JOURNAL OF BACTERIOLOGY, Aug. 1992, p. 5079–5085
0021-9193/92/155079-07$02.00/0

Vol. 174, No. 15

IncN Plasmid pKM101 and IncI1 Plasmid ColIb-P9 Encode Homologous Antirestriction Proteins in Their Leading Regions

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The IncN plasmid pKM101 (a derivative of R46), like the IncI1 plasmid ColIb-P9, carries a gene (*ardA*, for alleviation of restriction of DNA) encoding an antirestriction function. *ardA* was located about 4 kb from the origin of transfer, in the region transferred early during bacterial conjugation. The nucleotide sequence of *ardA* was determined, and an appropriate polypeptide with the predicted molecular weight of about 19,500 was identified in maxicells of *Escherichia coli*. Comparison of the deduced amino acid sequences of the antirestriction proteins of the unrelated plasmids pKM101 and ColIb (ArdA and Ard, respectively) revealed that these proteins have about 60% identity. Like ColIb Ard, pKM101 ArdA specifically inhibits both the restriction and modification activities of five type I systems of *E. coli* tested and does not influence type III (*Eco*P1) restriction or the 5-methylcytosine-specific restriction systems McrA and McrBC. However, in contrast to ColIb Ard, pKM101 ArdA is effective against the type II enzyme *Eco*RI. The Ard proteins are believed to overcome the host restriction barrier during bacterial conjugation. We have also identified two other genes of pKM101, *ardR* and *ardK*, which seem to control *ardA* activity and *ardA*-mediated lethality, respectively. Our findings suggest that *ardR* may serve as a genetic switch that determines whether the *ardA*-encoded antirestriction function is induced during mating.

Transfer of DNA between different gram-negative bacterial species may be mediated by self-transmissible plasmids and temperate bacteriophages (9, 11, 15, 16, 49). Some of them (R-plasmids) encode antibiotic resistance, and their dissemination among bacterial pathogens is a major problem worldwide in infectious diseases. However, when plasmid or phage DNA enters a new restriction-proficient host, it is often degraded by restriction endonucleases that recognize the methylation patterns of "guest" DNA (1, 7, 23, 41, 50, 54). It has been shown that some bacteriophages encode antirestriction functions which permit them to overcome host restriction (19, 33, 45, 55). Efficient transfer of large self-transmissible broad-host-range plasmids suggests that these plasmids may carry genes which express antirestriction functions. Our earlier experiments showed that two unrelated self-transmissible plasmids, pKM101 and ColIb-P9 (of the IncN and IncI1 incompatibility groups, respectively), carry *ard* (alleviation of restriction of DNA) genes that specifically affect type I restriction in *Escherichia coli* (6, 32). Recently, we determined the nucleotide sequence of the ColIb *ard* gene and identified the Ard product in *E. coli* maxicells (18). In this study, we examined in detail the *ard* region of pKM101 and report the nucleotide sequence of the *ardA* gene. Comparison of the deduced amino acid sequences of the antirestriction proteins of plasmids pKM101 and ColIb (ArdA and Ard, respectively) revealed that these proteins have about 60% identity. We have also identified two other genes of pKM101, *ardR* and *ardK*, that seem to control *ardA* activity and *ardA*-mediated lethality, respectively.

MATERIALS AND METHODS

Bacterial strains and media. AB1157 is an F^- derivative of *E. coli* K-12 having the genotype *thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 rpsL31 supE44*. BA556 is an r_K^- m_K^+ derivative of AB1157 (6). WA2377 (2), HB129 (40), and BZ216 (obtained from T. A. Bickle) are r_A^+ m_A^+, r_B^+ m_B^+, and r_D^+ m_D^+ derivatives of *E. coli* K-12, respectively. BA509 and BA2357 are r_{RI}^+ m_{RI}^+ and r_{R124}^+ m_{R124}^+ derivatives of AB1157, respectively, carrying the *Eco*RI plasmid (6) and plasmid R124, respectively. BA532 is an r_{P1}^+ m_{P1}^+ derivative of AB1157 that is lysogenic for P1CMts (6). JM109 (53) was used for maxicell analysis and as a host for plasmids. The media used in this study were described previously (18).

Plasmids and phages. The IncN plasmid pKM101, which is a 35.4-kb derivative of R46 (10, 34), was obtained from G. C. Walker. The IncFIV plasmid R124 (29, 37) was obtained from R. W. Hedges. Plasmids pBluescriptII (Stratagene) and pACYC184 (13) were used as vectors for cloning and DNA sequence analysis. The bacteriophage referred to as λ was λ*vir* (obtained from R. Devoret).

DNA manipulation and plasmid constructions. Standard procedures were used for DNA manipulation and plasmid construction (35). Plasmid DNA was prepared as described previously (18). Restriction endonuclease digests were carried out according to the manufacturer's recommendations (New England Biolabs). Deletions were prepared by exonuclease III and mung bean exonuclease treatment according to the supplier's instructions (Stratagene).

Plasmid pAB1 was made by ligating a 10.3-kb *Xho*I-*Bam*HI fragment of pKM101::Tn*5*Ω291 to pBluescriptII(KS−) DNA cut with *Xho*I and *Bam*HI. Note that pKM101 has no *Xho*I sites; all the *Xho*I sites of the pKM101::Tn*5* derivatives used are generated by Tn*5* insertions. pAB74 was made by inserting the Tn*903* Km^r gene cassette of pUC-4K (48) into the *Bam*HI site of pAB1 and deleting a 4.0-kb *Eco*RV-*Sma*I fragment from the resulting plasmid. For construction of pAB7 and pAB8, plasmid pAB1 was linearized with *Xho*I and *Kpn*I, treated with exonuclease III and mung bean nucleases for different times, and recircular-

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ized with T4 ligase, yielding deletion derivatives of pAB1. pAB7 and pAB8 were made by deleting three *Not*I fragments of appropriate deletion derivatives of pAB1 and transforming AB1157 harboring one of the compatible *ardK*$^+$ plasmids pOR36, pOR37, pOR69, or pOR80.

pOR36 and pOR37 were made by subcloning the appropriate *Xho*I-*Bam*HI fragments of pKM101::Tn*5*Ω130 and pKM101::Tn*5*Ω246 into pACYC184. Insertion derivatives of pKM101 were digested with *Xho*I, and pACYC184 was digested with *Hin*dIII. Digests were treated with DNA polymerase I Klenow fragment to generate blunt ends, and samples were digested with *Bam*HI. The desired plasmid fragments were purified by agarose gel electrophoresis and ligated with T4 DNA ligase.

To construct pOR80 and pOR84, a 3.3-kb *Acc*I-*Bgl*II fragment of pAB1 that had been filled in was ligated to *Eco*RV-cleaved pBluescriptII(KS+). Plasmids with fragments in the appropriate orientation were chosen. The resulting plasmid, pAB69, was linearized with *Eco*RI and *Pst*I, treated with exonuclease III and mung bean nuclease, and recircularized with T4 DNA ligase, yielding deletion derivatives of pAB69. pOR69, pOR80, and pOR84 were made by subcloning the inserts of pAB69 and its deletion derivatives into *Hin*dIII- and *Bam*HI-cleaved pACYC184.

DNA sequence analysis. The *ardA* gene region was sequenced on both strands by the dideoxy chain termination method (43) and the BluescriptII exonuclease III-mung bean nuclease sequencing system (Stratagene). Restriction fragments of pKM101 were subcloned into pBluescriptII vectors, and nested unidirectional deletions were created as described above. Deletion derivatives were rescued as single-stranded DNA and used for sequence analysis. Based on sequence information from the first strand, various oligodeoxynucleotides were synthesized and used as primers for sequencing the second strand. To locate Tn*5* insertions, primers derived from the sequence of Tn*5* terminal inverted repeats were used.

Measurement of antirestriction activity. The antirestriction activity of plasmids was defined as the efficiency of plating (EOP) of unmodified phage λ.0 on the experimental (plasmid-bearing) strain divided by the EOP on the plasmidless restricting strain (18). EOP was calculated as: phage titer on the restricting strain/phage titer on a nonrestricting strain (either BA556 or WA802). Unmodified phages, denoted by λ.0, were grown on *E. coli* C r_O m_O (7), which lacks restriction and modification functions. Modified phages, denoted by λ.K, were grown on the r_K^+ m_K^+ strain AB1157.

To test *E. coli* strains for their ability to restrict 5-methylcytosine-containing DNA (McrA$^+$ and McrBC$^+$ restriction phenotypes), the EOP for methylated and unmethylated λ.K phage was measured (38, 39). λ DNA was methylated by M · *Hpa*II and M · *Alu*I DNA methylases under the reaction conditions specified by the manufacturer (New England Biolabs), packaged, and tested with the resulting infective particles. AB1157, which is proficient in McrA and McrBC restriction (38) (unpublished data), was used as the restricting strain. WA802 (McrA$^-$BC$^-$), JM109 (McrA$^-$BC$^+$), and HB101 (McrA$^+$BC$^-$) were used as reference strains (38).

Nucleotide sequence accession number. The nucleotide sequence of the *ardA* gene has been deposited in GenBank and assigned accession number M81860.

RESULTS

Localization of the *ardA*, *ardK*, and *ardR* genes by Tn*5* insertion mutagenesis. To locate the *ard* gene more precisely, we obtained a number of independent insertion mutants of pKM101 with the transposable element Tn*5* inserted into the plasmid as described before (6). The positions of some Tn*5* insertions in the leading region of pKM101 are shown in Fig. 1A. All five Tn*5* insertions that completely abolished pKM101-mediated antirestriction activity were found in the region designated *ardA* (Fig. 1A and 2). Surprisingly, we also identified two neighboring Tn*5* insertions (only Ω64 is shown in Fig. 1A) that activated the ArdA function by a factor of 200 and completely inhibited *Eco*K restriction. This finding suggests that the Tn*5*Ω64 insertion lies within a region responsible for the regulation of ArdA function. We termed this locus *ardR*.

Unsuccessful attempts to subclone a 5.8-kb *Bgl*II fragment of pKM101 carrying the *ardA* region into multicopy vectors led us to the conclusion that the *ardA* locus might encode a *kil*-type gene(s) which was potentially lethal to the bacterial host. However, we were able to clone a 10.3-kb *Xho*I-*Bam*HI fragment of insertion derivative pKM101::Tn*5*Ω291, harboring the *ardA*$^+$ gene, on the high-copy-number vector pBluescriptII, yielding pAB1. Note that pKM101 has no *Xho*I sites, and all the *Xho*I sites used are generated by Tn*5* insertions (3, 34). Thus, a 10.3-kb insert of pAB1 contains both pKM101 DNA and a 489-bp end of Tn*5* resulting from *Xho*I digestion of the Tn*5*Ω291 insertion.

Further subcloning indicated that the presence of the *ardA* locus prevented survival of strains that did not also contain another locus of pKM101. Compatible plasmids pOR36, pOR37, pOR69, and pOR80, which carry pKM101 DNA between coordinates 30.5 and 31.8, are able to inhibit the lethality (Kil phenotype) caused by pAB7 (*ardA*$^+$) (Fig. 1). In contrast, a deletion derivative of pOR80, pOR84, is insufficient to prevent *ardA*-mediated lethality in pAB7. These results demonstrate that this locus, which seems to encode a *kil* override (Kor) function, is located at 31 kb. We termed it *ardK*.

Nucleotide sequence of the *ardA* gene and identification of its product. The data presented in Fig. 1A show that a 0.9-kb insert of pAB7 contains the *ardA* coding region, as judged by its ArdA$^+$ phenotype. On the other hand, a deletion derivative of pAB7, pAB8, failed to express Ard activity, suggesting that the deleted segment is needed for *ardA* expression. The nucleotide sequence of the *ardA* coding region revealed only one long open reading frame of 507 nucleotides that would code for a protein of 169 amino acids with a predicted molecular weight of 19,427 (Fig. 2). The initiation codon ATG at position 64 is preceded by a typical Shine-Dalgarno sequence. There are two additional potential initiation codons at positions 226 and 259. However, the spacing between them and appropriate Shine-Dalgarno-like sequences (14 and 12 nucleotides, respectively) is uncommon for *E. coli* ribosome-binding sites.

The proteins encoded by pAB7 (*ardA*$^+$) were examined on sodium dodecyl sulfate (SDS)-polyacrylamide gels with *E. coli* maxicells (Fig. 3). pAB7 (*ardA*$^+$) encodes a polypeptide of about 20 kDa (Fig. 3, lane 2) that is not encoded by the vector or by the coresident plasmid pOR80 (*ardK*$^+$) (Fig. 3, lane 1). Since the 20-kDa protein was absent in maxicells harboring the *ardA* mutant pAB8 (Fig. 3, lane 3) and its size agreed with that predicted from the nucleotide sequence of *ardA*, we concluded that it is the product of *ardA*.

Activity of *ardA* is controlled by the *ardR* gene. We also found that deletion of the *ardR* region (pOR37) drastically activated pAB7-directed ArdA function, while the presence of the *ardR* region in *trans* (pOR36) or *cis* (data not shown) markedly inhibited ArdA activity (Fig. 1A). Note that inhi-

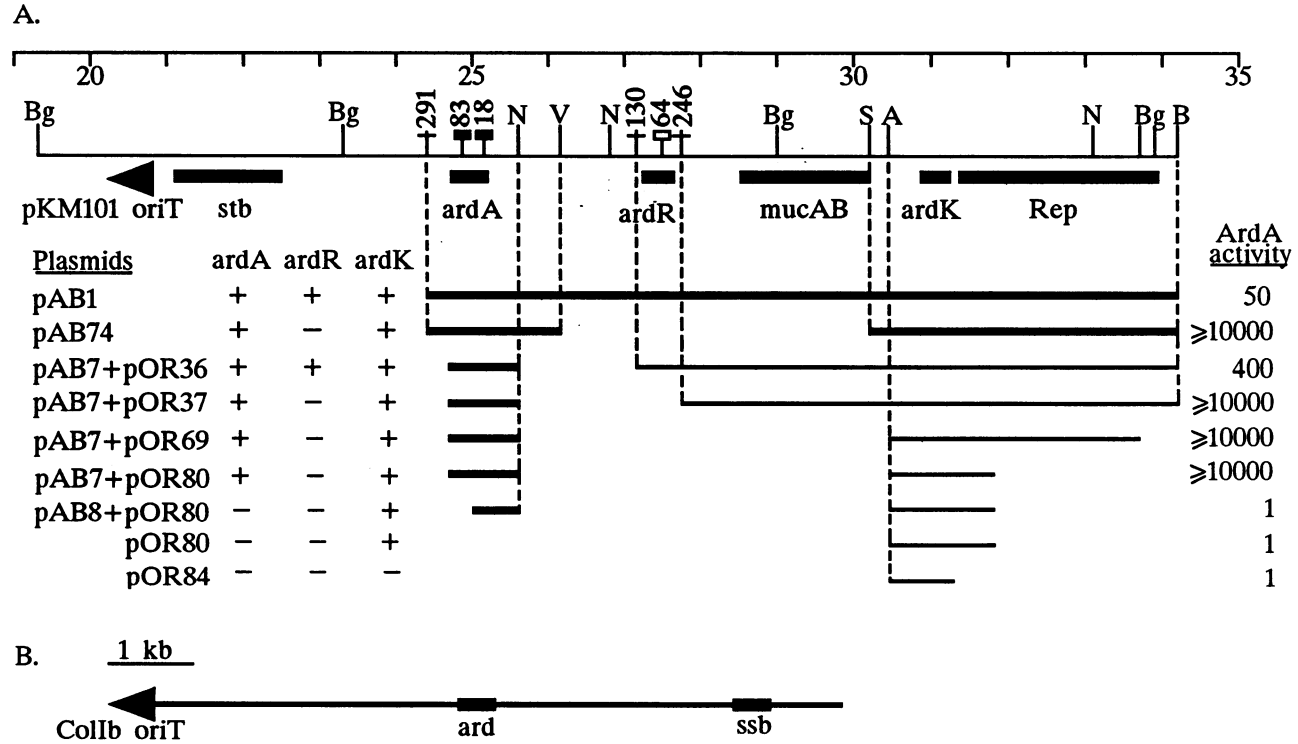

FIG. 1. Maps of the leading regions of pKM101 (A) and ColIb-P9 (B). (A) The top line indicates kilobase coordinates on the pKM101 map (51). The second line shows restriction sites and the locations of *oriT* (origin of transfer), Rep (origin of replication), *stb* (required for stability in recombination-proficient hosts), *mucAB* (mutagenesis enhancement) (10, 51), and the *ardA*, *ardR*, and *ardK* genes. Numbers in boldface refer to the positions of Tn*5* insertions which affect (■), activate (□), or do not influence (+) ArdA function. Three Tn*5* insertions (Ω291, Ω130, and Ω246) were also used for introducing *Xho*I sites into pKM101 DNA. Only the relevant restriction sites of pKM101 are indicated. The lines below the map indicate the regions of pKM101 subcloned into either vector pBluescriptII KS (pAB1, pAB7, pAB8, and pAB74; thick lines) or vector pACYC184 (pOR36, pOR37, pOR69, pOR80, and pOR84; thin lines). ArdA activity, expressed by these recombinant plasmids and indicated on the right, was measured in restricting strain *E. coli* K-12 AB1157 carrying one or two plasmids (indicated on the left) as described in Materials and Methods. None of the pOR plasmids represented express any antirestriction activity in the absence of pAB7. Abbreviations: A, *Acc*I; B, *Bam*HI; Bg, *Bgl*II; N, *Not*I; S, *Sma*I; V, *Eco*RV. (B) Location of the *oriT* region, *ssb* gene (30, 31), and *ard* gene (18) in the leading region of ColIb-P9.

bition of pAB7-directed ArdA function by pOR36 (*ardR*$^+$) was incomplete compared with that by pAB1. This presumably reflects a difference in copy number between pAB7 (a derivative of high-copy-number pBluescriptII) and pOR36 (a derivative of intermediate-copy-number pACYC184). These results, together with the Tn*5* insertion mutagenesis data, strongly indicate that the *ardR* locus controls the activity of *ardA*.

Antirestriction proteins of unrelated plasmids pKM101 and ColIb-P9 are homologous. A search in different data banks with the FASTA program failed to detect any extensive similarities with the nucleotide or deduced amino acid sequence of *ardA*. However, comparison of the ArdA protein of pKM101 with the Ard protein encoded by the IncI1 plasmid ColIb-P9 (18) revealed 60.2% identity over 169 amino acids (Fig. 4). Both proteins are very acidic and contain an excess of 25 (ColIb) and 27 (pKM101) negatively charged amino acids (Asp and Glu). It should be noted that little if any DNA homology is observed between plasmids of the IncI1 and IncN incompatibility groups (24, 28).

The data presented in Table 1 show that pKM101 ArdA, like ColIb Ard, efficiently inhibits all five type I restriction systems of *E. coli* tested (*Eco*A, *Eco*B, *Eco*D, *Eco*K, and *Eco*R124). It should be noted that the modification activity of type I systems is also susceptible to both plasmid-encoded antirestriction functions. This conclusion is based on the observations that the presence of either pED80 (18) or pAB7, encoding ColIb Ard and pKM101 ArdA, respectively, markedly inhibits *Eco*A, *Eco*B, *Eco*D, *Eco*K, and *Eco*R124 modification of unmodified phage λ (18) (unpublished results). In addition, both antirestriction functions seem to be ineffective against the type III (*Eco*P1) restriction and 5-methylcytosine-specific restriction systems McrA and McrBC (Table 1) (unpublished results). Despite these similarities, one notable difference exists between the pKM101 and ColIb antirestriction functions: in contrast to ColIb Ard, pKM101 ArdA slightly affects type II (*Eco*RI) restriction (Table 1) (18). Thus, these data indicate that two unrelated self-transmissible plasmids encode functionally similar and homologous antirestriction proteins in their leading regions.

DISCUSSION

We now show that two unrelated plasmids, pKM101 and ColIb-P9, encode antirestriction proteins in their leading regions, near the origin of transfer. These proteins are very acidic and have about 60% identity. The ArdA protein of pKM101 has a calculated molecular weight of 19,427, compared with 19,193 for ColIb Ard. The number of residues in pKM101 ArdA and ColIb Ard differs slightly, being 169 and

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                                                                                                    pAB34(-)  18(-)
                                                                                                                120
ATCATCGGGAGGCAATACCGCCACCCGAAAAATTGCCGGTATCGGCGCTTTAAGGAGTACACCATGACTGATATTACGACCCCTTCTGTTTATGTTGGCACTTACCATAAATACAACTGC
                                                  SD         MetThrAspIleThrThrProSerValTyrValGlyThrTyrHisLysTyrAsnCys

                                                                                                                240
GGAAGCATTGCGGGAGCCTGGCTCGATCTGACCGATTTTGATAGCTCAGAGGAGTTTTACGAGCGCTGCCGCGAGTTACACGCAAATGAGGCTGATCCAGAATTTATGTTCCAGGATTGG
GlySerIleAlaGlyAlaTrpLeuAspLeuThrAspPheAspSerSerGluGluPheTyrGluArgCysArgGluLeuHisAlaAsnGluAlaAspProGluPheMetPheGlnAspTrp
                388(-)                                       78(-)                               pAB8(-)          9(-)
                                                                                                                360
GAGGGTATTCCGTCTGATATGGCGTCAGAGTGCCATATCAACTGGGATTTTATTAACGGCTTTAAACAAGCCCGCGAGGAAGGAAACGAAGCGGCGTTTGTGGCCTTTGTTGATCTGTTT
GluGlyIleProSerAspMetAlaSerGluCysHisIleAsnTrpAspPheIleAsnGlyPheLysGlnAlaArgGluGluGlyAsnGluAlaAlaPheValAlaPheValAspLeuPhe
                                      83(-)
                                                                                                AccI            480
AATAGCACTGATTTCGACCTGTTTAGAGATGCCTATATGGGCGAAGCTAAGGACGAAGAAACTTTTGCAGAGGAATACCTGAATGATAGCGGTCTACTGAATGAAATTCCTGAATCCGTA
AsnSerThrAspPheAspLeuPheArgAspAlaTyrMetGlyGluAlaLysAspGluGluThrPheAlaGluGluTyrLeuAsnAspSerGlyLeuLeuAsnGluIleProGluSerVal
                                                                                                           pAB7(+)

GCCCGATATTTTGACATCGTAGCCTATGCGCGGGATCTGTTTATTGGGGATTTCAGTTTACATGACGGACACGTATTTAACATGACTTGCTGAAACCTAGTCCCCCGCCACGCGGGGG
AlaArgTyrPheAspIleValAlaTyrAlaArgAspLeuPheIleGlyAspPheSerLeuHisAspGlyHisValPheAsnMetThrCys***
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FIG. 2. Nucleotide and deduced amino acid sequences of the *ardA* gene. The sequence numbering begins at the first nucleotide following the stop codon TAA in the *ardA* reading frame. The proposed Shine-Dalgarno site (SD) is underlined. The bent arrows indicate the endpoints in the deleted clones. Arrowheads indicate the locations of Tn*5* insertions. The + and − signs represent the presence or absence of ArdA activity, respectively.

166, respectively. They are functionally similar in that both specifically inhibit both the restriction and modification activities of five type I systems of *E. coli* tested (*Eco*A, *Eco*B, *Eco*D, *Eco*K, and *Eco*R124) but do not influence type III (*Eco*P1) restriction or 5-methylcytosine-specific restriction (McrA and McrBC). However, in contrast to the ColIb Ard protein, the pKM101 ArdA protein was effective against the type II enzyme *Eco*RI. This may give the IncN plasmids an advantage in overcoming the host restriction barrier.

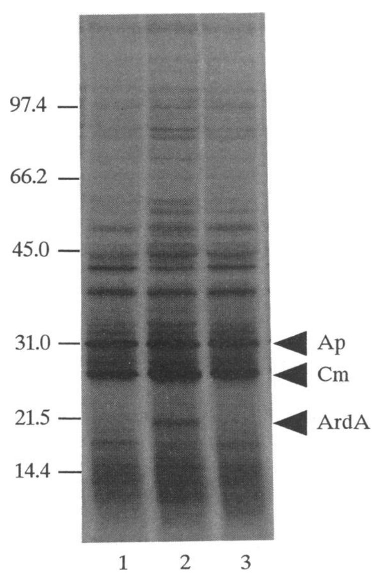

FIG. 3. Proteins produced by *ardA* derivative plasmids. The polypeptides encoded by the plasmids were specifically labeled with [^{35}S]methionine by using the maxicell system (42), analyzed by electrophoresis on a 5 to 25% gradient polyacrylamide–SDS gel, and visualized by autoradiography. Lanes: 1, vector pBluescriptII(KS−) plus pOR80 (*ardK*$^+$); 2, pAB7 (*ardA*$^+$) plus pOR80 (*ardK*$^+$); 3, pAB8 (Δ*ardA*) plus pOR80 (*ardK*$^+$). Arrowheads indicate the presumptive product of the *ardA* gene and the vector-encoded polypeptides β-lactamase (Ap) and chloramphenicol acetyltransferase (Cm). Positions of size standards (in kilodaltons) are indicated on the left.

Our results suggest that the *ardA* gene has a potentially lethal phenotype. A locus that inhibits this lethality, *ardK*, was also identified, and it was shown that the *ardA* gene could be cloned on multicopy vectors only in the presence (either in *cis* or in *trans*) of the *ardK* gene. *kil*- and *kor*-type functions appear to be common among broad-host-range self-transmissible plasmids (25). At least two pairs of *kil* and *kor* genes have been identified previously in pKM101 (52). These genes are located far away from *ardA*, within the region between 3 and 11 kb. One of them, *kilB*, is necessary for conjugal transfer; the other, *kilA*, decreases the growth rate of pKM101-containing strains on minimal medium. It is striking, however, that unlike the *ardA* gene of pKM101, the related *ard* gene of ColIb does not exhibit any *kil*-type activity (18). We have no obvious explanation for this

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pKM   1 MTDITTPSVYVGTYHKYNCGSIAGAWLDLTDFDSSEEFYERCRELHANEADPEFMFQDWE
        :.  ..  :.:::::.:::::::::::: : ::: ::    .:.   :: ::  ::::: ::::.:
Col   1 MSVVA-PAVYVGTWHKYNCGSIAGRWFDLTTFDDERDFFAACRALHQDEADPELMFQDYE

pKM  61 GIPSDMASECHINWDFINGFKQAREEGNEAAFVAFVDLFNSTDFDLFRDAYMGEAKDEET
        :  :   ::::::::: .. ::.:::.:: : :.   .:.      :::: ::::. :::  ::.
Col  60 GFPGNMASECHINWAWVEGFRQARDEGCEEAYRLWVEDTGETDFDTFRDAWWGEADSEEA

pKM 121 FAEEYLNDSGLLNEIPESVARYFDIVAYARDLFIDGFSLHDGHVFNMTC -169
        :: :.  :.::: ..::.:: :::   ::::::::.: :.  :::::
Col 120 FAVEFASDTGLLADVPETVALYFDYEAYARDLFLDSFTFIDGHVFRR   -166
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FIG. 4. Comparison of the predicted amino acid sequences of the pKM101 *ardA* and ColIb-P9 *ard* protein products. Alignment was done with the programs PALIGN and CLUSTAL (IntelliGenetics Inc.). Double dots indicate sequence identity, and single dots denote similar amino acids (in one-letter notation: A, S, and T; D and E; N and Q; R and K; I, L, M, and V; and F, Y, and W). Regions of identity with the 0.3 protein of phage T7 are shown in boldface and underlined.

TABLE 1. Effect of *ardA* on different *E. coli* restriction systems

Restriction system[a] (type)	EOP of test phage		Relief of restriction[b]	Antirestriction phenotype
	Without pAB7	With pAB7[c]		
*Eco*A (I)	4×10^{-3}	1.0	250	+
*Eco*B (I)	2×10^{-4}	1.0	5,000	+
*Eco*D (I)	2×10^{-4}	5×10^{-1}	2,500	+
*Eco*K (I)	1×10^{-4}	1.0	10,000	+
*Eco*R124 (I)	4×10^{-4}	1.0	2,500	+
*Eco*RI (II)	1×10^{-4}	1×10^{-2}[d]	100	±
*Eco*P1 (III)	5×10^{-5}	5×10^{-5}[d]	1	−
McrA	1×10^{-1}	1×10^{-1}	1	−
McrBC	1×10^{-1}	1×10^{-1}	1	−

[a] The restriction strains used were: *Eco*A, WA2377; *Eco*B, HB129; *Eco*D, BZ216; *Eco*K, AB1157; *Eco*R124, BA2357; *Eco*RI, BA509; *Eco*P1, BA532; and McrA and McrB, AB1157. Since strains BA509, BA532, and BA2357 contain two restriction systems, we used λ.K test phages in order to subject them only to *Eco*RI, *Eco*P1, and *Eco*R124 restriction, respectively. To test McrA and McrBC restriction, λ.K phages modified by M · *Hpa*II and M · *Alu*I methylases, respectively, were used. In other cases, we used λ.0 test phage.

[b] Relief of restriction refers to the EOP of unmodified test phages on the strain carrying pAB7 (*ardA*$^+$) relative to that on the same strain without a plasmid (or with the ArdA$^-$ plasmid pAB8). All strains tested carried the compatible plasmid pOR80 (*ardK*$^+$).

[c] Similar results were obtained for all restriction systems tested when instead of pAB7 plus pOR80, the ArdA$^+$ ArdK$^+$ ArdR$^-$ plasmid pAB74 was used as a carrier of the *ardA*$^+$ gene.

[d] Only pAB74 was used.

difference between the two homologous *ardA*-type genes. It is possible, however, that the *kil* function of *ardA* is distinct from the antirestriction function. This suggestion is based on the observation that the deletion derivative of pAB7 (*ardA*$^+$), pAB8, which is defective in the antirestriction function, exhibits a strong Kil$^+$ phenotype (data not shown).

Deletion and insertion studies and maxicell analysis of plasmid protein patterns suggest that the activity of *ardA* is controlled by the diffusible product of the *ardR* gene. It is possible, therefore, that *ardR* serves as a genetic switch that determines whether ArdA function is induced during mating. We view this as analogous to the establishment and maintenance modes in bacteriophage λ. From this model, we predict a zygotic induction effect on *ardA* expression after conjugation; the antirestriction ArdA protein should be synthesized in the new host until its repressor, the *ardR* product, accumulates. This suggestion is supported by the observation that plasmid pOR36, carrying the *ardR*$^+$ gene, abolishes pAB7-directed synthesis of ArdA protein in maxicells, while the ArdR$^-$ plasmid pOR37 does not (data not shown). Note that the *ardR* product seems to be a protein, because we have recently identified an open reading frame in the *ardR* coding region and an appropriate polypeptide in *E. coli* maxicells. We also identified the nucleotide sequence in the *ardA* promoter region that is needed for the function of ArdR as a regulatory protein (5a). Interestingly, a deletion of the upstream region of the ColIb *ard* gene also drastically increased antirestriction activity and ColIb Ard protein expression. However, we have failed to identify an appropriate regulatory gene of ColIb (unpublished data).

A question arises about the mechanism of action of ArdA-type antirestriction proteins. Binding to DNA and competition with the restriction-modification system for its recognition sites on DNA seem unlikely, given the strongly acidic nature of both antirestriction proteins. We have suggested that the acidic ColIb Ard protein might act like the acidic 0.3 protein of T7 phage (18) and inhibit the host-controlled restriction and modification systems by binding directly to an enzyme (5, 36, 44). It is possible that all three antirestriction proteins act as polyanions that inactivate the host restriction-modification complex by binding to their DNA-binding sites. Such a mode of action was suggested for the 0.3 antirestriction protein of T7 phage (20). Comparison of the deduced amino acid sequences of both Ard proteins and the 0.3 protein of T7 phage revealed only two small regions which are probably conserved for all three proteins (Fig. 4). It is possible that these protein regions are responsible for binding to the restriction-modification complex.

All three acidic antirestriction proteins are encoded by genes located in leading regions of the plasmid and phage genomes, which enter the host cell early during conjugation or infection (Fig. 1) (45). They specifically inhibit both the restriction and modification activities of the type I systems and influence type II restriction in *E. coli* only slightly or not at all (18, 36). These observations are consistent with the suggestion that antirestriction functions permit phages and self-transmissible plasmids to overcome the host restriction barrier during transfer into cells. In addition, it has been found that other IncN and IncI plasmids also express antirestriction functions (32). Interestingly, the leading regions of some plasmids also encode another protective function, Psi (plasmid SOS inhibition), that permits the transfer of single-stranded DNA without generating an SOS signal in the recipient cell during conjugation (4).

Type I restriction is also specifically alleviated in *E. coli* when the cell's DNA is damaged by agents such as UV radiation (17, 46, 47) or the base analog 2-aminopurine (22) or when the DNA is unmethylated in *dam* mutants deficient in adenine methylase activity (21). These findings are associated with the induction of some antirestriction functions in response to DNA damage or modification in cells. Thus, unlike type II restriction enzymes, the type I systems seem to be susceptible to both "guest" (phage and plasmid-encoded) and host antirestriction functions. It is possible, therefore, that host-controlled (type I) restriction is programmed to drop in certain circumstances as a result of the induction of some antirestriction functions. This in turn may facilitate foreign-gene transfer into the cell and make the bacterial cell more adaptable.

On the other hand, the type I systems sometimes are able to change their sequence specificity as a result of some recombination events within the *hsdS* genes, which control the specificity of DNA recognition by each system (12, 26, 27, 37). This ability is suggested to be advantageous to restriction-proficient cells because it may protect them from bacteriophages which lack susceptible sites in their genomes as a result of selection pressure (33, 37). These observations raise the possibility that changes in the activity and sequence specificity of host-controlled restriction may control gene flow between different populations of bacteria. This possible advantage may partly explain why the complicated type I restriction and modification systems have been retained when the much simpler type II systems are also effective in preventing foreign-DNA transfer.

ACKNOWLEDGMENTS

We thank G. C. Walker for providing plasmid pKM101 and S. M. Chigaeva and V. Zhurenkov for excellent technical assistance.

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