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Complete nucleotide sequence of *recD*, the structural gene for the α subunit of Exonuclease V of *Escherichia coli*

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ABSTRACT

Intracellular amplification of the Escherichia coli RecB and RecC proteins does not result in an increase in Exonuclease V activity unless the level of a third protein, encoded between the recB and argA genes, is also amplified. Nucleotide sequence analysis of this region reveals a 1,824 nucleotide open reading frame which would encode a protein of 608 amino acids with a calculated molecular weight of 66,973. This is assumed to be the structural gene for the α subunit of Exonuclease V, recently designated recD. The proposed initiation codon of the recD gene overlaps the termination codon of the upstream recB gene by one nucleotide, suggesting that these genes may form an operon. The deduced amino acid sequence of the RecD protein contains a region which is homologous to highly conserved sequences in adenine nucleotide binding proteins.

INTRODUCTION

Mutations in the recB and recC genes of E. coli lead to a reduced capacity for DNA repair and homologous genetic recombination (1,2), decreased viability (3) and deficiency in the enzyme known as Exonuclease V (4-6). Originally recognised as an ATP-dependent DNase, this enzyme has since been shown to exhibit a wide variety of catalytic activities, including ATP-dependent exonuclease, ATP-stimulated endonuclease, ATP-dependent DNA unwinding and DNA-dependent ATPase activities (see [7] for a review). Strand cleavage occurs during the unwinding of duplex DNA at Chi sequences, 5'-GCTGGTGG -3' (8,9), which locally stimulate recombination via the RecBC pathway (10,11).

The recB and recC genes, which code for proteins of 135 kDa and 125 kDa respectively (12-14), have been cloned into expression vectors, their products amplified and purified to homogeneity (15,16). Some ATP-dependent exonuclease activity characteristic of Exonuclease V (7) was obtained by adding the RecB and RecC proteins together, although the specific activity obtained was only about one tenth that of the native enzyme (16).

Lieberman and Oishi (17) reported that Exonuclease V could be dissociated

with 3.7 M NaCl into two fractions designated α and β. The β fraction complemented extracts from both recB and recC mutant strains for Exonuclease V activity but the α fraction failed to complement either extract, suggesting that the enzyme contained a protein component (α) that was not determined by either recB or recC (17).

We have found that amplification of intracellular levels of Exonuclease V requires amplification not only of the recB and recC genes but also of a third gene situated between recB and argA. This work will be described here together with an analysis of the nucleotide sequence of this region of the chromosome.

METHODS

Exonuclease assays

Exonuclease V was assayed in crude cell extracts by measuring the conversion of native λ DNA to acid soluble nucleotides, essentially as described (18).

Recombination assays

Strains to be tested for recombination proficiency were grown to OD_{650nm} 0.6 and mated with the strain N1617 (HfrC car::Tn10) for 40 minutes in LB medium. Following vortexing, the cultures were serially diluted in buffered saline and plated on selective plates in a 3 ml LB agar overlay. Recombinants were selected on LB medium containing 15 μg/ml tetracycline (Tc^r conferred by Tn10) and 50 μg/ml ampicillin (Ap^r conferred by the recombinant plasmid present in the recipient).

DNA Sequence analysis

DNA sequence analysis was performed by the dideoxy sequencing method (19) using single-stranded DNA from clones of M13 mp18 and mp19 phage (20). The source of the recD gene was pIDH201 which carries the entire thyA to argA region on a 19 kb BamHI fragment (21). The sequence of the recD gene was built up by determining the sequences of random clones of the 3.6 kb PstI fragment of pIDH201 (21). Storage and analysis of sequences was aided by computer programs (22,23). The entire sequence was determined on both strands.

RESULTS

Amplification of Exonuclease V

We previously fused the recB and recC genes individually to the phage λ leftward promoter in expression plasmids and purified the RecB and RecC

proteins (15,16). The expression vectors used, pPE237 and pPE223, are both derivatives of pBR322 (24) and are consequently incompatible in the same host. In order to amplify the RecB and RecC proteins together, we subcloned the *recB* and *recC* genes into compatible expression vectors in the hope of amplifying intracellular levels of Exonuclease V. To this end, an 8.7 kb EcoRI - XhoI fragment from pPE37 (15), which carries the *recC* gene and its own promoter (25), was cloned into the runaway replication vector pSY343 (26) to give the plasmid pPE5200. Following thermal induction, strains harbouring this plasmid synthesise greatly elevated levels of the RecC protein such that it can be detected in cell extracts by SDS PAGE (results not shown).

To achieve inducible expression of *recB*, the plasmid pPE536, which contains a 2.3 kb PstI-SalI fragment containing the coding sequence for the N-terminal portion of the RecB protein was cut with PstI and then digested with Bal-31. After restriction with SalI, deletion fragments of 2.3 kb or less were inserted between the SalI and SmaI sites of M13 mp9, and recombinants sequenced. An EcoRI-SalI fragment from one such plasmid, pPE556, in which the Bal-31 had deleted chromosomal sequences to within 25 nucleotides of the *recB* initiation codon, was cloned between the EcoRI-SalI sites of pAT153, to give pPE523. The complete *recB* structural gene was regenerated by cloning into pPE523 a 2.8 kb SalI fragment that contained the coding sequence for the C-terminal portion of the RecB protein, giving pPE505. Finally, a 76 bp EcoRI fragment from pDR720 that contains the *trp* promoter and operator (27) was cloned into pPE505 to give the plasmid pPE5100. This plasmid restores Exonuclease V activity and UV resistance to AB2470 (*recB21*). Elevated levels of the RecB protein can be observed by SDS

Table 1

Strain	Plasmid(s)	Specific activity (units/mg)	Amplification
W3110	pSY343	6.1	1.0
V186	pSY343	0.0	0.0
V186	pPE5100(*recB*$^+$)/pPE5200(*recC*$^+$)	14.7	2.4
V186	pPE5343(*recB*$^+$*C*$^+$)	20.7	3.4
V186	pPF307(*recB*$^+$*C*$^+$*D*$^+$)	245.5	40.0

Level of Exonuclease V activity in strain V186 (Δ(*thyA-argA*)) harbouring recombinant plasmids. The degree of amplification was normalised against the isogenic wild type strain W3110 harbouring the appropriate cloning vector.

PAGE in cell extracts of strains carrying pPE5100 after induction of the trp promoter with 10 μg/ml 3-indoyl acrylic acid.

The level of Exonuclease V activity was not increased following simultaneous derepression of the recB and recC genes in strain V186 (thyA-argA)$_{\Delta}$ harbouring plasmids pPE5100 (recB^{+}) and pPE5200 (recC^{+}) (Table 1). This is despite the presence of elevated levels of both the RecB and RecC proteins which could be observed by SDS PAGE. Also, no increase in enzyme activity is seen in strain V186 carrying the plasmid pPE5343 (Table 1). This plasmid was constructed from pSY343 by insertion of a 16.5 kb EcoRI-BamHI fragment from λIDH31 which carries thyA, recC and recB but not the neighbouring argA gene. However, the level of Exonuclease V activity was increased at least 40-fold by thermal induction of the strain V186 harbouring pPF307. This plasmid contains the 19 kb BamHI fragment from pIDH201, which carries the entire thyA-argA region of the chromosome, cloned into pSY343 (Table 1).

These results indicate that amplification of the RecB and RecC proteins alone does not lead to increased levels of Exonuclease V unless accompanied by increased expression of a third gene located between recB and argA. Amundsen et al. (28) recently reported that a certain class of mutations, previously designated recB‡, which inactivate Exonuclease V activity but are proficient at repair and recombination, inactivate the α subunit of Exonuclease V (17), and map between recB and argA in a gene designated recD. Biek and Cohen (49), in studies of mutants which reduce plasmid stability in dividing cells, have also recently identified recD as a gene mapping between recB and argA. Our results are in accord with those of Amundsen et al. (28) and Biek and Cohen (49) and we shall henceforth refer to this gene as recD.

Nucleotide sequence of the recB-argA region of the E. coli chromosome

The sequence of a 2,160 bp region of the E. coli chromosome situated between and linking the recB and argA genes is shown in Fig. 1. The sequence is numbered from the unique PstI site in the thyA gene (25) and is continuous with the numbering used for the recC (25), ptr (29) and recB genes (21). The region from bp 12,241 to bp 12,509 encodes the C-terminal portion of the RecB protein. The only other long open reading frame begins at the ATG codon, bp 12,509, and continues until the termination codon, TAA, at bp 14,333. This would direct the synthesis of a protein 608 amino acids long with a calculated molecular weight of 66,973 and is presumably the recD coding sequence. Preceding the ATG initiation codon by 4 nucleotides is the sequence AGGAGG, which is homologous to the consensus ribosome binding site (30).

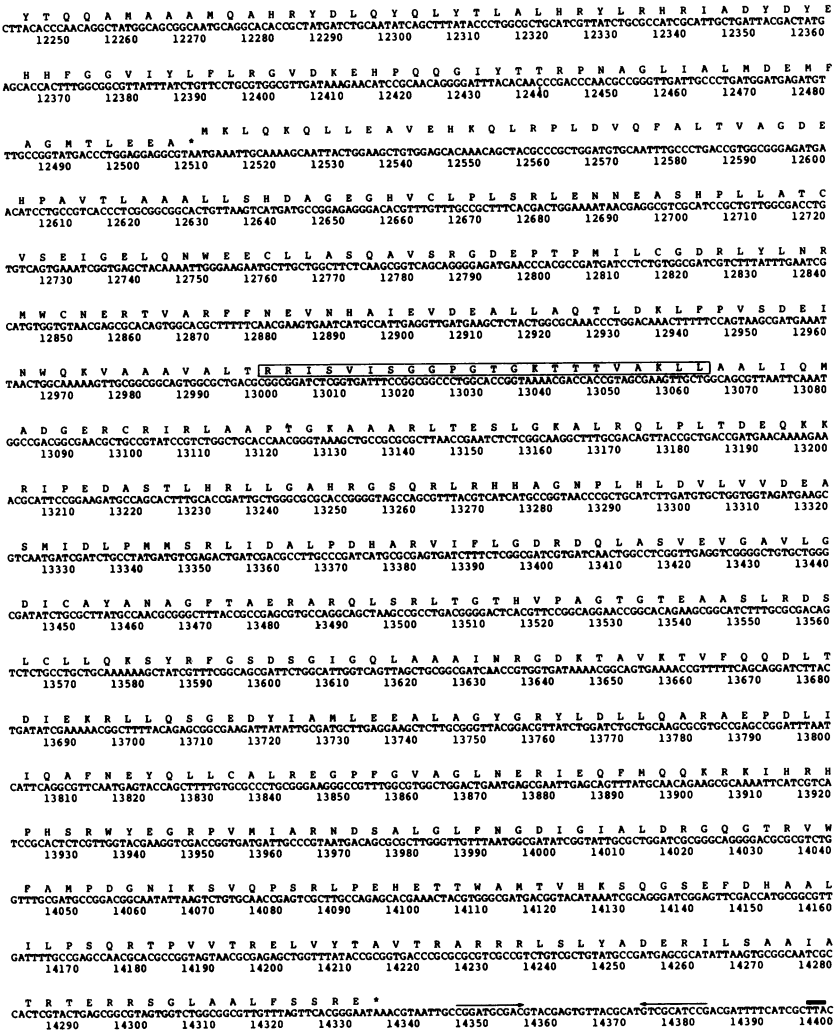
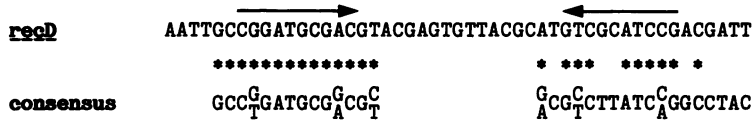


Figure 1
Nucleotide sequence of the recD gene. The numbering of the nucleotides is from the PstI site within the thyA gene (25) and is continous with that used for the recC (25), ptr (29) and recB genes (21). The recD gene and its deduced amino acid sequence begin at bp 12,509. The coding sequence for the C-terminal portion of the RecB protein extends from bp 12,241 to bp 12,509. The region of the RecD amino acid sequence that is homologous to the consensus found in adenine nucleotide binding proteins (residues 164 to 185) is boxed. The REP sequence is shown by facing arrows. The stop codon of the neighbouring argA gene which is transcribed from right to left (manuscript in preparation) is overlined.

There are ATG codons at bp 12806 and bp 12842 which would lead to the synthesis of proteins of 56.3 kDa and 54.8 kDa respectively. However, neither of these is preceded by a sequence resembling the consensus for a ribosome binding site.

Downstream of the recD coding sequence is a region of dyad symmetry consisting of the sequence CGGATGCGAC (bp 14,346 to bp 14,355) followed 16 bp later by GTCGCATCCG in a 10 bp inverted repeat. This sequence is homologous to the repetitive extragenic palindromic (REP) sequence which occurs frequently between genes in operons or at the ends of operons (31). The REP sequence found at the 3' end of recD is shown below along with the consensus REP sequence. Identical bases are indicated with an asterisk and diad symmetries are indicated by facing arrows:



Further downstream, at bp 14,397, is the triplet TTA which is complementary to the TAA stop codon at the end of a long open reading frame in the opposite direction. Evidence will be presented elsewhere that this is the argA gene.

Codon usage and amino acid composition

It has been estimated that there are only about ten molecules of Exonuclease V per cell (32), which is consistent with the low level of transcription of lacZ observed in studies with fusions of the Mud(lac bla) phage to the recB and recC promoters (15,16). It is possible that the level of Exonuclease V in the cell is also controlled at the level of translation. In E. coli there is a strong preference for those codons that correspond to the abundant tRNA species (33) and there is a bias against certain other codons, in particular ATA (Ile), TCG (Ser), CAA (Gln), AAT (Asn), CCT and CCC (Pro), ACG (Thr) and AGG (Arg) (34). In efficiently expressed genes, rare codons normally occur at a level of 4% in the coding frame versus 11% and 10% in the non-coding frames, whilst in genes which code for proteins present in low copy number, the rare codons are found at equal frequency in all three reading frames (34). Thus, a reduced rate of translation could be caused by a high frequency of these rare codons within a coding sequence. The rare codons occur at a frequency of 8.4% within recD, and at 10.2% and 8.7% in the

Table 2
Codon Usage in the recD Gene

TTT	Phe	11	TCT	Ser	6	TAT	Tyr	7	TGT	Cys	4
TTC	Phe	4	TCC	Ser	1	TAC	Tyr	3	TGC	Cys	5
TTA	Leu	10	TCA	Ser	3	TAA	End	1	TGA	End	0
TTG	Leu	17	TCG	Ser	7	TAG	End	0	TGG	Trp	6
CTT	Leu	10	CCT	Pro	3	CAT	His	12	CGT	Arg	18
CTC	Leu	5	CCC	Pro	2	CAC	His	7	CGC	Arg	17
CTA	Leu	4	CCA	Pro	3	CAA	Gln	15	CGA	Arg	7
CTG	Leu	33	CCG	Pro	16	CAG	Gln	14	CGG	Arg	7
ATT	Ile	14	ACT	Thr	6	AAT	Asn	9	AGT	Ser	7
ATC	Ile	13	ACC	Thr	14	AAC	Asn	7	AGC	Ser	10
ATA	Ile	1	ACA	Thr	2	AAA	Lys	13	AGA	Arg	1
ATG	Met	12	ACG	Thr	11	AAG	Lys	6	AGG	Arg	2
GTT	Val	7	GCT	Ala	11	GAT	Asp	26	GGT	Gly	11
GTC	Val	5	GCC	Ala	18	GAC	Asp	7	GGC	Gly	17
GTA	Val	6	GCA	Ala	9	GAA	Glu	23	GGA	Gly	8
GTG	Val	16	GCG	Ala	35	GAG	Glu	18	GGG	Gly	6

two non-coding frames (Table 2). This suggests that expression of recD may be partially controlled at the level of translation.

The level of expression of a gene can also be correlated with the choice between U and C in codon position 3. A preference exists in well expressed genes for nucleotides in the wobble position that yield a codon-anticodon binding interaction of intermediate strength. This interaction is optimised when a C follows AU, UA, UU or AA doublets and when a U follows GC, CG, CC and GG doublets (35,36). However, in genes encoding proteins present in the cell in low copy numbers this bias does not exist. In the recD coding sequence, AU, UA, UU and AA doublets are followed by a T in 63% of cases and by a C in 37%. Similarly, GC, CG, CC and GG doublets are followed by a T in 44% of cases and by a C in 56%. This is a further indication that translation of the recD message may not be very efficient.

From the predicted amino acid sequence, the RecD protein contains 145 (23.9%) charged residues, consisting of 74 (12.2%) acidic and 71 (11.7%) basic residues. This would give a net charge of -3, indicating that the isoelectric point of the RecD protein would be roughly neutral.

Identification of putative adenine nucleotide binding site in the RecD amino acid sequence

Walker et al. (37) identified a short consensus sequence which is present in a large number of adenine nucleotide binding proteins. Crystallographic

Table 3

Alignment of homologous sequences in the RecD protein and adenine nucleotide binding proteins. Identical or similar sequences are boxed.

Protein	Residues	Sequence	Ref.
Myosin (nematode)	162-183	N Q S M L [I] T [G] E S G A [G K T] E N T K K V [I]	37
Myosin (rabbit)	171-192	N Q S I L [I] T [G] E S G A [G K T] V N T K R V [I]	37
ATPase α (*E. coli*)	162-183	G Q R E L [I] I [G] D R Q T [G K T] A L A I D A [I]	37
AMP kinase (pig)	8-29	S K I I F [V] V [G] G P G S [G K G] T Q C E K I [V]	38
DnaB (*E. coli*)	223-244	S D L I I [V] A [A] R P S M [G K T] T F A M N L [V]	47
Rho (*E. coli*)	172-192	G Q R G L [I] V [A] P P K A [G K T] M L L Q N I [A]	48
RecD protein	164-185	R R I S V [I] S [G] G P G T [G K T] T T V A K L [L]	

analysis of adenylate kinase and several other enzymes has shown that this conserved sequence forms the phosphate binding region (38,39). There is such a sequence in the predicted amino acid sequence of the RecD protein from residues 164 to 185 (Table 3).

Properties of strains lacking recD

To determine the phenotype of a strain lacking the recD gene we transformed the strain V359(recB-argA)$_{\Delta}$, with the plasmid pPE399 (16), which contains the entire recB gene but only the first N-terminal 303 bp of the recD gene, and examined transformants for recombination proficiency and UV-sensitivity. Unlike recB and recC mutants, such strains are fully recombination proficient, (slightly hyper-rec, Table 4), as UV resistant as wild-type cells and are viable (data not shown).

DISCUSSION

We have found that a substantial increase in the intracellular levels of the RecB and RecC proteins only leads to amplified levels of Exonuclease V activity when accompanied by overproduction of a gene product encoded between recB and argA. Amundsen et al. (28) have recently shown that mutations, previously designated recB‡, inactivate a 58 kDa protein which copurifies

Table 4

Yield of recombinants expressed as a percentage of that obtained with the wild-type recipient, W3110. Strain V359 carries the deletion (recB-argA).

Donor	Recipient	Ap^r Tc^r recombinants
HfrC::Tn10(Tc^r)	W3110 [pPE523(recB$^-$ Ap^r)]	100
HfrC::Tn10(Tc^r)	V359 [pPE523(recB$^-$ Ap^r)]	6.9
HfrC::Tn10(Tc^r)	V359 [pPE399(recB$^+$ Ap^r)]	159

with Exonuclease V activity. These mutations map between recB and argA and have been assigned to a new gene designated recD (28). Furthermore, biochemical evidence (28) suggests that the recD gene product is the α subunit of Exonuclease V, first described by Lieberman and Oishi (17). Our results indicate that overexpression of recD as well as of recB and recC is necessary in order to amplify Exonuclease V levels in vivo. We have sequenced the region of the E. coli chromosome between recB and argA and shown that the recD gene encodes a protein of 608 amino acids with a calculated molecular weight of 66,973, in rough agreement with the estimates of 60 kDa from sedimentation analysis (17) and 58 kDa (28) to 63 kDa (our unpublished results) from SDS PAGE.

We have previously shown that some ATP-dependent exonuclease activity can be obtained by mixing the RecB and RecC proteins but the specific activity so obtained is only about one tenth that of Exonuclease V purified form wild type cells (16). Lieberman and Oishi (17) found that purified β fraction of Exonuclease V, which presumably consists of the RecB and RecC proteins alone, contains a low level of ATP-dependent DNase activity. Furthermore, results presented in this paper (Table 1) indicate that cells which contain elevated levels of the RecB and RecC proteins, but which lack RecD protein, do possess some Exonuclease V activity. Taken together, these results suggest that there is a residual low level of nuclease activity contained within the complex of the RecB and RecC proteins. Binding of the α subunit may stimulate the β complex to become a more potent nuclease. However, it cannot be discounted that some of the activity seen in the strains harbouring either pPE5343 (thyA$^+$ recB$^+$ recC$^+$), or both pPE5100 (recB$^+$) and pPE5200 (recC$^+$), is due to partial activity of the truncated RecD protein, which consists of the first 497 (out of 609) amino acid residues. This estimate of the size of the truncated RecD protein is based on DNA sequence analysis.

The termination codon for the recB gene, TAA, overlaps the ATG initiation codon for recD by one nucleotide. Also, the 3' end of the structural gene for Protease III, ptr, overlaps the start of the recB coding sequence (29,21). A comparison of the codon usage within these three genes reveals that codons which occur at an average frequency of 4% in efficiently expressed E. coli genes (34) occur at a frequency of 8.4, 7.2 and 8.2%, respectively, in the ptr, recB and recD genes. Furthermore, the usual bias found in the efficiently expressed genes for codons that give an intermediate level of codon-anticodon interaction (35,36) is reversed. These results suggest that all three genes may be translated inefficiently. The similarities in codon

usage, the finding that ptr, recB and recD partially overlap, and the presence of a REP sequence downstream of the recD gene, suggests that ptr, recB and recD may form an operon. REP sequences are normally found in extragenic nontranslated regions either between two genes which are cotranscribed or within the 3' untranslated region at the ends of operons (31). Amundsen et al. (28) found that Tn1000 insertions within recB are polar on recD, suggesting that these two genes at least are cotranscribed.

Although the function of REP sequences is unclear, they appear to affect the rate of mRNA degradation and can affect the expression of both upstream and downstream genes within an operon to a small extent (31). An interesting feature of the REP sequence that we have identified is that it lies between the recD and argA genes which are convergently transcribed. Since we have been unable to identify any obvious termination signals in this short intergenic region, it seems possible that this particular REP sequence may serve as a transcription terminator, perhaps by enabling the two transcripts to assume stem and loop structures.

It has been proposed that the recD gene alone has a weak promoter (28), which must presumably be located within the recB structural gene. However, we have been unable to detect any sequences upstream of the recD coding sequence that reasonably fit the consensus for E. coli promoters (40).

The predicted primary structure of the RecD protein contains a consensus adenine nucleotide binding sequence (37). However, Lieberman and Oishi (17) reported that the α subunit does not possess DNA-dependent ATPase activity. We have shown previously that the isolated RecB subunit has DNA-dependent ATPase activity roughly equivalent to that of the holoenzyme (16). An analogous situation may be found in the case of the UvrABC endonuclease, which is required for nucleotide excision repair (see [41] for a review). It has been shown that purified UvrA protein is an ATPase which binds to ss DNA and UV-irradiated duplex DNA with a higher affinity than to unirradiated duplex DNA (42). The UvrB protein is not itself an ATPase but stimulates the ATPase activity of the UvrA protein by a factor of 2.5 in the presence of UV-irradiated duplex DNA (43). However, both the UvrA and UvrB proteins contain consensus ATP binding sequences (44-46). It is possible that RecD protein has a similar role to that of the UvrB protein in that it may stimulate the ATPase activity of the RecB subunit within the RecBCD enzyme complex. Further studies are needed to determine whether the RecD protein binds ATP, and whether only the RecB subunit or both the RecB and RecD subunits function as ATPases in the holoenzyme.

Although the RecD protein is a subunit of Exonuclease V (17,28), the phenotype of recD mutants is surprisingly different from that of recB and recC mutants. The observation that recD mutants are recombination proficient (indeed, slightly hyper-rec) and UV resistant (28, this work) suggests that the complex formed between the RecB and RecC proteins alone is able to carry out the functions of Exonuclease V in repair and recombination.

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REFERENCES

1. Emmerson, P.T. (1968) Genetics 60, 19-30.
2. Willets, N.S. and Mount, D.W. (1969) J. Bacteriol. 100, 923-934.
3. Capaldo-Kimball, F. and Barbour, S.D. (1971) J. Bacteriol. 106, 204-212.
4. Buttin, G. and Wright, M. (1968) Cold Spring Harbour Symp. Quant Biol. 33, 259-264.
5. Oishi, M. (1969) Proc. Natl. Acad. Sci. USA 64, 1292-1299.
6. Goldmark, P.J. and Linn, S. (1970) Proc. Natl. Acad. Sci. USA 67, 434-441.
7. Muskavitch, K.M.T. and Linn, S. (1981) in The Enzymes (Boyer, P.D., ed) Vol. 14, pp233-250, Academic Press, New York.
8. Ponticelli, A.S., Schultz, D.W. Taylor, A.F. and Smith, G.R. (1985) Cell 41, 145-151.
9. Taylor, A.F., Schultz, D.W., Ponticelli, A.S. and Smith, G.R. (1985) Cell 41, 153-163.
10. Smith, G.R. (1983) in Lambda II (Hendrix, R.W., Roberts, J.W., Stahl, F.W. and Weisberg, R.A., eds). pp 175-209, Cold Spring Harbor, New York.
11. Gillen, J.R. and Clark, A.J. (1974) in Mechanisms In Recombination (Grell, R.F., ed) p123, Plenum Press, New York.
12. Hickson, I.D. and Emmerson, P.T. (1981) Nature 294, 578-580.
13. Umeno, M., Sasaki, M., Anai, M. and Takagi, Y. (1983) Biochem. Biophys. Res. Commun. 116, 1144-1150.
14. Dykstra, C.C., Prasher, D. and Kushner, S.R. (1984) J. Bacteriol. 157, 21-27.
15. Hickson, I.D., Atkinson, K.E., Hutton, L., Tomkinson, A.E. and Emmerson, P.T. (1984) Nucl. Acids Res. 12, 3807-3819.
16. Hickson, I.D., Robson, C.R., Atkinson, K.E., Hutton, L. and Emmerson, P.T. (1985) J. Biol. Chem. 260, 1224-1229.
17. Lieberman, R.P. and Oishi, M. (1974) Proc. Natl. Acad. Sci. USA 71, 4816-4820.
18. Barbour, S.D. and Clark, A.J. (1970) Proc. Natl. Acad. Sci. USA 65, 955-961.
19. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
20. Yanisch-Perron, C., Vieira, J. and Messing J. (1985) Gene 33, 103-119.
21. Finch, P.W., Storey, A., Chapman, K.E., Brown, K., Hickson, I.D. and Emmerson, P.T. (1986) Nucl. Acids Res., 14, 8573-8582.

22. Queen, C. and Korn, L.J. (1984) Nucl. Acids Res. 12, 581-599.
23. Staden, R. (1984) Nucl. Acids Res 12, 551-567.
24. Remaut, E., Stanssens, P. and Fiers, W. (1981) Gene 15, 81-83.
25. Finch, P.W., Wilson, R.E., Brown, K., Hickson, I.D., Tomkinson, A.E. and Emmerson, P.T. (1986) Nucl. Acids Res. 14, 4437-4451.
26. Yasuda, S. and Takagi, T. (1983) J. Bacteriol. 154, 1153-1161.
27. Russell, D.R. and Bennet, G.N. (1982) Gene 20, 231-
28. Amundsen, S.K., Taylor, A.F., Chaudhury, A.M. and Smith, G.R. (1986) Proc. Natl. Acad. Sci. USA 83, 5558-5562.
29. Finch, P.W., Wilson, R.E., Brown, K., Hickson, I.D. and Emmerson, P.T. (1986) Nucl. Acids Res., submitted.
30. Shine, J. and Dalgarno, L. (1974) Proc. Natl. Acad Sci. USA 21, 1342-1346.
31. Stern, M.J., Ames, G.F.I., Smith, N.H., Robinson, E.C. and Higgins, C.F. (1984) Cell 37, 1015-1026.
32. Taylor, A.F. and Smith, G.R. (1980) Cell 22, 447-457.
33. Ikemura, T. (1980) J. Mol. Biol. 151, 389-409.
34. Konigsberg, W. and Godson, G.N. (1983) Proc. Natl. Acad. Sci. USA 80, 687-691.
35. Grosjean, H. and Fiers, W. (1982) Gene 18, 199-209
36. Gouy, M. and Gautier, C. (1982) Nucl. Acids Res. 10, 7055-7075.
37. Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) EMBO J. 1, 645-951.
38. Pai, E.F., Sachsenheimer, W., Schirmer, R.H. and Schultz, G.E. (1977) J. Mol. Biol. 114, 37-45.
39. Fry, D.C., Ruby, S.A. and Mildvan, A.S. (1986) Proc. Natl. Acad. Sci. USA 83, 907-911.
40. Hawley, D.K. and McClure, W.R. (1983) Nucl. Acids Res. 11, 2237-2255.
41. Friedberg, E.C. (1985) DNA Repair, Freeman Publications, San Francisco.
42. Seeberg, E. and Steinum, A.L. (1982) Proc. Natl. Acad. Sci. USA 79, 988-992.
43. Thomas, D.C., Levy, M. and Sancar, A. (1985) J. Biol. Chem. 260, 9875-9883.
44. Husain, I., Van Houten, B., Thomas, D.C. and Sancar, A. (1986) J. Biol Chem. 261, 4895-4901.
45. Arikan, E., Kulkarni, M.S., Thomas, D.C. and Sancar, A. (1986) Nucl. Acids Res. 14, 2637-2651.
46. Backendorf, C., Spaink, H., Barbeiro, A.P. and van de Putte, P. (1986) Nucl. Acids Res. 14, 2877-2890.
47. Nakayama, N., Arai, N., Bond, M.W., Kaziro, Y. and Arai, K. (1984) J. Biol. Chem. 259, 97-101.
48. Pinkham, J.L. and Platt, T. (1983) Nucl. Acids Res. 11, 3531-3545.
49. Biek, D.P. and Cohen, S.N. (1986) J. Bacteriol. 167, 594-603.