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Proteins Responsible for Lysogenic Conversion Caused by Coliphages N15 and φ80 Are Highly Homologous

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Lysogenic conversion caused by lambdoid bacteriophage φ80 and that caused by coliphage N15 have similar characteristics, suggesting that similarities in their *cor* genes and Cor proteins are responsible for this effect. Here we present the nucleotide sequence of the N15 *cor* gene. The N15 *cor* gene homolog was found in the φ80 *cor* region, but in the opposite direction of that of the open reading frame to which the φ80 *cor* gene had previously been assigned (M. Matsumoto, N. Ichikawa, S. Tanaka, T. Morita, and A. Matsushiro, Jpn. J. Genet. 60:475–483, 1985).

Bacteriophage φ80 is a member of the lambda phage family (16), and bacteriophage N15 is a temperate phage of *Escherichia coli* isolated by Ravin in 1964 (13). It was shown that N15 prophage exists in a plasmid state (15). Later, we found that N15 plasmid prophage is a double-stranded linear DNA molecule with covalently closed ends (19).

Lysogenization by some temperate bacteriophages changes the host cell phenotype. The characteristics of this phenomenon, named lysogenic conversion, are similar for phages φ80 and N15. Neither φ80 lysogens (4) nor N15 lysogens (14) adsorb superinfecting phages φ80, N15, and T1. We previously described in detail this phenomenon for phage φ80 (7) and subsequently cloned and mapped the gene of conversion, which we named *cor* (5, 6). The phenotype induced by this gene was designated Cor^+. The nucleotide sequence of the φ80 *cor* gene region was determined by Matsumoto et al. (9). The N15 *cor* gene was also cloned and mapped. By using a minicell system, N15 Cor protein was shown to have a molecular mass of about 8 kDa (8).

Further localization, sequencing, and analysis of the N15 *cor* gene. Elsewhere, it was shown that *E. coli* cells acquired the Cor phenotype after being transformed with plasmid pNC304 or pNC3044, which contain the N15 plasmid DNA *Pst*I restriction fragment (coordinates 39.43 to 41.68 kb) and *Sal*I-*Pst*I restriction fragment (coordinates 40.98 to 41.68 kb) (8), respectively.

A series of deletions was introduced into pNC304, producing plasmids pNC3041, pNC3042, and pNC3043 (Fig. 1). Transformation with plasmid pNC3041 or pNC3042, but not with plasmid pNC3043, permitted host *E. coli* HB101 cells to grow on plates covered with φ80 *vir* phage (10^8 PFU/cm^2), i.e., caused the Cor^+ phenotype. The results suggested that the *cor* gene resides between the *Cla*I and *Pvu*II restriction sites.

We determined the nucleotide sequence of the N15 plasmid DNA *Sal*I-*Pst*I restriction fragment. The data obtained are summarized in Fig. 2.

An open reading frame (ORF) was found in the *Cla*I-*Pst*I direction, starting at nucleotide 196 and terminating at nucleotide 432 (ORF78; Fig. 2). This ORF encodes a protein with a molecular mass of 8.6 kDa. Putative −35 and −10 promoter elements were found upstream of this ORF. The sequences of both elements as well as the putative Shine-Dalgarno sequence are very close to the consensus sequence. Both the reading direction of ORF78 and the size of the protein it encodes are consistent with the results reported earlier for the *cor* gene and its product (8). No other extensive ORF was found in either direction of the *Cla*I-*Pst*I restriction fragment. Therefore, we assigned the N15 *cor* gene to ORF78.

Computer-assisted analysis based on the Argos and Rao method (12) revealed that the 21 N-terminal amino acids of the deduced N15 Cor protein are capable of forming a transmembrane helix. This suggests that the protein could be integrated into the membrane, where it may interact with common receptors for phages N15 and φ80.

Comparison of the N15 and φ80 *cor* gene and protein sequences. Initially, the φ80 *cor* gene was mapped near gene *13*, the last gene of the late operon (5). Later, it was found that transformants harboring a *Sma*I-*Pst*I restriction fragment from this region are resistant to φ80 and retain the inhibitory effect to φ80 adsorption (9). The nucleotide sequence of this fragment was determined, and the Cor^+ phenotype was ascribed to ORF92, which is the longest ORF found on the *Hae*III-A–*Hae*III-B part of the fragment (Fig. 3). This assignment was verified by introducing a frameshift mutation into the *Pvu*II site within this ORF, which resulted in the loss of the adsorption inhibition function (9). Experimental evidence for the direction of gene transcription was also provided. However, later results that were inconsistent with this assignment were reported; the insertion of transposon Tn*1000* into a site that lies outside this ORF (Fig. 3), close to its end, produces the Cor^- phenotype (6).

Comparison of the nucleotide sequence established for the N15 *cor* gene with the one suggested for the φ80 *cor* gene (9) shows neither DNA nor protein homology. This fact by itself is not surprising, since the same function may be fulfilled by different proteins with unrelated primary structures. Nevertheless, taking into account the discrepancies in previously published results, we decided to look for another possible interpretation of the φ80 *cor* gene sequencing data (9).

There is an additional ORF (ORF77) within the *Hae*III-A–*Hae*III-B restriction fragment. This ORF, not discussed by Matsumoto et al. (9), lies between nucleotides 326 and 556 and is in the direction opposite of that of ORF92 (Fig. 3). We compared the N15 *cor* gene nucleotide sequence with that of

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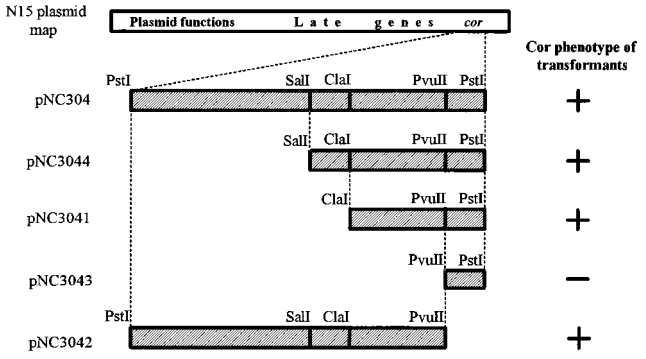

FIG. 1. Position of the *cor* gene on the N15 genome. The N15 plasmid map is shown at the top. The N15 DNA fragments cloned in different plasmids are indicated by shaded boxes. The Cor phenotype of the transformant carrying the corresponding plasmid is marked. +, present; −, absent.

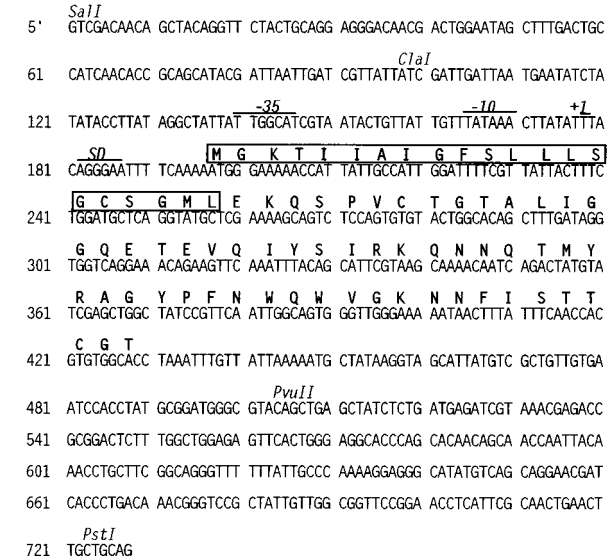

FIG. 2. Nucleotide sequence of the N15 *cor* gene region and deduced amino acid sequence of the Cor protein. Putative −10 and −35 promoter elements, the transcription start site (+1), and the Shine-Dalgarno sequence (SD) of the *cor* gene are overlined. The predicted transmembrane helix domain of the Cor protein is boxed.

ORF77 and found 64.5% similarity. Computer analysis revealed at least three possible weak promoters for ϕ80 ORF77, with −35 and −10 boxes placed around nucleotides (i) 227 and 250, (ii) 241 and 262, and (iii) 248 and 277, which would result in transcriptional start sites at nucleotides 261, 276, and 287, respectively. The transposon insertion which inactivates this gene (6) lies within the putative promoter area, disrupting its sequence. ORF77 encodes an 8.6-kDa protein, which shows 75% homology (62% identity and 13% similarity) with the amino acid sequence deduced from the N15 *cor* gene sequence (Fig. 4). The sequence homology is relatively weak in the N-terminal area of these proteins. However, the 21 N-terminal amino acids of the protein encoded by ORF77 are capable of forming a transmembrane helix. This was found by the same method of computer-assisted analysis that predicted a domain with a similar secondary structure in the corresponding region of N15 Cor protein. As proposed above for N15 Cor protein, such a domain might be responsible for the positioning of proteins in the cell for interaction with phage receptors.

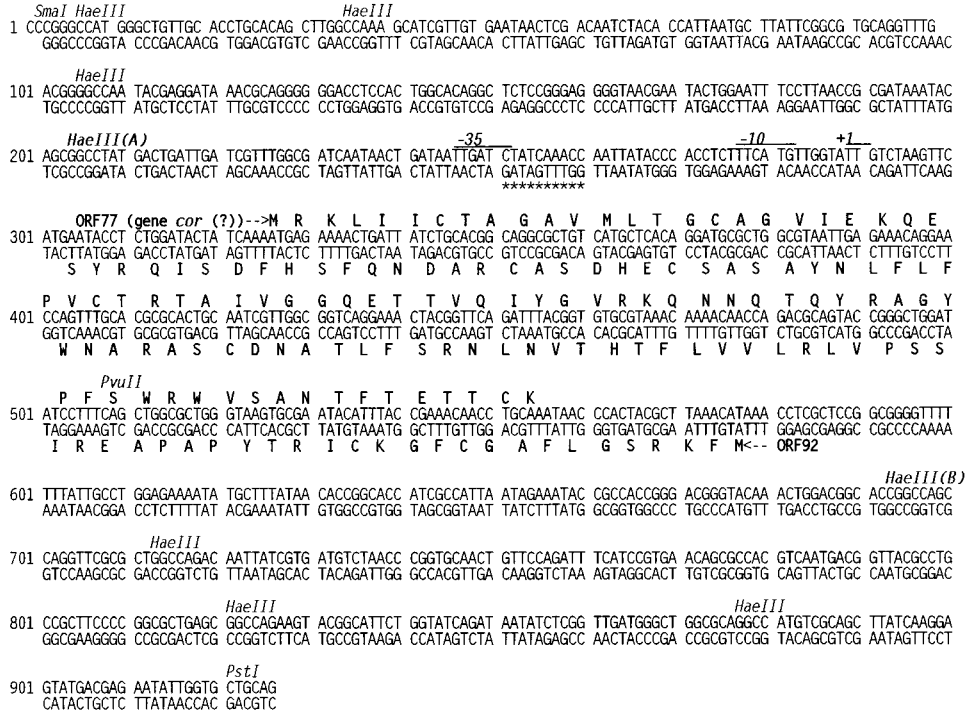

FIG. 3. Nucleotide sequence of the bacteriophage ϕ80 *cor* gene region (9), presented in inverted complementary order. The deduced amino acid sequence for ORF77 is shown above the upper DNA strand, and that for ORF92 is shown below the complementary strand. One set of potential −35 and −10 promoter boxes and the transcription start site (+1) for ORF77 are overlined. The position of the transposon Tn*1000* insertion, which inactivates the ϕ80 *cor* gene (6), is indicated by asterisks.

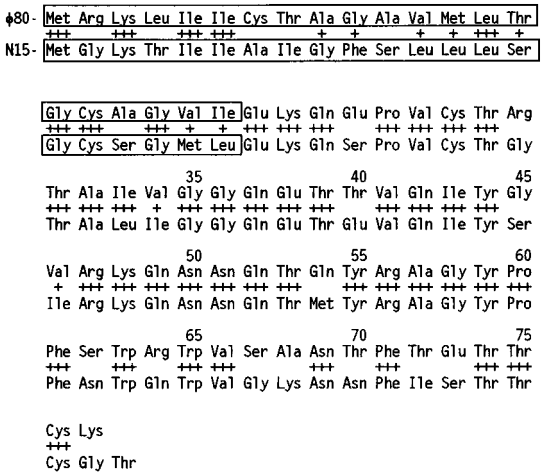

FIG. 4. Comparison of the amino acid sequences of N15 Cor protein (N15) and the protein encoded by ϕ80 ORF77 (ϕ80). +++, identical amino acids; +, amino acids with similar chemical properties. The predicted transmembrane helix domains of these proteins are boxed.

The combination of all these facts led us to hypothesize that the ϕ80 *cor* gene could be assigned to ORF77 rather than to ORF92. This assignment is consistent with the results from the transposon insertion experiment (6) as well as with all the data presented by Matsumoto et al. (9), except for the experiment showing the direction of ϕ80 *cor* gene transcription. We suppose that additional experiments should be carried out to resolve this controversy and to test our assignment of the ϕ80 *cor* gene to ORF77. Meanwhile, we hereafter refer to the proteins encoded by ORF77 and ORF92 as the ϕ80 Cor and ϕ80 ORF92 proteins, respectively.

Potential targets of ϕ80 and N15 Cor protein action. Both ϕ80 and N15 bacteriophages, as well as phages T1 and T5, require the multifunctional FhuA (formerly designated TonA) receptor protein in the outer membrane of *E. coli* for adsorption. FhuA is essential for the uptake of the iron carrier ferrichrome, colicin M, and antibiotics albomicin and microcin 25 (1, 3, 17, 21). The transport of ferrichrome, albomicin, and colicin M through the outer membrane, as well as infection by T1, ϕ80, and N15, depends additionally on the TonB inner membrane protein. TonB is anchored in the cytoplasmic membrane by an uncleaved hydrophobic amino-terminal signal sequence, with the remainder of the protein protruding into the periplasmic space (11). TonB is postulated to function as an energy transmitter, coupling inner membrane energy to active transport across the outer membrane (10). In the periplasmic space, energized TonB protein interacts with outer membrane receptors (including FhuA [2]), causing a conformational change which allows directed release of the bound ligand from the receptor into the periplasmic space. The Cor protein may interact directly with FhuA protein or indirectly via TonB protein.

Outer membrane receptors that use TonB as an energy transmitter have a conserved sequence motif, Glu-Thr-Val-Ile-Val, designated the TonB box (18). ϕ80 phage adsorption and other TonB-dependent activities are inhibited in vivo by a synthetic TonB box consensus pentapeptide (20). The 30th through 34th amino acids of both Cor proteins (ϕ80, Arg-Thr-Ala-Ile-Val; N15, Gly-Thr-Ala-Leu-Ile) strongly resemble the TonB box consensus. Therefore, in principle, the Cor^+ phenotypes of ϕ80 and N15 lysogens could be caused by direct interaction between Cor and TonB proteins.

Some observations indicate that ϕ80 Cor^+ lysogens have a $FhuA^-$ phenotype. (i) It is known that ϕ80*h* (host range mutant) does not adsorb on a *fhuA* mutant but can grow on *tonB* cells; the hybrid phage λ*h*80*h* (immunity of λ and the host range of ϕ80*h*) does not infect ϕ80 lysogenic strains that have the Cor^+ phenotype (7). (ii) Colicin B is a TonB-dependent and FhuA-independent factor; it kills Cor^+ lysogens (7). (iii) ϕ80 lysogens do not allow TonB-independent infection by phage T5 via the FhuA receptor (20). These facts point to FhuA protein as the Cor protein target. Alternatively, if Cor proteins interact with TonB, then this interaction should occur in such a way that it prevents a normal TonB-FhuA relationship and imparts an FhuA phenotype to lysogenic cells, but their $TonB^+$ phenotype remains unchanged or only slightly altered.

GenBank and EMBL accession numbers. The nucleotide sequence of the N15 *cor* gene was deposited in the GenBank database under accession number U28834 and in the EMBL nucleotide sequence database under accession numberZ49847.

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