. (415)642-5951; Fax (415)643-9290.

Abbreviations: aa, amino acid(s); am, amber mutant/mutation; AMV, avian myeloblastosis virus; bp, base pair(s); DTT, dithiothreitol; Exo, exonuclease; nt, nucleotide(s); Old, overcoming lysogenization defectiveness; oligo, oligodeoxyribonucleotide; ORF, open reading frame; PolIk, Klenow (large) fragment of *E. coli* DNA polymerase I; RBS, ribosome-binding site; RT, reverse transcriptase; *tsp*, transcription start point(s); wt, wild type; [ ], denotes plasmid-carrier state.

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synthesis (Sironi et al., 1971). The *old* gene product requires the *E. coli pin* (*P2 in*terference) genes for its action (Ghisotti et al., 1983). The first λ mutants selected for their ability to make plaques in a P2-lysogenic strain were λ *bio* transducing phages. They had lost their *gam* gene, which is responsible for inactivating *recBC* nuclease (Karu et al., 1975), and their recombination genes *exo* and β (Zissler et al., 1971). Selection for genetically engineered λ transducing phages is often performed by plating on *E. coli* that carries P2 prophage (Murray, 1983) or the P2 *old* gene on a plasmid (Finkel et al., 1986). To be able to

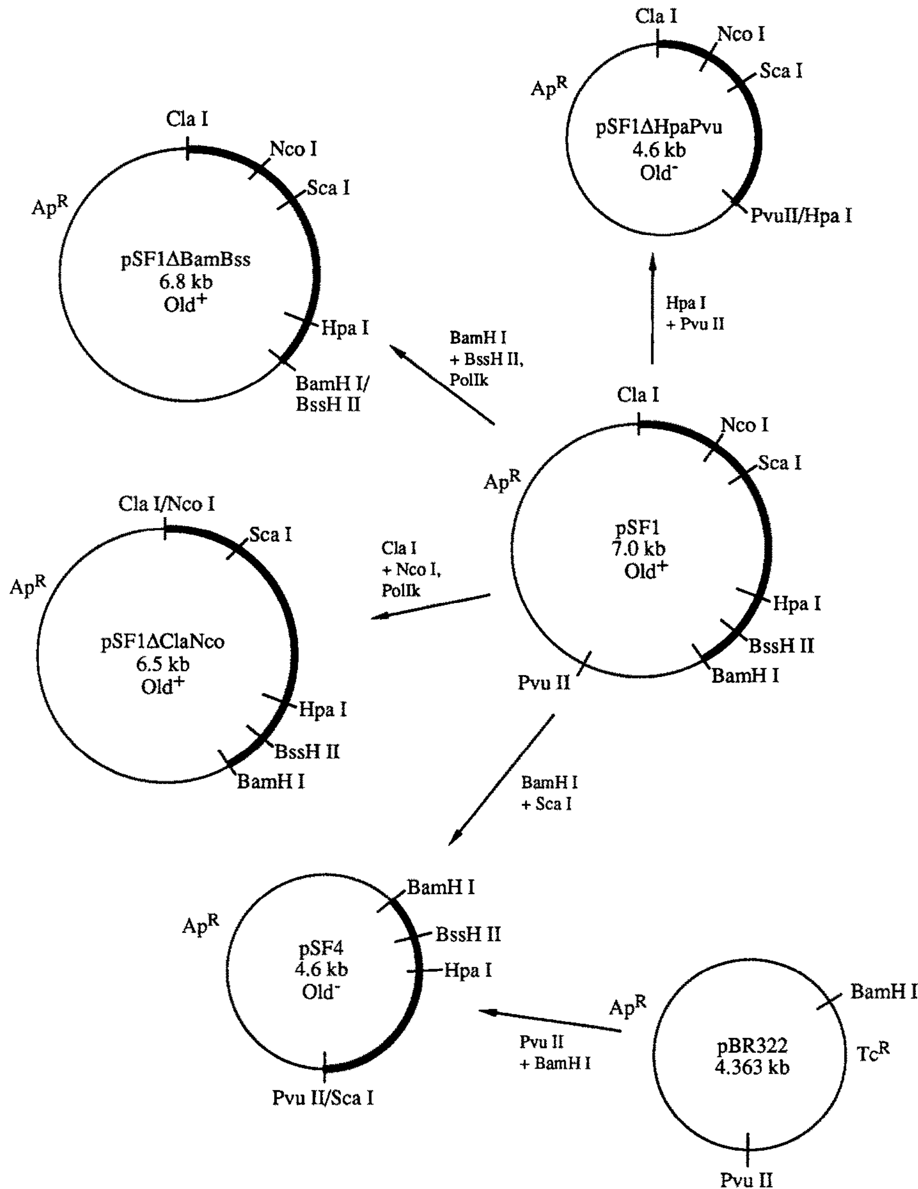

Fig. 1. Plasmids containing deletions of the *old* region. The Old$^+$ phenotype was determined by interference with λ phage growth as described by Finkel et al. (1986). The thin lines represent DNA from plasmids, whereas the thick lines are DNA from P2 phage. Plasmid pBR322 is described by Bolivar et al. (1977); pSF1 is described by Finkel et al. (1986). Deletions of pSF1 were constructed as follows. Cleavage with *Hpa*I + *Pvu*II followed by ligation yielded pSF1ΔHpaPvu. Cleavage with *Bam*HI + *Bss*HII was followed by filling-in with PolIk (Jacobsen et al., 1974) and ligation, to give pSF1ΔBamBss. Cleavage with *Cla*I + *Nco*I, followed by filling and ligation yielded pSF1ΔClaNco. pSF1 was cleaved with *Bam*HI + *Sca*I; the fragment containing only P2 DNA was purified by electrophoresis in low melting agarose and was ligated to the large fragment of pBR322 cut with *Bam*HI + *Pvu*II, to give pSF4. Plasmid pUC18 (Norrander et al., 1983) was used as the vector for nt sequence analysis.

clone the *old* gene more precisely, to overproduce its product and to study its mechanism of action, we have determined its location by deletion and sequence analyses and have identified the *old* gene product.

RESULTS AND DISCUSSION

(a) Deletion analysis

To locate the *old* gene more precisely we deleted DNA from the plasmid pSF1, which contains an active *old* gene in a 3-kb *Cla*I-*Bam*HI fragment of P2 DNA (Figs. 1 and 2). We deleted 500 bp from the left side using *Nco*I without loss of the Old$^+$ phenotype. When we removed another 650 bp from the left side using *Sca*I we lost the Old$^+$ phenotype. From the right side of the P2 DNA fragment we deleted 240 bp using *Bss*HII without loss of the Old$^+$ phenotype. Removal of 700 bp from the right side using *Hpa*I destroyed the Old$^+$ phenotype. Thus all of the *old* gene lies between the *Nco*I (93.1%) and *Bss*HII (100%) sites. The *Sca*I (95.1%) and *Hpa*I (98.6%) sites must lie within the *old* gene.

(b) Sequence analysis

As shown in Figs. 2 and 3, the *Nco*I-*Bss*HII region contains only one long ORF, going from right to left on the P2 genetic map. It begins at nt 214 with an AUG start codon and ends at nt 1971 with an *ochre* stop codon, giving a polypeptide of 586 aa. The AUG codon is preceded by a potential RBS (Steitz and Jakes, 1975) centered 9 nt upstream from the start codon, and as the next in-frame Met codon is located 149 aa further downstream and has no potential RBS, the first Met is the likely start codon.

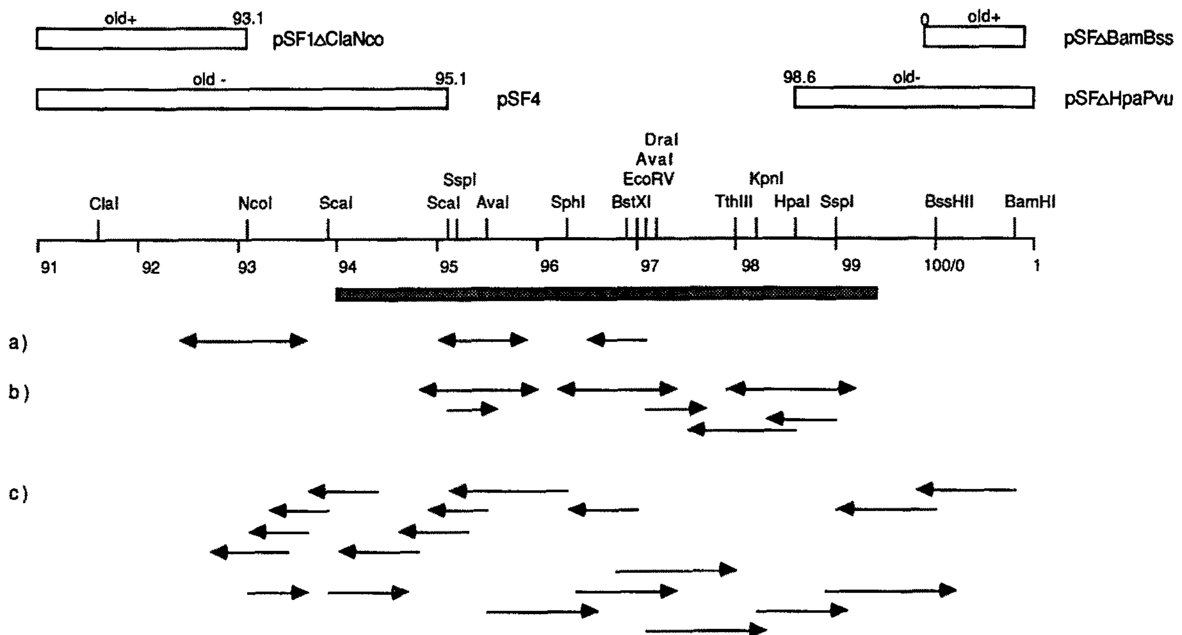

Fig. 2. Physical map of the *old* region. The open boxes above the line represent the deletions of P2 DNA from pSF1 in the plasmids shown in Fig. 1. The restriction sites used for sequencing are indicated above the line, and their distances in percentage from the left end of the linear P2 genome are indicated below. The total length of P2 is assumed to be 33 kb (Chattoraj, 1977). The shaded bar below the line shows the location of the *old* gene. The arrows below the line indicate the regions sequenced using the chemical cleavage method of Maxam and Gilbert (1980) with 5′-end labeled DNA (**a**) or 3′-end-labeled DNA (**b**) or the chain-termination method of Sanger et al. (1977) (**c**). In the Maxam–Gilbert method purified phage DNA was cleaved with appropriate restriction enzymes, and the 3′ termini were labeled with [α-^{32}P]ddATP and terminal deoxynucleotidyl transferase or the 5′ termini with [γ-^{32}P]ATP and T4 polynucleotide kinase. After cleavage with a second restriction enzyme, the fragments were separated by gel electrophoresis, isolated and chemical sequencing was performed. In the method of Sanger et al. (1977), the DNA was subcloned into pUC18 (Norrander et al., 1983), and when necessary the insert was reduced by digestion with exonuclease III. [α-^{35}S]dATP and T7 Sequenase were used for the sequencing reaction, using the 'universal' or 'reverse' primers of M13.

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    <------- P2 right end

5'-GTGCTTTCCCCGCCTCGCCCGCCCGCTTCACGGGGCGGTTTTAATGCACCTTAATAAAACTAATGAGCCATCAGTATTTCCAGTCAAAAGCCAAGCCATATCACAGT
   1        cos
                                                                                                                  1
                                                                                                                Met
TTCCCTTAATGCAATCTCATGCACCCTTGAATGCAACGACTATAAAATGTAAAAAAGCGTTTCGTATGAACTCATTGCATACAAATAGCACATTTAAGGAAAAATA ATG
108                  -35    -35                -10   -10                                             RBS

2
Thr Val Arg Leu Ala Ser Val Ser Ile Ser Asn Phe Arg Ser Cys Lys Ser Thr Ser Ala Ile Leu Arg Pro Phe Thr Ala Leu
ACT GTA CGT CTT GCT TCA GTT TCA ATT AGC AAC TTT CGT TCT TGT AAG TCA ACA TCG GCT ATT TTG CGC CCC TTC ACT GCT TTA
217                                             -----------------------------
                                                GCAAGAACATTCAGTTGTAGCCG-5'
30
Val Gly Tyr Asn Asn Ala Gly Lys Ser Asn Ile Ile Leu Ala Ile Lys Trp Leu Leu Asp Gly Ser Leu Ile Ser Glu Ser Asp
GTT GGT TAT AAT AAC GCA GGA AAA TCA AAT ATT ATC TTA GCA ATT AAG TGG CTG TTA GAT GGC TCT TTG ATT TCA GAA TCA GAT
301                                 SspI 99.0

58
Val Tyr Asp Pro Thr His Pro Val Ser Val Glu Gly Val Ile Gln Gly Ile Thr Asp Asp Thr Leu Ser Leu Leu Thr Glu Glu
GTG TAC GAT CCT ACA CAC CCC GTA TCC GTT GAA GGC GTT ATT CAA GGT ATA ACA GAT GAT ACT CTG TCT TTG TTA ACA GAA GAG
385                                                                                          HpaI 98.6

86
Asn Gln Gln Lys Ile Ala Pro Phe Ile Ile Asp Gly Thr Leu Thr Phe Ala Arg Arg Gln Glu Phe Asn Lys Glu Thr Gly Lys
AAT CAA CAA AAA ATA GCT CCG TTT ATA ATT GAC GGG ACT CTT ACC TTT GCA AGA AGA CAG GAA TTT AAT AAG GAA ACA GGA AAA
469

114
Ala Lys Lys Ser Leu Asp Val Tyr Asp Gly Thr Thr Trp Lys Lys Asn Pro Gly Gly Ile Asp Gly Ala Ile Ser Asn Ile Phe
GCC AAA AAA AGT CTG GAT GTA TAT GAC GGT ACC ACG TGG AAG AAA AAC CCT GGC GGC ATA GAT GGT GCA ATC TCC AAT ATA TTT
553                                 KpnI 98.2

142
Pro Glu Pro Ile His Ile Pro Ala Met Ser Asp Ala Val Glu Asp Ser Thr Lys Cys Lys Asn Thr Thr Thr Ile Gly Lys Ile
CCG GAA CCA ATT CAT ATC CCT GCA ATG TCT GAC GCT GTC GAA GAC TCA ACA AAA TGC AAA AAC ACG ACC ACA ATA GGA AAG ATA
637                                     TthIII 98.0

170
Leu Ser Ala Ile Val Ser Glu Ile Lys Gln Glu His Glu Glu Lys Phe Ser Lys Asn Ile Ser Glu Ile Gly Lys Tyr Leu Ser
CTT TCC GCA ATT GTT TCT GAA ATA AAA CAA GAA CAC GAA GAA AAA TTT TCA AAA AAC ATA TCA GAA ATA GGT AAA TAT CTT TCT
721

198
His Asn Gly Glu Asn Arg Leu Glu Ser Leu Asn Lys Ile Asp Ser Gly Val Asn Lys Lys Val Asn Gln Phe Phe Pro Asp Val
CAC AAC GGT GAG AAC AGA TTA GAA AGC CTT AAT AAA ATA GAC TCA GGT GTA AAT AAA AAA GTA AAC CAA TTT TTT CCT GAC GTA
805

226
Ser Val Lys Leu His Phe Pro Thr Pro Thr Leu Asp Glu Ile Phe Lys Ser Gly Thr Leu Lys Val Phe Glu Ser Arg Glu Asp
AGT GTA AAG TTA CAC TTT CCC ACA CCG ACA TTA GAT GAG ATA TTT AAA TCC GGC ACT CTG AAA GTT TTT GAG TCT CGG GAA GAT
889                                                     DraI 97.2                        AvaI 97.1

254
Glu Pro Val Met Arg Asp Ile Ser Arg Phe Gly His Gly Thr Gln Arg Ser Ile Gln Met Ala Leu Ile Gln Tyr Leu Ala Glu
GAA CCG GTA ATG AGA GAT ATC AGC CGA TTT GGT CAC GGA ACA CAA CGT TCC ATT CAA ATG GCA TTA ATT CAA TAC CTG GCC GAA
973                 EcoRV 97.0                              ↓        BstXI 96.9
                                                        A,old1,His
282
Ile Lys Lys Glu Asn Ser Glu Ser Lys Lys Ser Asn Thr Leu Ile Phe Ile Asp Glu Pro Glu Leu Tyr Leu His Pro Ser Ala
ATA AAA AAA GAA AAC AGC GAA TCA AAA AAA TCA AAC ACT TTG ATT TTC ATT GAT GAA CCT GAG TTA TAT TTA CAC CCT TCA GCC
1057

310
Ile Asn Ser Val Arg Glu Ser Leu Val Thr Leu Ser Glu Ser Gly Tyr Gln Val Ile Ile Ser Thr His Ser Ala Ser Met Leu
ATT AAT TCT GTC AGA GAA TCA CTT GTC ACA TTA AGT GAA TCA GGG TAT CAG GTT ATA ATA TCA ACT CAC TCA GCC AGT ATG CTT
1141

338
Ser Ala Lys His Ala Ala Asn Ala Ile Gln Val Cys Lys Asp Ser Asn Gly Thr Ile Ala Arg Lys Thr Ile Ser Glu Lys Ile
TCT GCA AAG CAT GCA GCA AAT GCG ATT CAG GTT TGT AAG GAT TCT AAT GGA ACC ATA GCA AGG AAG ACT ATA TCT GAA AAA ATC
1225     SphI 96.3

366
Glu Glu Leu Tyr Lys Ser Ser Ser Pro Gln Leu His Ser Ala Phe Thr Leu Ser Asn Ser Ser Tyr Leu Leu Phe Ser Glu Glu
GAA GAA TTA TAT AAA TCT TCA TCA CCG CAA TTG CAC TCA GCA TTC ACG CTT TCT AAT TCA TCA TAC CTT TTA TTT TCT GAA GAA
1309

394
Val Leu Leu Val Glu Gly Lys Thr Glu Thr Asn Val Leu Tyr Ala Leu Tyr Lys Lys Ile Asn Gly His Glu Leu Asn Pro Ser
GTT TGG CTT GTT GAA GGG AAA ACA GAG ACA AAC GTT CTA TAT GCA CTT TAT AAA AAA ATT AAC GGA CAT GAA CTC AAC CCG AGT
1393                                                                                              AvaI 95.5

422
Lys Ile Cys Ile Val Ala Val Asp Gly Lys Gly Ser Leu Phe Lys Met Ser Gln Ile Ile Asn Ala Ile Gly Ile Lys Thr Arg
AAA ATC TGC ATT GTT GCC GTT GAC GGT AAG GGT AGT TTA TTT AAG ATG TCA CAA ATC ATC AAT GCC ATC GGC ATA AAA ACA AGG
1477

450
Ile Leu Ala Asp Cys Asp Phe Leu Ser Asn Ile Leu Leu Thr Glu His Lys Asp Leu Leu Ser Thr Glu Cys Asp Asn Leu Leu
ATT CTA GCT GAT TGT GAT TTC TTA TCA AAT ATT CTA TTA ACA GAG CAT AAA GAC TTA CTT AGT ACT GAA TGT GAT AAT CTA TTG
1561                                SspI 95.2        ↓                     ScaI 95.1
                                                 T, am11
478
Thr Ala Leu Ile Glu Ser Ile Asn Ser Gly Glu Leu Ser Leu Asn Thr Lys Val Thr Thr Phe Glu Ser Phe Lys Ser Ile Ser
ACT GCT TTG ATT GAA TCA ATC AAC TCA GGA GAG CTT AGT TTA AAT ACA AAA GTT ACT ACT TTT GAG TCA TTC AAA AGC ATT TCA
1645

506
Ser Lys Asp Phe Ile Lys Ile Cys Asn His Glu Lys Thr Gln Lys His Ile His Glu Ile His Gln Lys Leu Lys Asp Asn Gly
AGC AAA GAC TTC ATC AAA ATA TGT AAC CAT GAA AAA ACA CAA AAG CAT ATA CAT GAA ATA CAT CAA AAA TTG AAA GAT AAC GGA
1729

534
Ile Tyr Ile Trp Lys Ser Gly Asp Ile Glu Ala Val Tyr Gly Phe Gly Lys Lys Gln Thr Glu Trp Asp Ser Leu Leu Asp Cys
ATC TAT ATA TGG AAA TCC GGT GAT ATA GAG GCT GTT TAT GGA TTT GGT AAA AAA CAA ACT GAA TGG GAT AGT CTT TTA GAT TGT
1813

562
Leu Cys Asp Glu Ser Lys Asp Val Arg Ala Val Ile Lys Lys Tyr Asp Glu Met Glu Asp Phe Ile Lys Trp Ile TER
TTA TGT GAT GAA AGC AAA GAT GTT AGA GCA GTA ATA AAA AAA TAT GAT GAA ATG GAA GAT TTC ATA AAA TGG ATT TAA CTTAATC
1897

TGATACTATTACAGCACAGTACT-3'
1982          ScaI 93.9
                                 P2 left end ------->
```

The ORF is confirmed as that coding for the *old* gene product by the locations of the *old*1 missense mutation and the *old*am11 mutation. The *old*1 mutation causes the C-1018 → A transversion (Fig. 3), with an Arg-269 to His replacement. The *old*am11 mutation causes the normal G-1603 to be replaced with a T, giving the am codon UAG.

The *old* gene is expressed constitutively and is therefore expected to have a promoter that is recognized by the *E. coli* RNA polymerase. Between the *cos* sequence and the presumed start codon of *old* there are two promoter-like sequences (Rosenberg and Court, 1979): at nt 126 there is a potential −35 region with the sequence ATGCAC followed by a potential −10 region with the sequence TAAAAT 18 nt further down; at nt 135 there is an alternative −35 region with the sequence TTGAAT followed by a potential −10 region with the sequence TAAAAA 17 nt further down, although the rightward −10 region lacks the highly conserved terminal T (Fig. 3).

(c) Location of the 5′ termini of the *old* transcript

To map the *tsp* of the *old* gene transcript, a 5′ labeled synthetic 23-mer, located about 90 nt from the presumed 5′ end of the *old* transcript (Fig. 3) was hybridized to RNA extracted from the P2 lysogenic strain C-117 or as a control from the non-lysogenic strain C-1a and elongated with RT. Using RNA from the P2 lysogenic strain C-117, two major extension products were detected (Fig. 4, lanes 3 and 4). The appearance of bands at these positions is consistent with the *tsp* of the *old* mRNA being 46 or 44 nt upstream from the start codon at nt 214, i.e., at nt 168 and 170, respectively (Fig. 3). Thus the −10 and −35 regions located farthest away from the start codon seem to be preferred in the P2-lysogenic strain. Using RNA from cells carrying the *old* gene on a plasmid (pSF1), we obtained the same extension products (Fig. 4, lanes 11 and 12), but the bands are more intense due to the increased gene dosage.

(d) Levels of the *old* gene transcript are reduced by the *Escherichia coli pin*3 mutation

Bacterial mutants able to suppress the effects of the *old* gene are named *pin* (Ghisotti et al., 1983). To test if the suppression is due to an absence of *old* mRNA, a primer extension analysis was performed comparing the RNA from *pin*3 mutant strains, carrying the *old* gene on a prophage (C-5024) or on a plasmid (pSF1), using the same 5′ labeled primer as above. As can be seen in Fig. 4, the *old* transcript is not detectable in strain C-5024 and very reduced in the *pin*3 strain carrying plasmid pSF1. Therefore, either *old* transcription decreased in *pin*3 strains or the *old* mRNA is rapidly degraded.

(e) The effect of the *pin*3 mutation is suppressed by the wild-type *argU* gene

The *pin*3 mutation maps at 12.2 min on the *E. coli* map (Ghisotti et al., 1983). This position corresponds to that of *argU*, which encodes the precursor of a $tRNA^{Arg}$ that reads the rare codon AGA and probably also the rare codon AGG (Garcia et al., 1986). Thus we tested the effect of wt *argU* gene (plasmid pBR1824; Mullin et al., 1984) on *E. coli pin*3 strain C-5024. This *argU* plasmid restored the ability of the *old* gene to interfere with phage λ growth. Inspection of the *old* gene sequence shows that the AGA codon appears six times (103, 104, 203, 258, 314, 570), and the AGG codon appears twice (358, 449). The other Arg codons, which begin with CG, are used only six times (4, 14, 24, 251, 262, 269). Thus the majority of Arg codons are those rare ones read by *argU* tRNA, whereas these codons represent only 4% of the Arg codons in *E. coli* (Aota et al., 1988). Grosjean and Fiers (1982) suggested that the appearance of rare codons slows down translation and Spanjaard and Van Duin (1988) showed that the sequence AGG AGG causes ribosomal frameshifting. We suppose that, when the

Fig. 3. Nucleotide sequence of the P2 wt *old* gene region and deduced aa sequence. The proposed promoters are indicated with the underlined −10 and −35 regions. The location of the 5′ termini (*tsp*) of the *old* gene transcript is indicated by the arrow above the line. The restriction sites used for the nt sequence determination are underlined, along with the nt and aa changes resulting from the *old*1 and 11 mutations. The primer used for the primer extension analysis is shown in outlined letters below the line. The GenBank accession number for this sequence is M27131. P2 *old*am11 was isolated by screening for a phage that would make plaques on *E. coli recB sup*° but not on *E. coli recB supF*. These bacterial strains are WM1200 *hsdR araD*Δ*ara-leu*Δ*lac galU galK rpsL recB recC* [a derivative of MC1061 of Casadaban and Cohen (1980)], and WM1200 carrying *supF* on the plasmid pGFYπAN7 (Lutz et al., 1987; H.V. Huang and J. Schwedock, unpublished).

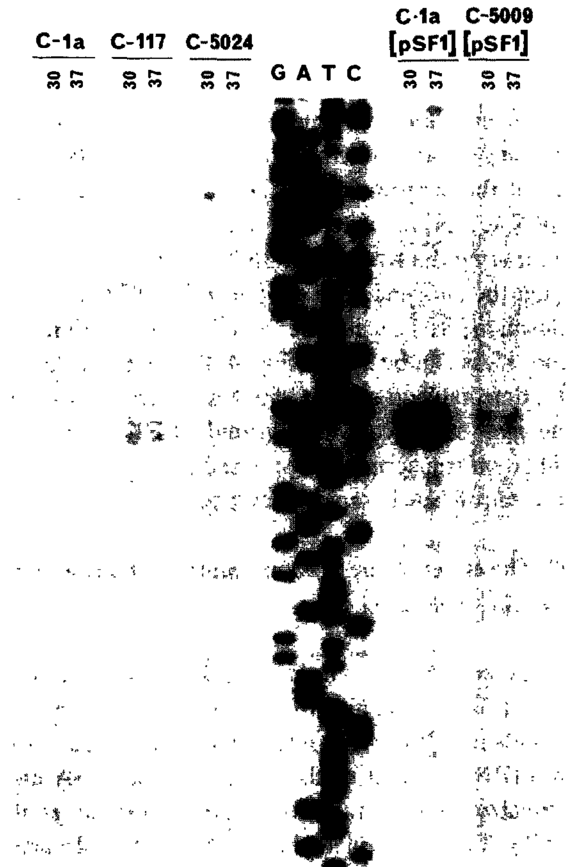

Fig. 4. Analysis of the *old* gene transcript by primer extension. RNA was extracted from the cells listed above the respective lane, hybridized to the 5′-labeled primer (Fig. 3) at the temperatures (in °C) indicated above the lanes, extended by RT and analyzed by gel electrophoresis. G, A, T, and C indicate the respective sequencing reactions (Fig. 2). C-1a is a prototrophic *E. coli* C strain (Sasaki and Bertani, 1965). C-117 is C-1a lysogenized with P2 (Bertani, 1968). C-5009 is a *pin*3 (Ghisotti et al., 1983) derivative of C-1a and C-5024 is a P2 lysogenic derivative of C-5009. Total RNA was extracted from 20-ml cultures containing 4×10^8 bacteria per ml as described by Hagen and Young (1978). The primer (Fig. 3) was labeled at the 5′ termini with ^{32}P as follows: 50 pmol of the oligo was incubated with 10 units of T4 polynucleotide kinase and 200 μCi [γ-^{32}P]ATP (3000 Ci/mmol), using the reaction conditions recommended by the supplier (Promega). Polynucleotide kinase was inactivated by incubation at 65°C for 15 min, and the labeled oligo was precipitated with ethanol until no radioactivity could be detected in the supernatant after centrifugation. The 5′ end-labeled oligo was thereafter used to prime synthesis as follows: 5 μg RNA was incubated with 5×10^{-2} pmol 5′ end-labeled oligo in 40 mM Pipes pH 6.4/0.4 M NaCl/1 mM EDTA/80% formamide in a total volume of 30 μl at 30°C or 37°C overnight. The primed RNA was ethanol-precipitated, redissolved in 10 μl 2 × AMV buffer (100 mM Tris·HCl pH 8.3/16 mM $MgCl_2$/60 mM KCl/2 mM DTT) and 4 μl dNTP mix (containing each of the four dNTPs at 10 mM), 0.5 μl RNasin (40 units/μl), 4.5 μl H_2O and 1 μl AMV RT (10 units/μl) were added and the sample was incubated at 42°C for 1 h. The sample was precipitated with ethanol, dried and dissolved in formamide-dye buffer described by Maxam and Gilbert (1980). Dideoxy sequencing ladders were generated using the 5′ end-labeled oligo hybridized to the 99–100% region of P2 DNA cloned into pUC18 and T7 Sequenase using standard conditions. All samples were heated to 90°C for 3 min, chilled on ice and loaded onto an 8% polyacrylamide/7 M urea sequencing gel.

ribosome reaches the two tandem AGA codons at positions 103 and 104 in the *old* gene, translation stalls or shifts frame and the *old* mRNA is degraded.

(f) Identification of the Old protein and comparison to other proteins

To identify the Old protein we performed two-dimensional gel electrophoresis of proteins made in *E. coli* strain C carrying either pSF1 or pSF5, which carries the *old*am11 allele (Fig. 5). We identified a basic protein that is present in cells carrying pSF1 (*old*$^+$) but which is absent in cells carrying pSF5 (Fig. 5). The mobility of this protein during electrophoresis on SDS-polyacrylamide gels is between the mobilities of the GroEL protein (63 kDa) and the DnaK protein (69 kDa). Its electrophoretic position suggests that the Old protein bands at about 65 kDa, in agreement with the M_r 65 373 deduced from the nt sequence. The protein that we have identified as the *old* gene product is among the basic proteins found in *E. coli*, having a pI of about 7.

Gibbs et al. (1983) assigned the P2 Old protein an M_r of 29 500. However, the *old* gene is followed by an ORF that might code for a 27-kDa protein (E.H.-L., unpublished). Thus, the protein found by Gibbs et al. (1983) using a deletion mutation might be the product of this ORF.

We compared the P2 Old polypeptide sequence with the proteins in the EMBL Swissprotein database, using the FASTP program (Lipman and Pearson, 1985) but found no significant similarities.

(g) The mechanism of Old protein action

The *recB*, *recC*, and *recD* genes code for exonuclease V (ExoV), a complex enzyme that has multiple activities and which plays an important role in homologous genetic recombination and DNA repair in *E. coli* (Amundsen et al., 1986). Infection of *E. coli*

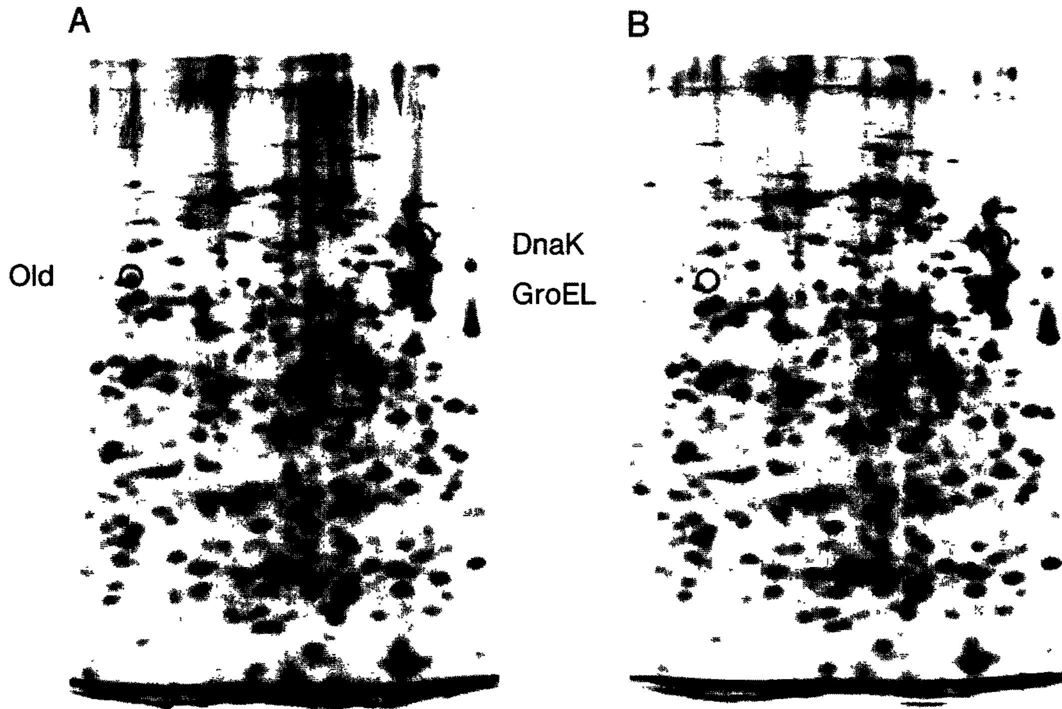

Fig. 5. Identification of the P2 *old* gene product synthesized in vivo: a comparison of the two-dimensional gel profiles of *E. coli* C-1a carrying pSF1 (old^+, left) or pSF5 (*old*am11, right). The circles denote the positions of the proteins encoded by genes *old*, *groEL* (M_r 62883) and *dnaK* (M_r 69121; upper of the two major spots). Exponential growth phase cultures were labeled for 1 min with [^{35}S]methionine (Hoyt et al., 1982). Cells were harvested, lysed and subjected to two-dimensional gel electrophoresis (O'Farrell, 1975).

lacking ExoV by P2 old^+ causes cell death (Sironi, 1969; Sironi et al., 1971). Bacteriophage λ is unable to grow on P2 old^+-lysogenic strains because the λ *gam* gene product inactivates ExoV, making the cells into RecBCD$^-$ phenocopies that are then killed by the P2 Old protein (Sakaki et al., 1973). The killing of *E. coli* by the Old protein has not been studied in *recB* or *recC* cells but only in P2-lysogenic cells infected by phage λ (Brégégère, 1974). Under these conditions protein synthesis stops and tRNA is functionally inactivated. The absence of functional tRNA stops translation, presumably causing λ mRNA to be degraded.

To begin studying the activities of the Old product, we can modify Brégégère's system and study *old* function in the absence of phage λ, i.e., in an *E. coli recB* strain where the *old* gene can be expressed from a plasmid. In cells lacking ExoV, synthesis of the Old protein would need to be controlled. This could be accomplished by cloning the *old* gene downstream from an inducible promoter that can be tightly repressed. Experimentation with this system will allow us to determine whether any other genes of P2 or λ are necessary for the phenomena observed by Brégégère (1974).

A specific explanation of Brégégère's findings is that the Old protein activates or is a nuclease that attacks tRNA from the 3′ end, removing the accessible CCA. Such a RNase would be similar to ExoI, which removes 5′-phosphoryl mononucleotides from the 3′ end of single-stranded DNA (Lehman and Nussbaum, 1964). Mutations that inactivate the ExoI-encoding gene (*xonA*) partially restore the recombination proficiency of *recB* and *recC* strains and cause the cells to grow faster (Kushner et al., 1972). Thus, ExoI is deleterious for *E. coli recB* mutants, and if the P2 Old protein had an Exo I-like activity it might be expected to kill *recB* mutant cells. To test these hypothetical enzyme activities of the *old* product, we can assay an *E. coli* strain that has been deleted for the *xonA* gene and which carries the old^+ gene on a plasmid.

ACKNOWLEDGEMENTS

This work was supported by grants 72 and 7078 from the Swedish Medical Research Council; by research grant AI-08722 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health; and by a Biotechnology Training Grant from the State of California. D.M.K. is an Investigator in the Howard Hughes Medical Institute at Ann Arbor, MI. The primer for analysis of the mRNA *tsp* was the generous gift of KabiGene. Strains C-5009 and C-5024 were a generous gift from Gianpiero Sironi. Thanks are due to Steven Finkel for characterizing some of the plasmids described; to Conrad Halling for helpful discussions on deletion analysis and analysis of the sequence data, and to Julie Schwedock for assistance in the isolation of P2 *old* amber mutants.

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