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Nucleotide sequence of the cro - cII - oop region of bacteriophage 434 DNA

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ABSTRACT

The nucleotide sequence of a 869 bp segment of phage 434 DNA including the regulatory genes cro and cII is presented and compared with the corresponding part of the phage λ DNA sequence. The 434 cro protein as deduced from the DNA sequence is a highly basic protein of 71 amino acid residues with a calculated molecular weight of 8089. While the cro gene sequences of phage 434 and λ DNA are very different, the nucleotide sequences to the right of the λimm434 boundary show differences only at 11 out of 512 positions. Nucleotide substitutions in the cII gene occur with one exception in the third positions of the respective codons and only one out of several DNA regulatory signals located in this region of the phage genomes is affected by these nucleotide substitutions.

INTRODUCTION

Bacteriophage 434 is genetically very similar to phage λ and together with several others belongs to the group of lambdoid coliphages. All of them share as common characteristics comparable DNA lengths and a similar structural and functional organization of their genomes. Genes coding for biologically related functions quite often are clustered into contiguous groups such as the immunity and replication regions. Because of their functional equivalence, these groups are exchangeable among the various lambdoid phages, resulting in the formation of hybrid bacteriophages such as λimm434 (1), λimm21 (2) and others. Within these regions, genes and DNA target sites interacting with the encoded gene products are often structurally linked or even overlapping, as has been shown for example for the O gene/ origin of replication (3,4)

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and the cII gene/ cy site (5,6) in phage λ .
The different degrees of homology between the lambdoid phages are also reflected by their capacities for genetic complementation. For the closely related phages λ and 434, complementation has been demonstrated for the two phage coded gene O and P proteins (7), necessary for initiation of phage DNA replication, for the proteins coded by genes cII and cIII (8), which act positively on establishment of repression, and for the gene N product (9), which controls the expression of most other phage genes on the level of RNA transcription by its ability to overcome rho-mediated transcriptional termination. Specific for each phage and not exchangeable are the products of genes cI and cro. The former functions as repressor to maintain the lysogenic state, while the latter acts as its antagonist and therefore plays a crucial role for lytic phage growth. In phage λ, both cI and cro repressor proteins act by binding to the same operator structures O_R and O_L. At O_R, the λ repressor regulates transcription starting from the promoters P_R and P_{RM}, which overlap O_R, by binding to the three repressor binding sites with an affinity $O_R1 > O_R2 > O_R3$ (10,11), whereas the cro protein has an inversed order of affinity $O_R3 > (O_R1, O_R2)$ (12). It is likely, that in phage 434 the cI and cro gene products control transcription in a very similar way by binding to the 434 operator structures.

The relationship between λ and 434 in terms of sequence homology of their DNA molecules has been analyzed by DNA heteroduplex mapping (13). One nonhomologous region of λ /434 heteroduplexes includes genes cI and cro both lying in the 434 immunity region surrounded to its left and right by segments of sequence homology which have been assigned to genes N, cII, O and P.

A more detailed comparison of the sequences of the genomes of related phages at the nucleotide level should help to characterize the respective genes and regulatory signals either by analysis of common structural features of gene products or DNA signal structures exerting analogous functions, or by analysis of parts of structural genes or regulatory signals, that can be altered without influencing its function(s). For this purpose we have determined the nucleotide sequence of the portion of phage 434 DNA comprising gene cro and the DNA region to the right of the λimm434 boundary extending to the N-ter-

minal part of the 434 O gene and have compared it with the corresponding λ sequence.

MATERIALS

DNA of the plasmids λ dvimm434 (14) and pHL81 (15), which contains a 9.4 kb HindIII : BamH1 fragment of 434 DNA inserted into pBR313, were kindly provided by G. Hobom.

Restriction enzymes HhaI and BglII were prepared by a modification of the method of Sharp et al. (16) and Smith and Wilcox (17). HhaI was further purified according to Bickle et al. (18). HinfI, HpaII, HindII and MboII were gifts of G. Scherer and H. Sommer. SalI, AluI, MboII and HaeII were purchased from New England Biolabs (USA), EcoRI from Boehringer Mannheim. Bacterial alkaline phosphatase and T4 polynucleotide kinase were obtained from Worthington and from Biogenics Research Corporation, respectively.

[γ^{32}P]ATP with a specific activity of 1000 - 1500 Ci/mmole was prepared as described (19) using HCl-free, carrier-free ^{32}P-phosphate from New England Nuclear. Agfa Gevaert Osray T4 or Dupont Cronex 2 films in connection with Dupont Lightning intensifying screens were used for autoradiography.

METHODS

After constructing a physical map of 434 DNA by restriction analysis (data not shown), restriction fragments were prepared and separated on polyacrylamide slab gels. Fragments were isolated from the gels either by a diffusion method (19) or by an electrophoretic elution procedure (20).

Restriction fragments were labeled at their 5'ends using T4 polynucleotide kinase and [γ^{32}P]ATP (19). Separation of the labeled ends was either achieved by recutting the DNA fragments with a second restriction enzyme, followed by separation of the cleavage products on 7.5% or 10% polyacrylamide gels or by strand separation on polyacrylamide gels (19). DNA sequencing was performed essentially as described (19) using the G-, A>C-, C>T- and C+T-reactions. Products were fractionated on denaturing polyacrylamide gels with gel concentrations of 10%, 12%, 15% and 20%.

Gel dimensions used were 23 cm x 41 cm x 0.2 cm, 16.5 cm x 90 cm x 0.1 cm and 30 cm x 100 cm x 0.1 cm. With this set of different gel electrophoresis conditions and running the gels for different time intervals, it was possible to read up to 200 positions from the labeled 5'end of a given fragment. Before loading of samples, gels were aged for one day and pre-electrophoresed for 6 h at 1000 V. Electrophoresis was carried out at 1200 - 3000 V depending on the polyacrylamide concentrations and gel dimensions used. These high voltages were necessary in order to achieve gel temperatures of 50° - 60°C necessary to minimize the formation of DNA secondary structure which can result in artefacts in the band patterns.

RESULTS AND DISCUSSION

434 cro gene and cro protein

For determination of the 434 cro gene sequence, DNA of the plasmids λ dvimm434 and, to a minor extent, pHL81 was used. Plasmid λ dvimm434, derived from the hybrid phage λimm434 (1) contains the 434 immunity region including genes cI and cro and the 434 $O_R P_R$ operator and promoter structures (see Fig.1A). The 434 promoter P_R is located to the right of an EcoRI cut (G. Hobom, personal communication) which was used as a reference point for the construction of a restriction map. Further supporting evidence for the position of the P_R promoter was obtained from binding experiments of RNA polymerase (according to ref.21) to restriction fragments obtained by double digestion of λ dvimm434 DNA with enzymes EcoRI and HhaI (data not shown).

The order of the various restriction fragments and the strategy used for sequence analysis of the 434 cro gene region is shown in Fig.1B. Fig.2A gives an example of a sequence autoradiogram and the resulting DNA sequence is presented in Fig.3.

From cells carrying the λdvimm434 plasmid a protein had been isolated which by its specific binding to 434 DNA was concluded to be the 434 cro gene product (22). The molecular weight of the protein as determined by SDS gel electrophoresis was given as 11000.

From the nucleotide sequence shown in Fig.3 the amino acid sequence of the 434 cro protein can be predicted. Screening of the l-strand

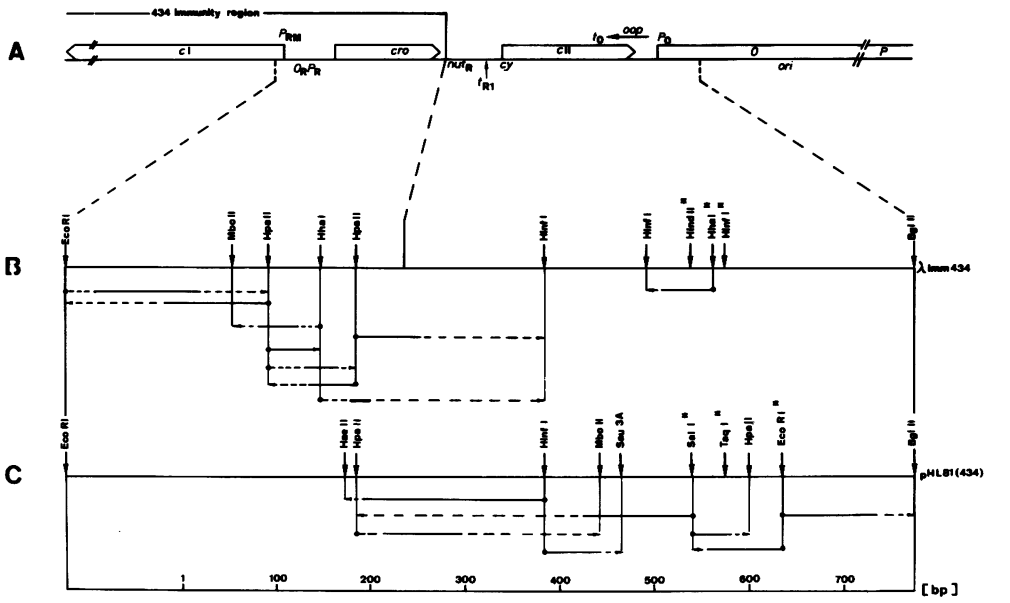


Fig.1A. Partial genetic and physical map of the phage 434 genome extending from the right part of the immunity region to the replication region. The right-hand boundary of the 434 immunity region and the location of the presumptive 434 oop RNA are indicated. B and C. Enlargement of the 869 bp EcoRI ;BglII fragment of λ imm434 DNA (B) and 434 DNA (C). Restriction endonuclease cleavage sites used for sequence analysis are indicated by vertical arrows. Cleavage sites only present in either λimm434 DNA or 434 DNA are marked by asterisks. 5'terminally labeled restriction fragments used for sequence analysis are shown by horizontal arrows, the circles indicating the labeled 5'end. The sequenced part of each restriction fragment is set off by a solid line.

sequence (which corresponds to the mRNA-sequence) for terminator codons leaves a single reading frame not interrupted by termination signals up to the UAA codon at position 229-231. As the most likely initiation codon for cro protein translation appears the AUG codon at position 16-18, i.e. situated 16 nucleotides beyond the startpoint for rightward transcription (ref.23; chosen as position 1 and located 125 nucleotides to the right of the EcoRI cut). This AUG codon is preceded by the sequence GGAGG complementary to the 3'end of ribosomal 16S RNA (24, 25).

The cro protein as deduced from the DNA sequence consists of 71

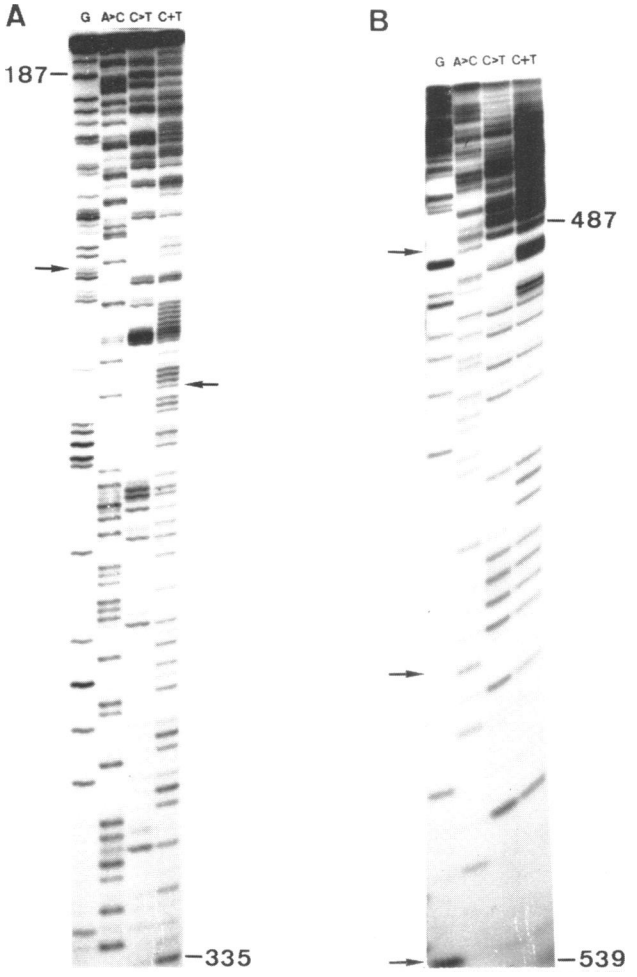
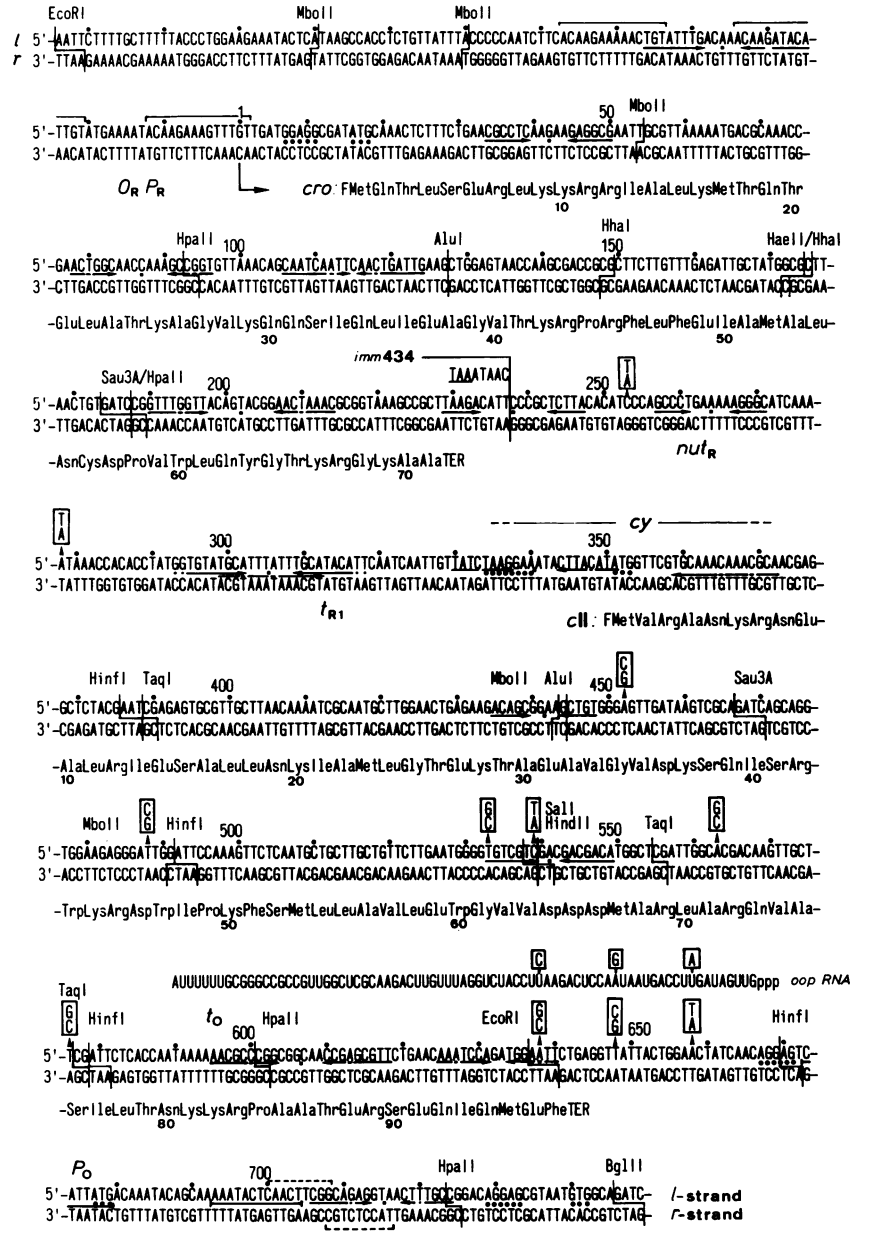


Fig.2. Representative sequence autoradiograms obtained from phage 434 DNA. The numbers beside the autoradiograms refer to the corresponding positions in the DNA sequence shown in Fig.3. Nucleotide positions to the right of the imm434 boundary which differ from those in phage λ DNA are marked by arrows. A. Sequence pattern of the 217 bp HaeII : HinfI restriction fragment, ^{32}P-labeled at the 5'end of the HinfI site. The sequence represents part of the cro/ cII intercistronic region and the C-terminal portion of the cro gene of 434 DNA. Separation of the products obtained after partial base specific cleavages (19) was achieved on a 10% polyacrylamide gel containing 7M urea. B. Patterns obtained from the 350 bp HpaII : SalI fragment, labeled at the 5'end of the SalI cut. The sequence constitutes part of the 434 cII gene. Fractionation was carried out on a denaturing 20% polyacrylamide gel.

amino acids (Figs.3 and 4) giving the molecular weight of 8089, smaller than the molecular weight reported (22). However, a similar disagreement with earlier measurements from SDS gels (26) has also been observed for the λ cro protein. At positions 210 and 228 the sequence deviates from an independently determined sequence of the λimm434 cro gene (27). Fig.2A shows an autoradiogram of the r-strand sequence including these two positions, also confirmed by information obtained for the complementary l-strand sequence of this region (compare Fig.1B). The nucleotide sequence of the $O_R P_R$ containing EcoRI : HpaII fragment has also been determined by V. Pirrotta (23) showing a difference of one base-pair regarding positions -14 to -17 in Fig.3.

With the reservation that for the 434 cro protein no supporting amino acid sequence data are at present available, a comparison of the cro gene and cro protein sequences of phages 434 and λ (6, 28, 29) shows that (a) both proteins are of similar size (434 cro: 71 amino acids, MW 8089 - λ cro: 66 amino acids, MW 7360), (b) both proteins are highly basic (434 cro: 14 basic and 5 acidic amino acids - λ cro: 11 basic and 6 acidic amino acids) and (c) in both cases the termination signal for translation is a UAA codon with alanine as the C-terminal amino acid confined into the immunity regions in a very similar location relative to the imm434 boundary (see Fig.3). Very striking and more pronounced than in the cro protein of phage λ is the strong clustering of basic amino acids in the N-terminal part of the 434 cro protein (Figs.3 and 4). This is a feature also found in the N-terminal regions of the lac and λ cI repressor proteins (30, 31, 10) and it has been suggested that the specific contact between repressor protein and operator DNA is mediated by the N-terminal residues (31, 32). As in λ O_R the phage 434 operator O_R also contains three potential repressor binding sites, which however are quite different in their nucleotide sequence from those of λ (ref.23; see Fig.3). This difference is not surprising, as both cro proteins only recognize their own phage specific operator structures. On the nucleotide level no obvious homologies between the 434 and λ cro gene sequences can be observed, which is in agreement with the results of λ/434 DNA heteroduplex analysis (13). Can we, nevertheless, detect any homology between the amino acid sequences of the two cro protwins? An alignment as depicted in Fig.4 reveals a


EcoRI
MboII
MboII
l 5'-AATTCTTTTGCTTTTTACCCTGGAAGAAATACTCATAAGCCACCTCTGTTATTTACCCCCAATCTTCACAAGAAAAACTGTATTTGACAAACAAGATACA-
r 3'-TTAAGAAAACGAAAAATGGGACCTTCTTTATGAGTATTCGGTGGAGACAATAAATGGGGGTTAGAAGTGTTCTTTTTGACATAAACTGTTTGTTCTATGT-
1
50
MboII
5'-TTGTATGAAAATACAAGAAAGTTTGTTGATGGAGGCGATATGCAAACTCTTTCTGAACGCCTCAAGAAGAGGCGAATTTGCGTTAAAAATGACGCAAACC-
3'-AACATACTTTTATGTTCTTTCAAACAACTACCTCCGCTATACGTTTGAGAAAGACTTGCGGAGTTCTTCTCCGCTTAACGCAATTTTTACTGCGTTTGG-
OR PR
cro: FMetGlnThrLeuSerGluArgLeuLysLysArgArgIleAlaLeuLysMetThrGlnThr
10
20
HpaII
100
AluI
HhaI
150
HaeII/HhaI
5'-GAACTGGCAACCAAAGCCGGTGTTAAACAGCAATCAATTCAACTGATTGAAGCTGGAGTAACCAAGCGACCGCGCTTCTTGTTTGAGATTGCTATGGCGCTT-
3'-CTTGACCGTTGGTTTCGGCCACAATTTGTCGTTAGTTAAGTTGACTAACTTCGACCTCATTGGTTCGCTGGCGCGAAGAACAAACTCTAACGATACCGCGAA-
-GluLeuAlaThrLysAlaGlyValLysGlnGlnSerIleGlnLeuIleGluAlaGlyValThrLysArgProArgPheLeuPheGluIleAlaMetAlaLeu-
30
40
50
imm434
Sau3A/HpaII
200
TAAATAAC
250
5'-AACTGTGATCCGGTTTGGTTACAGTACGGAACTAAACGCGGTAAAGCCGCTTAAGACATTCCCGCTCTTACACATCCCAGCCCTGAAAAAGGGCATCAAA-
3'-TTGACACTAGGCCAAACCAATGTCATGCCTTGATTTGCGCCATTTCGGCGAATTCTGTAAGGGCGAGAATGTGTAGGGTCGGGAACTTTTTCCCGTCGTTT-
nutR
-AsnCysAspProValTrpLeuGlnTyrGlyThrLysArgGlyLysAlaAlaTER
60
70
cy
300
350
5'-ATAAACCACACCTATGGTGTATGCATTTATTTGCATACATTCAATCAATTGTTATCTAAGGAAATACTTACATATGGTTCGTGCAAACAAACGCAACGAG-
3'-TATTTGGTGTGGATACCACATACGTAAATAAACGTATGTAAGTTAGTTAACAATAGATTCCTTTATGAATGTATACCAAGCACGTTTGTTTGCGTTGCTC-
tR1
cII: FMetValArgAlaAsnLysArgAsnGlu-
HinfI
TaqI
400
MboII
AluI
450
Sau3A
5'-GCTCTACGAATCGAGAGTGCGTTGCTTAACAAATCGCAATGCTTGGAACTGAGAAGACAGCGAAGCTGTGGGAGTTGATAAGTCGCAGATCAGCAGG-
3'-CGAGATGCTTAGCTCTCACGCAACGAATTGTTTAGCGTTACGAACCTTGACTCTTCTGTCGCCTTCGACACCCTCAACTATTCAGCGTCTAGTCGTCC-
-AlaLeuArgIleGluSerAlaLeuLeuAsnLysIleAlaMetLeuGlyThrGluLysThrAlaGluAlaValGlyValAspLysSerGlnIleSerArg-
10
20
30
40
MboII
HinfI
500
SalI
HindII
550
TaqI
5'-TGGAAGAGGGATTGGATTCCAAAGTTCTCAATGCTGCTTGCTGTTCTTGAATGGGGTGTCGTCGACGACAGACATGGCCTCGATTGGCACGACAAGTTGCT-
3'-ACCTTCTCCCTAACCTAAGGTTTCAAGCGTTACGACGAACGACAAGAACTTACCCCACAGCAGCTGCTGCTGTACCGAGCTAACCGTGCTGTTCAACGA-
-TrpLysArgAspTrpIleProLysPheSerMetLeuLeuAlaValLeuGluTrpGlyValValAspAspMetAlaArgLeuAlaArgGlnValAla-
50
60
70
AUUUUUUGCGGGCCGCCGUUGGCUCGCAAGACUUGUUUAGGUCUACCUUAAGACUCCAAUAAUGACCUUGAUAGUUGppp oop RNA
TaqI
HinfI
tO
600
HpaII
EcoRI
650
HinfI
5'-TCGATTCTCACCAATAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTC-
3'-AGCTAAGAGTGGTTATTTTTTGCGGGCCGCCGTTGGCTCGCAAGACTTGTTTAGGTCTACCTTAAGACTCCAATAATGACCTTGATAGTTGTCCTCAG-
-SerIleLeuThrAsnLysLysArgProAlaAlaThrGluArgSerGluGlnIleGlnMetGluPheTER
80
90
PO
700
HpaII
BglII
5'-ATTATGACAAATACAGCAAAAATACTCAACTTCGGCAGAGGTAACTTTGCCGGACAGGAGCGTAATGTGGCAGATC- l-strand
3'-TAATACTGTTTATGTCGTTTTTATGAGTTGAAGCCGTCTCCATTGAAACGGCCTGTCCTCGCATTACACCGTCTAG- r-strand

similar arrangement of certain amino acids, particularly in the N-terminal part. Moreover, an approximately comparable distribution of the basic amino acid residues along the two sequences can be observed. The functional importance, however, of both observations, if any, is not clear.

Intercistronic region between genes cro and cII

In phage λ DNA the noncoding region between the end of the cro gene and the presumptive start of the cII gene is 118 nucleotides in length (5,6). It contains the two regulatory signals for transcription of P_R-mRNA, the rho-dependent termination signal t_{R1} and the recognition site nut_R for protein N interaction, which allows transcriptional readthrough beyond t_{R1} (5). Sequence analysis of the 434 intercistronic region (for the sequencing strategy see Fig.1C) shows that beyond the imm434 nonhomology boundary it differs only in the two positions 253 and 278 from the respective sequence in phage λ DNA (Fig.3). These nucleotide ex-

Fig.3. Nucleotide sequence of the 869 bp EcoRI : BglII restriction fragment of phage 434 DNA. The startpoint for rightward transcription (23) is indicated by an arrow and chosen as position 1. The location of the three repressor binding sites of the 434 operator O_R (23) is indicated by brackets on top of the l-strand. The sequence of the first 30 nucleotides to the right of the EcoRI cut is from ref.23. Putative translational initiator codons as well as the preceding sequences complementary to the 3'end of ribosomal 16S RNA are indicated by dotted lines below the messenger like l-strand. Amino acid sequences of the 434 cro and cII proteins are written below the DNA sequence. The locations of the signals nut_R, t_R1, cy, t_0 and P_0 as determined in phage λ DNA are marked. The P_0 related pseudo-operator sequence is indicated by dashed brackets (see text for discussion). The presumptive 434 oop RNA sequence is included on top of the DNA sequence. The right-hand boundary of the imm434 immunity region as present in λ imm434 is indicated. To its left, eight nucleotides of the l-strand sequence of λDNA are included on top of positions 230 to 237 with the termination codon of the λ cro gene underlined. To the right of the imm434 boundary nucleotide positions which are different in λDNA from those in 434 DNA are given above the 434 DNA sequence (position 238 corresponds to position 225 in the λ DNA sequence (6) and so on for all further nucleotide positions). Restriction endonuclease cleavage sites are indicated at the respective positions. The arrows between the DNA strands designate inverted sequence repetitions.

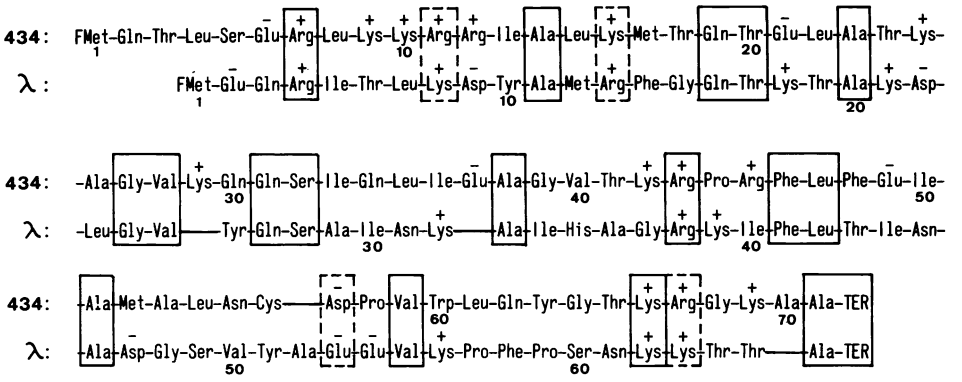


Fig.4. Amino acid sequences of the cro proteins of phages 434 and λ. The sequences are aligned to show maximal homology Positively or negatively charged amino acid residues are marked by + or −. Solid boxes enclose identical amino acids in the sequences of both cro proteins at the respective positions. Dashed boxes indicate equally charged amino acid residues.

changes do not affect either of the two regulatory signals nut_R and t_{R1}, but are located in regions with apparently little or no functional importance. An identical nut_R signal in 434 DNA is in agreement with the earlier observation that phage 434 can complement for the N function in λ (9).

In the hybrid phage λ imm434 (λdvimm434) the sequence beyond the imm434 boundary is clearly that of λ, because it is lacking the first nucleotide substitutions at positions 253 and 278 observed in 434 DNA. Thus one of the recombinational events which gave rise to this hybrid phage actually must have taken place in the segment defined by the right nonhomology boundary of the 434 immunity region and 16 nucleotides further to the right by the first sequence deviation between λ and 434 DNA. In contrast to this, the right-hand boundary of the λ/λ imm21 nonhomology region as defined by DNA heteroduplex analysis (33) does not likewise represent the place of the recombinational event resulting in the formation of hybrid phage λ imm21, because the λimm21 nucleotide sequence to the right of the imm21 nonhomology boundary is identical with the sequence of phage 21 DNA and different from λ at

all the positions deviating between phage 21 and λ DNA in this region (34).

434 cII gene and cy region

By genetic analysis it has been shown that recombination between phages λ and 434 is possible within their cII genes (35). The DNA homology in this area could be further characterized by λ/434 heteroduplex analysis (13). On the protein level a mutual exchangeability of the λ and 434 cII gene products has been demonstrated (8). From the 434 DNA sequence presented here and the known λ DNA sequence (6) we are able to confirm this homology on the nucleotide level. The sequence identity of λ and 434 DNA in the cII gene region is interrupted by nucleotide substitutions at seven positions only (Fig.3). Based on this extensive sequence homology and on the genetic data it is possible to extrapolate the known reading frame of the λ cII gene (6) to that of the phage 434 gene. The 434 cII protein as deduced from the DNA sequence consists of 97 amino acids differing from the λ cII amino acid sequence only by an Ala^{λ} - Ser^{434} exchange at position 76. The sequence data of the 434 cII gene also confirm the nucleotide sequence of the λ cII gene (6). In particular, this applies to the segment between positions 529 and 534, GGGGTG in 434 DNA (Fig.3) and GGGGGG in λ DNA (6) regarding the l-strand sequences, which deviates from an independently determined nucleotide sequence of the λ cII gene obtained from λ imm434 DNA (27). Sequence data concerning this region have been obtained by determination of the l-strand sequence from λdvh93 and λcIIam60am41 DNA (6), of the r-strand sequence derived from λdvimm434 DNA (see Fig.1B) and of the r-strand sequence of 434 DNA (see Fig.1C and Fig.2B).

The cy region defined in phage λ by cis-dominant mutations (35-37) and partly overlapping with the N-terminal part of the cII gene (5,6) is thought to be the binding site of the cII protein, which acts positively on repressor est-ablishment transcription (8). The perfect 434/λ homology observed in the cy region as well as the overall homology of the cII proteins of both phages is in agreement with this hypothesis.

Recently a second DNA signal, proposed to be involved in lambdoid phage DNA replication and designated as "replicational inceptor" was

found within the cII genes of phages λ and 434 (15,38). The nucleotide substitutions at positions 533 and 539 of 434 DNA (Fig.3) can be interpreted to improve the length of the inverted repeat located in this DNA region, which is thought to constitute an essential part of the inceptor signal structure. Parallel to these nucleotide substitutions a different initiator protein requirement has been observed in a hybrid plasmid replication system containing 434 DNA instead of λ DNA (15,38).

434 oop RNA region

In the cII - O intercistronic region of lambdoid phages a short RNA, called oop RNA, is initiated at the P_0 promoter and transcribed from the l-strand in leftward direction (39). The exact biological function of this RNA is still unknown. It was thought to have a dual role as leader for repressor establishment transcription and as RNA primer for DNA replication (40). However, recent experiments showed that the deletion of the P_0 promoter does not reduce the overall λ DNA replication rate (41).

Because of the very close similarity of the 434 DNA sequence to the λ oop DNA sequence (42) and the completely identical P_0 and t_0 signal structures we deduced the sequence of a 434 oop RNA from the DNA sequence as indicated in Fig.3. The 434 oop DNA sequence deviates from that of phage λ at three nucleotide positions (Fig.3). The G^{λ} - A^{434} exchange at position 638 constitutes an EcoRI cleavage site in 434 DNA. The C^{λ} - T^{434} substitution at position 648 is interesting with regard to a hypothetical translation of the oop RNA, since the latter has not yet been disproven by biochemical data and cannot be ruled out by knowledge of the λ oop RNA sequence (42,43). Translation of oop RNA would provide a possible mechanism for regulating antitermination at the terminator t_0, in a similar way as has been proposed for some bacterial katabolic operons (44-47). If oop RNA serves indeed as a leader for repressor establishment transcription, antitermination to overcome the termination signal t_0 is required. For phage λ it was shown by in vitro experiments that in the presence of the host factor rho no elongation of the oop RNA is detectable (48). The substitution at position 648 converts the single potential AUG initiation codon in the λ oop RNA

into an AUA triplet in the 434 RNA (see Fig.3). Since an AUA codon has not been found so far to function *in vivo* as an initiation triplet, translation of lambdoid *oop* RNA is unlikely to occur. A third difference between λ and 434 DNA sequences in the *oop* region is located at position 658.

The DNA sequence of the 434 P_0 promoter region is completely identical to that of λ. This argues against a λ O_R like sequence located in this area to function as a binding site for the λ *cro* protein (42), because the λ and 434 *cro* proteins and also the O_R operator sequences are phage specific. Therefore it seems unlikely that the 434 *cro* protein recognizes the pseudo-operator sequence in the P_0 region, identical in the phage λ and 434 DNA. Since the same regulatory principles are used in all lambdoid phages, we may extrapolate the above conclusion to phage λ, i.e. that the λ *cro* protein does not bind to the λ P_0 region and thereby regulate *oop* RNA synthesis. This suggestion is supported by *in vitro* experiments showing that the λ *cro* protein does not repress λ *oop* RNA synthesis (49).

The N-terminal part of the 434 *O* gene sequenced so far is identical to that of λ (4). Therefore no decision between the two possible initiation sites of the λ and 434 *O* genes (pos. 678-680 and 740-742 in Fig.3) can be made from comparison of the 434 and λ DNA sequences.

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REFERENCES

1. Kaiser,A.D. and Jacob,F. (1957) Virology 4, 509-521.

2. Liedke-Kulke,M. and Kaiser,A.D. (1967) Virology 32, 475-481.
3. Furth,M.E., Blattner,F.R., McLeester,C. and Dove,W.F. (1977) Science 198, 1046-1051.
4. Scherer,G. (1978) Nucl. Ac. Res. 5, 3141-3156.
5. Rosenberg,M., Court,D., Shimatake,H., Brady,C. and Wulff,D.L. (1978) Nature 272, 414-423.
6. Schwarz,E., Scherer,G., Hobom,G. and Kössel,H. (1978) Nature 272, 410-414.
7. Dove,W.F. (1968) Ann. Rev. Genetics 2, 305-340.
8. Reichardt,L.F. (1975) J. Mol. Biol. 93, 267-288.
9. Thomas,R. (1970) J. Mol. Biol. 49, 393-404.
10. Ptashne,M., Backman,K., Humayun,M.Z., Jeffrey,A., Maurer,R., Meyer,B. and Sauer,R.T. (1976) Science 194, 156-161.
11. Walz,A., Pirrotta,V. and Ineichen,K. (1976) Nature 262, 665-669.
12. Johnson,A., Meyer,B.J. and Ptashne,M. (1978) Proc. Natl. Acad. Sci. 75, 1783-1787.
13. Simon,M.N., Davis, R.W. and Davidson, N. (1971) in The Bacteriophage Lambda, Hershey,A.D.,Ed., 313-328, Cold Spring Harbor, New York.
14. Matsubara,K. and Otsuji,Y. (1978) Plasmid 1, 284-296.
15. Lusky,M. and Hobom,G., Gene, submitted for publication.
16. Sharp,P., Sugden,B. and Sambrook,J. (1973) Biochemistry 12, 3055-3063.
17. Smith,H.O. and Wilcox,K.W. (1970) J. Mol. Biol. 51, 379-391.
18. Bickle,T.A., Pirrotta,V. and Imber,R. (1977) Nucl. Ac. Res. 4, 2561-2572.
19. Maxam,A. and Gilbert,W. (1977) Proc. Natl. Acad. Sci. 74, 560-564.
20. Wienand,U., Schwarz,Z. and Feix,G., FEBS Letters, in press.
21. Seeburg,P.H. and Schaller,H. (1975) J. Mol. Biol. 92, 261-277.
22. Aono,J. and Horiuchi,T. (1977) Molec. Gen. Genet. 156, 195-201.
23. Pirrotta,V., Nucl. Ac. Res., submitted for publication.
24. Shine,J. and Dalgarno,L. (1974) Proc. Natl. Acad. Sci. USA 71, 1342-1346.
25. Steitz,J.A. and Jakes, K. (1975) Proc. Natl. Acad. Sci. USA 72, 4734-4738.
26. Folkmanis,A., Takeda,Y., Simuth,J., Gussin,G. and Echols,H. (1976) Proc. Natl. Acad. Sci. USA 73, 2249-2253.
27. Ovchinnikov,Y.A., Guryev,S.O., Krayev,A.S., Monastyrskaya,G.S., Sverdlov,E.D., Skryabin,K.G., Zaharyev,V.M. and Bayev,A.A. (1978) in Frontiers in Bioorganic Chemistry and Molecular Biology, Moscow/Tashkent, in press.
28. Roberts,T.M., Shimatake,H., Brady,C. and Rosenberg,M. (1977) Nature 270, 274-275.
29. Hsiang,M.W., Cole,R.D., Takeda,Y. and Echols,H. (1977) Nature 270, 275-277.
30. Beyreuther,K., Adler,K., Fanning,E., Murray,C., Klemm,A. and Geisler, N. (1975) Europ. J. Biochem. 59, 491-509.

31. Beyreuther,K. and Gronenborn,B. (1976) Molec. Gen. Genet. 147, 115-117.
32. Müller-Hill,B. (1975) Progr. Biophys. molec. Biol. 30, 227-252.
33. Westmoreland,B.C., Szybalski,W. and Ris,H. (1969) Science 163, 1343-1348.
34. Grosschedl,R. and Hobom,G., Nature, submitted for publication.
35. Kaiser,A.D. (1957) Virology 3, 42-61.
36. Eisen,H., Pereira da Silva,L. and Jacob,F. (1968) C. hebd. Seanc. Acad. Sci. Paris 266, 1176-1178.
37. Brachet,P. and Thomas,R. (1969) Mutat. Res. 7, 257-260.
38. Hobom,G., Grosschedl,R., Lusky,M., Scherer,G., Schwarz,E. and Kössel, H. (1978) Cold Spring Harb. Symp. Quant. Biol. 43, in press.
39. Hayes,S. and Szybalski,W. (1973) Molec. Gen. Genet. 126, 275-290.
40. Honigman,A., Hu, S.-L., Chase,R. and Szybalski,W. (1976) Nature 262, 112-116.
41. Moore,D.D., Denniston-Thompson,K., Kruger,K.E., Furth,M.E., Williams, B.G., Daniels,D.L. and Blattner,F.R. (1978) Cold Spring Harb. Symp. Quant. Biol. 43, in press.
42. Scherer,G., Hobom,G. and Kössel,H. (1977) Nature 265, 117-121.
43. Dahlberg,J.E. and Blattner,F.R. (1973) in Virus Research, Fox,C.F. and Robinson,W.S.,Eds., 533-543, Academic, New York.
44. Lee,F., Bertrand,K., Bennett,G. and Yanofsky,C. (1978) J. Mol. Biol. 121, 193-217.
45. Zurawski,G., Brown,K., Killingly,D. and Yanofsky,C. (1978) Proc. Natl. Acad. Sci. USA 75, 4271-4275.
46. Di Nocera,P.P., Blasi,F., Di Lauro,R., Frunzio,R. and Bruni,C.B. (1978) Proc. Natl. Acad. Sci. USA 75, 4276-4280.
47. Barnes,W.M. (1978) Proc. Natl. Acad. Sci. USA 75, 4281-4285.
48. Howard,B., de Crombrugghe, B. and Rosenberg,M. (1977) Nucl. Ac. Res. 4, 872-842.
49. Takeda,Y., Folkmanis,A. and Echols,H. (1977) J. Biol. Chem. 252, 6177-6183.