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# Two DNA Antirestriction Systems of Bacteriophage P1, *darA*, and *darB*: Characterization of *darA*$^-$ Phages

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**Bacteriophage P1 is only weakly restricted when it infects cells carrying type I restriction and modification systems even though DNA purified from P1 phage particles is a good substrate for type I restriction enzymes *in vitro*. Here we show that this protection against restriction is due to the products of two phage genes which we call *darA* and *darB* (*dar* for *d*efense *a*gainst *r*estriction). Each of the *dar* gene products provides protection against a different subset of type I restriction systems. The *darA* and *darB* gene products are found in the phage head and protect any DNA packaged into a phage head, including transduced chromosomal markers, from restriction. The proteins must, therefore, be injected into recipient cells along with the DNA. The proteins act strictly in *cis*. For example, upon double infection of restricting cells with *dar*$^+$ and *dar*$^-$ P1 phages, the *dar*$^+$ genomes are protected from restriction while the *dar*$^-$ genomes are efficiently restricted.** 

## INTRODUCTION

Many bacteriophages have evolved means of avoiding the worst effects of the DNA restriction and modification systems that they encounter in their hosts. One striking feature of these antirestriction mechanisms is their variety. They range from chemical modifications to the DNA that render it resistant to restriction through the production of proteins that physically inhibit the restriction enzymes to, perhaps the simplest of them all, the elimination of restriction enzyme recognition sites in their genome by mutation (reviewed by Krueger and Bickle, 1983).

In this paper we show that bacteriophage P1 also has an antirestriction mechanism which is different from any other yet described in that it is mediated by two proteins found in the phage head. We call the structural genes for the two proteins *darA* and *darB* (the symbol *dar* is derived from *d*efense *a*gainst *r*estriction). These Dar proteins do not promote antirestriction in the cells in which they are synthesized; rather, they are active in newly infected cells, where they are injected along with the DNA. Even in newly infected cells, the Dar proteins act in *cis*. When restricting hosts are coinfected with Dar$^+$ and Dar$^-$ phages, only the Dar$^+$ genomes escape restriction. Moreover, any DNA packaged in a P1 phage head, including transduced chromosomal DNA, is protected from restriction.

The Dar antirestriction mechanism is effective only against type I restriction systems. These are the chromosomally coded restriction and modification systems of the Enterobacteriaceae. The type I restriction enzymes are multifunctional and have a complex subunit structure (reviewed in Bickle, 1982). The features of their reaction mechanisms relevant for this paper are that these enzymes require ATP to restrict DNA and once an enzyme molecule has cleaved DNA it is irreversibly transformed into a DNA-dependent ATPase. This ATP hydrolysis forms the basis of a useful assay for the enzyme. A peculiar feature of type I restriction enzymes is that although they recognize specific sequences in DNA, they cut DNA randomly and far from the recognition sites. They do this by forming looped intermediates in which the enzyme is simultaneously bound to both the recognition site and the cleavage site. The enzyme first binds to the recognition site and then uses the energy of ATP hydrolysis to "pump" the DNA past it until the cleavage site is reached. The signal for the enzyme to stop pumping and start cutting is unknown (Yuan *et al.*, 1980; Endlich and Linn, 1985).

It has recently been shown that the type I restriction systems belong to several allelic families. Within a family, mutational defects in one member can be complemented by wild-type alleles from another and the structural genes of all family members are homologous by hybridization analysis. Between families no DNA homology is found and complementation does not occur (Murray *et al.*, 1982; Suri *et al.*, 1984; Fuller-Pace *et al.*, 1985; Suri and Bickle, 1985). We have found that the *darA* gene product protects against just one of these families, that represented by the enzyme *Eco*A. The *darB* gene product, perhaps in conjunction with the *darA* product, protects against another family of type I systems which includes *Eco*K.

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## MATERIALS AND METHODS

### Nomenclature

The nomenclature used for restriction and modification systems is that recommended by Smith and Nathans (1973). In particular, systems originating in *Escherichia coli* have the prefix *Eco* and those originating in *Salmonella typhimurium* and its relatives are prefixed *Sty*. The modification state of a phage genome is indicated as follows: P1.0, no modification; P1.B, modified by the *Eco*B system; P1.K modified by the *Eco*K system, etc. Bacterial strains that have lost the ability to restrict but which are still modification proficient are denoted $r^-m^+$ and strains that have lost both restriction and modification are $r^-m^-$. The particular system is indicated by subscripts where necessary; i.e., a strain which is $r_K^-m_K^+$ has lost *Eco*K restriction but still confers *Eco*K modification; in this case the *Eco* or *Sty* prefix is omitted (Arber and Linn, 1969). The nomenclature used for phage-coded proteins is that generally used for phage T4; thus, gp*darA* is the *darA* gene product.

### Microbiological materials and methods

The bacterial strains used are listed in Table 1 and the bacteriophages in Table 2. All of the media used and the preparation of phage stocks were described previously (Iida, 1977, 1984; Iida and Arber, 1977). To determine the efficiency of restriction of phage P1 derivatives, the host strains to be tested were grown in tryptone broth to $2 \times 10^8$ cells/ml, $CaCl_2$ was added to 10 m*M*, and the bacteria were further incubated for 30 min. These cells were used immediately for P1 infection and titers of plaque-forming units (PFU) and of drug-resistant specialized transductants were measured separately.

### Biochemical methods

The *Eco*K restriction enzyme was purified as described (Meselson and Yuan, 1968; Bickle *et al.*, 1977). The ATPase activity of the enzyme was assayed according to Hadi *et al.* (1975). Proteins were analyzed on sodium dodecyl sulfate–polyacrylamide gels according to Laemmli (1970) and were stained with Coomassie blue.

## RESULTS

### Bacteriophage P1 is resistant to DNA restriction

Phage P1 is only weakly restricted by many of the restriction systems found in *E. coli* and other members of the Enterobacteriaceae. As shown in Table 3, phage mutants that are far more severely restricted than the

TABLE 1

BACTERIAL STRAINS

| Designation | Relevant characteristics and comments | Source and references |
|---|---|---|
| *Escherichia coli* K12 strains | | |
| WA921 | $r^-m^-$ | Wood (1966) |
| W3110 | $r_K^+m_K^+$ | Our collection |
| SI1065 | $r_K^-m_K^+$ | Our collection |
| WA960 | $r_B^+m_B^+$ | Wood (1966) |
| WA837 | $r_B^-m_B^+$ | Wood (1966) |
| WA2379 | $r_A^+m_A^+$ | Arber and Wauters-Willems (1970) |
| CC4617 | $r_{SA}^+m_{SA}^+$, $r_{SB}^+m_{SB}^+$ | Van Pel and Colson (1974) |
| CC4619 | $r_{SA}^+m_{SA}^+$ | Van Pel and Colson (1974) |
| CC4620 | $r_{SB}^+m_{SB}^+$ | Van Pel and Colson (1974) |
| CC4621 | $r^-m^-$ | Van Pel and Colson (1974) |
| L4001 | $r_{SB}^+m_{SB}^+$ | Bullas and Colson (1975) |
| L4002 | $r_{SP}^+m_{SP}^+$ | Bullas and Colson (1975) |
| L4004 | $r_{SQ}^+m_{SQ}^+$ | Bullas *et al.* (1976) |
| WA3366 (pRI) | $r_B^+m_B^+$, $r_{RI}^+m_{RI}^+$ (*Eco*RI restricting) | Our collection |
| C600 (pRTF2) | $r_K^+m_K^+$, $r_{RII}^+m_{RII}^+$ (*Eco*RII restricting) | Our collection |
| C600 (pRTF1) | $r_K^+m_K^+$, $r_{RII}^-m_{RII}^+$ | Our collection |
| K140 (P1Cm *cry*) | $r_K^+m_K^+$, $r_{P1}^+m_{P1}^+$, P1 sensitive | Rosner (1973) |
| *Salmonella typhimurium* LT2 strains | | |
| CC4515 | $r_{SA}^+m_{SA}^+$, $r_{SB}^+m_{SB}^+$, $r_{LT}^+m_{LT}^+$, P1 sensitive | Bullas and Colson (1975) |
| CC4526 | $r_{SA}^-m_{SA}^+$, $r_{SB}^+m_{SB}^+$, $r_{LT}^-m_{LT}^+$, P1 sensitive | Bullas and Colson (1975) |

**TABLE 2**

BACTERIOPHAGES USED IN THIS WORK

| Designation | Relevant characteristics and comments | Source and references |
|---|---|---|
| Bacteriophage λ | | |
| λ*vir* | | Our collection |
| λ*ind*$^-$ | | Our collection |
| Bacteriophage Mu | | |
| Mucts62 *mom*$^+$ | | Toussaint (1976) |
| Mucts62 *mom*3457 | | Toussaint (1976) |
| Bacteriophage P1 and P1-15[a] | | |
| P1kc | Wild-type P1 | Our collection |
| P1Cm0 *sus*50 | Produces DNA-filled free heads | Yamamoto (1982) |
| P1Cm1 | Dar$^+$ | Arber *et al.* (1978) |
| P1Cm13 *mod*(P1)$^-$ | Dar$^+$, no P1 restriction or modification | Iida *et al.* (1983b) |
| P1Tc1 | Dar$^+$, carries Tn*10* | Mise and Arber (1976) |
| P1CmTc1 Δ*darA* | Dar$^-$ | Iida *et al.* (1982) |
| P1Tc18 Δ*darA* | Cm$^s$ derivative of P1CmTc1, Dar$^-$ | |
| P1CmTc1318 Δ*darA mod*(P1)$^-$ | Dar$^-$, no P1 restriction or modification, recombinant between P1Cm13 and P1Tc18 | |
| P1Cm2 Δ*darA* | Dar$^-$ | Iida *et al.* (1985) |
| P1Cm$^s$202 Δ*darA* | Cm$^s$ derivative of P1Cm2, Dar$^-$ | |
| P1-15::Tn*10* D4 | Tn*10* inserted in the *darA* gene, Dar$^-$ | Our collection |
| P1-15::Tn*10* D15 | Tn*10* inserted in the *darA* gene, Dar$^-$ | Our collection |
| P1-15::Tn*2653* WI1 | Tn*2653* inserted in the *lyd* gene, Dar$^-$ | Iida *et al.* (1980) |
| P1-15::IS*30* A20 | IS*30* inserted in the *darB* gene, DarB$^-$ | Caspers *et al.* (1984) |
| P1-15::IS*30* B3 | IS*30* inserted in the *darB* gene, DarB$^-$ | Caspers *et al.* (1984) |

[a] P1-15 is P1-15 hyb 2, a hybrid between phage P1 and plasmid p15B (Meyer *et al.*, 1983). All P1 and P1-15 derivatives except P1kc carry the *c*1ts225 allele.

wild type have been found. These mutations define a phenotype, Dar (for *d*efense *a*gainst *r*estriction). The data shown in Table 3 resulted from measurements of efficiency of plating. Efficiencies of specialized transduction of drug resistance markers on nonrestrictive and restrictive hosts were similar and severe restriction of P1 Dar$^-$ phages in specialized transduction was also observed (data not shown). It is thus clear that Dar

**TABLE 3**

RESTRICTION OF Dar$^+$ AND Dar$^-$ PHAGES[a]

| Restricting/nonrestricting hosts | Restriction system | e.o.p. of P1Dar$^+$ | e.o.p. of P1*darA*$^-$ | Ratio *darA*$^-$/Dar$^+$ | e.o.p. of P1*darB*$^-$ | Ratio *darB*$^-$/Dar$^+$ |
|---|---|---|---|---|---|---|
| W3110/WA921 | *Eco*K | 0.2 | $7 \times 10^{-4}$ | $3.5 \times 10^{-3}$ | $4 \times 10^{-4}$ | $2 \times 10^{-3}$ |
| WA960/WA921 | *Eco*B | 0.03 | $7 \times 10^{-5}$ | $2 \times 10^{-3}$ | $10^{-4}$ | $3 \times 10^{-3}$ |
| WA2379/WA921 | *Eco*A | 0.8 | $6 \times 10^{-4}$ | $1.5 \times 10^{-4}$ | 0.75 | 0.9 |
| CC4619/CC4621 | *Sty*SA | 0.8 | $3 \times 10^{-5}$ | $4 \times 10^{-5}$ | 0.8 | 1 |
| CC4620/CC4621 | *Sty*SB | 0.9 | $7 \times 10^{-3}$ | $8 \times 10^{-3}$ | $9 \times 10^{-3}$ | 0.01 |
| L4001/W3110 | *Sty*SB | 0.6 | $4 \times 10^{-3}$ | $6 \times 10^{-3}$ | $8 \times 10^{-3}$ | 0.01 |
| L4002/W3110 | *Sty*SP | 1 | 0.01 | 0.01 | $2 \times 10^{-3}$ | $2 \times 10^{-3}$ |
| L4004/W3110 | *Sty*SQ | 0.4 | $10^{-4}$ | $3 \times 10^{-3}$ | $6 \times 10^{-4}$ | $2 \times 10^{-3}$ |
| WA3366(pRI)/WA960 | *Eco*RI | $10^{-6}$ | $8 \times 10^{-7}$ | 0.7 | Not determined | |
| C600(pRTF2)/C600(pRTF1) | *Eco*RII | $6 \times 10^{-3}$ | $6 \times 10^{-3}$ | 1 | Not determined | |
| CC4515/CC4526 | *Sty*LT | $3 \times 10^{-6}$ | $2 \times 10^{-6}$ | 0.7 | Not determined | |

[a] The Dar$^+$ phages were P1Cm1 and P1Tc1. *DarA*$^-$ phages were P1Cm2 and P1CmTc1, while *darB* phages were P1-15::IS*30* A20 and B3 derivatives carrying Tn*9*. The table shows e.o.p. values. Transduction of the drug markers measured in the same experiment gave similar values. Phage stocks were prepared in the nonrestricting strain indicated except for the experiment testing *Eco*RII where W3110 was used and that testing *Sty*LT where CC4617 was used.

protects infecting phage genomes in both the lytic and lysogenic pathways. The P1-related phage P7 (Yarmolinsky, 1982) is also Dar$^+$. The Dar function provides protection only against type I restriction systems, both the *Eco*K family (*Eco*K, *Eco*B, *Sty*SB, *Sty*SP, and *Sty*SQ) and the *Eco*A family enzymes, *Eco*A, and probably *Sty*SA (Van Pel and Colson, 1974; Bullas *et al.*, 1976; Murray *et al.*, 1982; Fuller-Pace *et al.*, 1985; Suri and Bickle, 1985). Dar does not protect against the type II restriction enzymes *Eco*RI and *Eco*RII or against the unclassified *Sty*LT (Table 3) and only a slight effect is seen with the type III *Eco*P1 and *Eco*P15 (data not shown). The Dar phenotype is not affected by the procedure used to prepare the phage stocks, namely, by infection or after induction of lysogens. This is in contrast to the antirestriction function of phage Mu, Mom, which is much more strongly expressed after induction than following infection (Toussaint, 1976). In addition, *dam* mutations in the host genome have no effect on the Dar phenotype (data not shown) but they abolish the Mom phenotype of phage Mu (Toussaint, 1977).

## Additional phenotypic characterization of P1 Dar$^-$ mutants

The first Dar$^-$ phages that we isolated were P1CmTc1 and P1Cm2 (Mise and Arber, 1976). Physical analysis of their genomes revealed that they carry deletions and substitutions between the resident IS*1* element of P1 and the invertible C segment resulting in a loss of about 10 kb of the P1 genome (Iida *et al.*, 1982). Although both phages are proficient for lytic growth and for the establishment of stable lysogeny, they show several discernible phenotypic differences from the wild type apart from being Dar$^-$. Among these are the Lyd (for *ly*sis *d*elayed), Vad (for *v*iral *a*rchitecture *d*eterminant), Gta (for *g*eneralized *t*ransduction *a*ffected), and Tsu (for *t*ransduction *s*timulated by *u*ltraviolet irradiation) phenotypes. Lyd$^-$ phages only begin to lyse cells 60 min after induction and lysis is still not complete after 120 min, whereas a wild-type phage has completely lysed the cells by 90 min after induction (Iida and Arber, 1977). Therefore, P1 Lyd$^-$ phages make minute plaques. Vad$^-$ phage lysates contain a large number of phages with abnormally small heads (Iida and Arber, 1977; Walker *et al.*, 1979). Gta$^-$ phages transduce chromosomal markers at a frequency about 20-fold higher than wild-type phages (e.g., see Table 4). Transduction of chromosomal markers by wild-type P1 phage is stimulated by ultraviolet irradiation (Arber, 1960); this effect is not seen in Tsu$^-$ mutants (S. Iida, unpublished; cited in Yarmolinsky, 1982).

A number of other plaque-forming P1Cm phages carrying deletions between the IS*1* and the C segment were subsequently isolated and called type B P1Cm phages (Iida and Arber, 1977; Iida *et al.*, 1982). All of them show the same range of phenotypes as P1Cm2 and P1CmTc1 (data not shown).

## Mapping of mutations providing the Dar$^-$ phenotype

We screened a number of plaque-forming insertion derivatives of P1 and the related P1-15 (see Meyer *et*

**TABLE 4**

EFFECTS OF Dar ON GENERALIZED TRANSDUCTION

| Marker transduced | Restriction system (strain) | e.o.t. for P1Cm1 Dar$^+$ | Relative efficiency | e.o.t. for P1CmTc1 Dar$^-$ | Relative efficiency |
|---|---|---|---|---|---|
| *leu*[a] | None (WA921) | $1.7 \times 10^{-4}$ | = 1 | $2.4 \times 10^{-3}$ | = 1 |
| | *Eco*B (WA960) | $8.6 \times 10^{-5}$ | 0.5 | $1.9 \times 10^{-8}$ | $8 \times 10^{-6}$ |
| | *Eco*A (WA2379) | $1.9 \times 10^{-4}$ | 1.1 | $3.7 \times 10^{-8}$ | $1.5 \times 10^{-5}$ |
| λ*ind*$^-$[b] | None (WA921) | $2.3 \times 10^{-3}$ | = 1 | $4.4 \times 10^{-3}$ | = 1 |
| | *Eco*K (W3110) | $5.3 \times 10^{-4}$ | 0.23 | $2.8 \times 10^{-6}$ | $6.4 \times 10^{-4}$ |
| | *Eco*B (WA960) | $6.8 \times 10^{-4}$ | 0.3 | $6.2 \times 10^{-6}$ | $1.4 \times 10^{-3}$ |
| | *Eco*A (WA2379) | $1.4 \times 10^{-3}$ | 0.6 | $9.7 \times 10^{-5}$ | $2.2 \times 10^{-2}$ |

[a] W3110 lysogenic for P1Cm1 or P1CmTc1 was thermoinduced. The resulting lysates served to infect WA921, WA960, and WA2379 at a m.o.i. of 0.15. Infected bacteria were plated on selective media to measure Cm$^r$ specialized transductants and Leu$^+$ generalized transductants. The e.o.t. values here are defined as generalized transductants per specialized transductants. The relative efficiency normalizes measured e.o.t. values to the e.o.t. on WA921. Due to the Gta$^-$ (for *g*eneral *t*ransduction *a*ffected) phenotype, the e.o.t. value of P1CmTc1 on WA921 is higher than that of P1Cm1 on WA921.

[b] Either P1Cm1 or P1CmTc1 was thermally induced from WA921 (λ*ind*$^-$) lysogen and the resulting lysate was used to infect WA921 at m.o.i. 0.08 and W3110, WA960, and WA2379 at m.o.i. 0.2. After 30 min at 30°, aliquots were plated with the indicated bacteria and incubated at 41°. The e.o.t. values given are the ratios of λ plaques scored on the indicated strain to P1 plaques scored on WA921.

*al.*, 1986) for their Dar phenotype and found six phages that had a Dar$^-$ phenotype. Three of them, P1-15::Tn*10* D4, P1-15::Tn*10* D10, and P1-15::Tn*2653* WI1 carry their insertion in the region of the P1-15 genome homologous to the region between the IS*1* element and the C segment of phage P1. The first two are Lyd$^+$ Dar$^-$ while P1-15::Tn*2653* WI1 is Lyd$^-$ Dar$^-$. This suggests the presence of a single operon, which we call the *darA* operon, that is transcribed from right to left (Fig. 1), assuming that insertion of the IS*1*-flanked transposon Tn*2653* inactivated the Dar function by its polar effect (Iida *et al.*, 1983a).

The remaining three Dar$^-$ phages are independently isolated P1-15 derivatives that have IS*30* inserted at what is most likely the same site in the genome (Caspers *et al.*, 1984), clearly separate from the *darA* operon (Fig. 1). These phages are Lyd$^+$, Vad$^+$, Gta$^+$, and Tsu$^+$ and identify a second *dar* gene, *darB*. Interestingly, P1-15 *darB*$^-$ phages, while sensitive to the *Eco*K family of restriction enzymes, are still protected against *Eco*A and *Sty*SA; i.e., they provide a DarA$^+$ phenotype for these enzymes (Table 3). Experimental data given below indicate that gp*darB* fails to function in *darA* mutant phages, which explains why *darA* mutants are phenotypically DarA$^-$ DarB$^-$. In the following studies we characterize the Dar function against *Eco*A, *Eco*B, and *Eco*K using *darA* mutant phages, thus providing the DarA$^-$ DarB$^-$ phenotype.

## Dar protects host DNA transduced in P1 phage particles

Table 4 shows that P1 Dar$^+$-mediated transduction of the *leu* genes from *E. coli* K12 (carrying the *Eco*K restriction-modification system) into cells expressing either *Eco*A or *Eco*B is very efficient. This is in sharp contrast to transduction of the same marker by Dar$^-$ phage, which is severely restricted in cells carrying *Eco*A and *Eco*B. Similar results were obtained for transduction of the *trp* and *his* chromosomal markers (data not shown).

General transduction by P1 of a λ*ind*$^-$ prophage to restrictive recipients is also protected by the Dar function to about the same extent as the *leu* markers (Table 4). The relatively low level of restriction by *Eco*A of λ*ind*$^-$ after transduction by the Dar$^-$ phage reflects the fact that the λ genome contains only one site for this enzyme (Arber *et al.*, 1972).

The results clearly show that any DNA packaged into a wild-type P1 virion is protected from restriction.

## Dar does not act on phage DNA packaged in λ or Mu capsids

Since λ DNA carried in transducing phage particles is protected from restriction, we wondered whether the same effect could be seen for λ DNA packaged into λ phage heads. To test this, cultures of WA921 r$^-$m$^-$

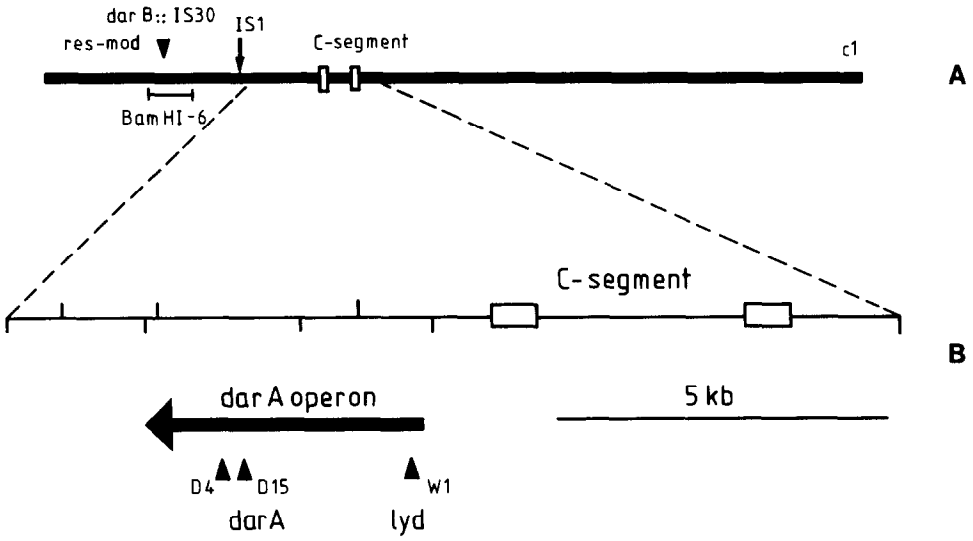


FIG. 1. Location of the *darA* and *darB* mutations on the P1 and P1-15 genome. (A) The landmarks shown are the *res-mod* region, the resident IS*1* element of phage P1, the C segment (with the flanking inverted repeat sequences drawn as open boxes), and the *c1* gene (Yarmolinsky, 1982). The IS*30* insertion in the *Bam*HI-6 fragment which inactivates the *darB* gene is indicated (Caspers *et al.*, 1984). (B) The *darA* region. Vertical lines above the map indicate *Eco*RI sites, and those below the line indicate *Bgl*II sites (Baechi and Arber, 1977; Iida *et al.*, 1980). The *darA* operon is shown as a horizontal arrow pointing in the direction of transcription. The sites of insertion of the transposons in P1-15::Tn*10* D4, P1-15::Tn*10* D15, and P1-15::Tn*2653* WI1 are shown by arrowheads with D4, D15, and W1, respectively. The order of the *darA* and *lyd* genes is indicated.

lysogenized by P1Cm1 Dar$^+$ *c*1ts and WA921 r$^-$m$^-$ lysogenized by P1CmTc1 Dar$^-$ c1ts were induced by placing them at 43° and then infected with P1 modified λ*vir* phages at different times (up to 30 min) after the temperature shift. After incubation at 43° for 30 min the cultures were further incubated at 38° and phage were harvested 120 min later. The λ burst size varied from 120 phages per cell when the infection was done immediately after shifting the cultures to 43° to eight per cell when the infection was 30 min later. The lysates were titrated on WA921, W3110, and WA960 derivatives made lysogenic for P1kc so that only the λ phages in the lysates make plaques. In no case was there any evidence for protection of the λ genome against B or K restriction, indicating that Dar will protect λ DNA only when it is packaged in a P1 head.

Possible *trans*-acting effects of Dar were also tested with phage Mu by experiments similar to those described above and also by induction of double lysogens. Mu has its own antirestriction system which is based on acetimidation of adenosyl residues in DNA by the Mu-encoded Mom function (Toussaint, 1977; Swinton *et al.*, 1983). Although the Mom function of Mu protects Dar$^-$ P1 phages to some extent, no protection of the Mu genome by Dar was seen.

These results indicate that Dar protection against restriction is not due to a stable chemical alteration of the DNA. This can be tested directly with purified phage DNA.

## Purified P1 phage DNA shows no Dar phenotype

DNA was purified from both Dar$^+$ and Dar$^-$ P1 phages grown in the r$^-$m$^-$ host, WA921, and the ability of these DNAs to serve as a substrate for the purified type I enzyme, *Eco*K, was examined using the restriction-dependent ATPase activity of the enzyme as an assay. Both substrates supported the same level of ATPase activity, while *Eco*K-modified DNA controls have only background values (Fig. 2). We conclude therefore that Dar does not alter the DNA, at least not in such a way as to render it resistant to restriction *in vitro.*

Figure 2 also shows comparative experiments done with DNA isolated from wild-type and *mom*$^-$ Mu phage particles. While the Mu *mom*$^-$ DNA is sensitive to restriction by *Eco*K, the wild-type DNA is much more resistant, although it is still more sensitive than *Eco*K-modified DNA. The result is an *in vitro* confirmation that the *mom* alteration does indeed protect DNA against *Eco*K.

In another series of experiments, nonmodified DNA from P1Cm1 Dar$^+$ and P1Cm1 Dar$^-$ phages was used to transfect nonrestricting and *Eco*K-restricting hosts,

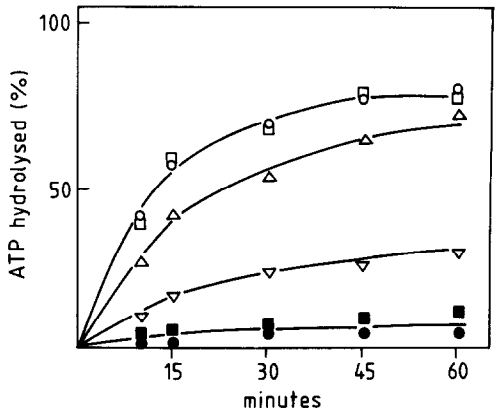


FIG. 2. Test of DNA from P1 Dar$^+$ and Dar$^-$ and Mu Mom$^+$ and Mom$^-$ phages as a substrate for *Eco*K ATPase activity. DNA was isolated from phage particles by extraction with phenol and was then dialyzed against 10 m*M* Tris–HCl, pH 8.0, 0.1 m*M* EDTA. ATPase activity was assayed according to Hadi *et al.* (1975). Reaction mixtures (50 μl) containing 10 μg/ml of the DNA to be tested were incubated at 30° and reactions were initiated by the addition of *Eco*K to a concentration of 10 μg/ml. Aliquots of 2 μl were removed at the indicated times and the amount of ATP hydrolyzed was determined. ○, P1Cm1 Dar$^+$.0; ●, P1Cm1 Dar$^+$.K; □, P1CmTc1 Dar$^-$.0; ■, P1CmTc1 Dar$^-$.K; ▽, Mu Mom$^+$.0; △, Mu Mom$^-$.0.

and phage plaques as well as Cm$^r$ transformants were assayed. Although the transformation efficiency was low for these 100-kb linear molecules (about $10^3$/10 μg DNA) and the difference between restricting and nonrestricting hosts was only on the order of $10^2$, no difference between the Dar$^-$ and the Dar$^+$ DNA could be detected.

If the Dar phenotype is not caused by a chemical modification of the DNA, it could result from the action of a factor sensitive to the DNA purification procedure, but specifically associated with P1 phage particles. One such factor might well be a phage head internal protein. Supporting evidence for this view comes from the following experiments.

## Complementation of Dar$^-$ phages by Dar$^+$ phages

When P1 Dar$^+$ and Dar$^-$ phages are propagated in the same cell, all of the phage particles produced are phenotypically Dar$^+$, independent of their genotype. Line 1 in Table 5 illustrates this for the nonmodifying strain WA921 lysogenic for the Dar$^+$ phage P1Cm1, superinfected with the Dar$^-$ phage P1Tc18 and then induced. The progeny were assayed for Cm$^r$ and Tc$^r$ transductants on both nonrestricting and *Eco*K-restricting hosts. It is apparent that the Dar$^-$ Tc$^r$ transducers are as well protected against restriction as the Dar$^+$ Cm$^r$ transducers. Similar results were obtained when strains carrying the *Eco*A or *Eco*B restriction systems were used as the recipients. The same result is

TABLE 5

P1 Dar⁻ GROWN IN THE PRESENCE OF P1 Dar⁺ ACQUIRES A Dar⁺ PHENOTYPE[a]

| Prophage | Superinfecting phage | Relative yield | e.o.t. for Cm$^r$ | e.o.t. for Tc$^r$ |
|---|---|---|---|---|
| P1Cm1 Dar⁺ | P1Tc18 Dar⁻ | 0.67 | 0.09 | 0.08 |
| P1Tc18 Dar⁻ | P1Cm1 Dar⁺ | 0.71 | 0.09 | 0.12 |
| P1Cm2 Dar⁻ | P1Tc18 Dar⁻ | n.d.[b] | 0.002 | 0.002 |

[a] Lysogenic derivatives of WA921 with the indicated prophage were grown at 30° to $10^8$ cells/ml, supplemented with 10 m*M* $CaCl_2$, and further incubated for 30 min. The bacteria were then superinfected at a m.o.i. of 4 and phage reproduction was thermally induced. The resulting lysates were titrated for Cm$^r$ and Tc$^r$ transductants on both WA921 and W3110. The transduction efficiency (e.o.t.) is the ratio of transductants on WA921 to those on W3110. The relative yield expressed as prophage genotype/infecting phage genotype in the progeny was determined from transduction in WA921. About 1% of the progeny in these experiments were P1CmTc recombinants.

[b] n.d.: not determined.

found in the reciprocal experiment when the prophage is Dar⁻ and the superinfecting phage is Dar⁺ (Table 5, line 2). Complementation of P1 Dar⁻ phages is also obtained with the P1-related phage P7 (data not shown). For each of the experiments shown in Table 5, 15 Tc$^r$ and 5 Cm$^r$ transductants of the restricting strain were induced and the Dar phenotype of the progeny phages was examined. All of the Cm$^r$-transducing phages were still Dar⁺ and all of the Tc$^r$ transducers were Dar⁻. Thus, the Dar⁺ phenotype acquired by P1 Dar⁻ phages during mixed growth with P1 Dar⁺ phages is not inherited, suggesting that it is due to the packaging of Dar⁻ DNA into P1 Dar⁺ heads. This notion of phenotypic mixing is also consistent with the observation that any DNA packaged into a P1 Dar⁺ virion is protected against restriction.

## The Dar function does not work in *trans* upon coinfection of restricting hosts with Dar⁺ and Dar⁻ phages

Next, we asked whether P1 Dar⁺ phages could provide protection for P1 Dar⁻ phages upon coinfection of a restricting host. The *Eco*K-restricting strain, W3110, was simultaneously infected with nonmodified Dar⁻ P1Tc18 and *Eco*K-modified Dar⁺ P1Cm1 phages and Tc$^r$ transductants were assayed. As a control, the Dar⁺ P1Cm1 was replaced by the Dar⁻ P1Cm2. As Table 6 shows, the efficiency of transduction by P1Tc18 was not significantly affected by coinfection with a Dar⁺ phage and therefore the *dar* gene products act only in *cis* in newly infected, restrictive hosts.

The fact that protection by the Dar product in an infected recipient strain is limited to the DNA of the phage with which Dar is associated makes it unlikely that Dar inactivates intracellular restriction enzyme activity, at least not rapidly enough to be measurable. This notion was confirmed by using the unrelated phage λ as a superinfecting phage.

## The Dar function does not inactivate restriction activity in infected bacteria

An *Eco*K-restricting strain was infected with either P1 Dar⁺.K or P1 Dar⁻.K phages at different multiplicities. These bacteria were then used 15 min later as indicators to plate λ.P1, that is, P1 modified but *Eco*K unmodified λ. The restriction of λ was identical to that with the non-P1-infected control, and this is the case for all P1 multiplicities and for both the Dar⁺ and the Dar⁻ P1 strains (Fig. 3). Control experiments with the $r^-m^-$ strain WA921 showed that the efficiency of plating of λ is not affected by the previous infection with P1. Thus P1 Dar⁺.K phage fails to inactivate restriction activity in infected cells.

That the experimental conditions used could detect a possible inactivation of *Eco*K is seen when a similar experiment is done with nonmodified P1.0. At high multiplicity the infecting P1 is seen to titrate out the restriction activity so that the superinfecting λ phage is only weakly restricted (Fig. 3). This effect has previously been well documented under different experimental conditions (Paigen and Weinfeld, 1963; Heip *et al.*, 1974). Interestingly, the effect is more pronounced with P1 Dar⁻ than with P1 Dar⁺. Thus, the loss of *Eco*K activity correlates with restriction of the P1 DNA: P1 Dar⁻ is strongly restricted while P1 Dar⁺ is only weakly restricted.

TABLE 6

Dar ACTS IN *cis* UPON INFECTION OF RESTRICTING HOSTS[a]

| Infecting phages | e.o.t. to Cm$^r$ | e.o.t. to Tc$^r$ |
|---|---|---|
| P1Tc1 Dar⁺.0 alone | — | 0.1 |
| P1Tc18 Dar⁻.0 alone | — | $10^{-4}$ |
| P1Cm1 Dar⁺.K + P1Tc1 Dar⁺.0 | 0.6 | 0.6 |
| P1Cm2 Dar⁻.K + P1Tc1 Dar⁺.0 | 0.9 | 0.5 |
| P1Cm1 Dar⁺.K + P1Tc18 Dar⁻.0 | 0.55 | $9 \times 10^{-3}$ |
| P1Cm2 Dar⁻.K + P1Tc18 Dar⁻.0 | 0.8 | $7 \times 10^{-3}$ |

[a] Phage P1.0 was prepared on WA921, and phage P1.K on W3110. The $r_K^+m_K^+$ strain W3110 was used for all the infections which were carried out with a m.o.i. of 8 for P1.K and of 0.05 for P1.0. The infected bacteria were scored for Cm$^r$ and for Tc$^r$ transductants. The e.o.t. is the ratio of specialized transductants on WA921 to those on W3110. Cm$^r$Tc$^r$ recombinant phage were also scored and amounted to less than 4% of all transductants.

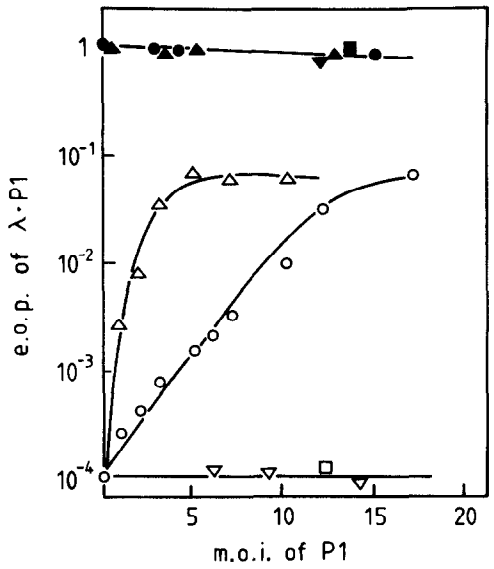


FIG. 3. Dar does not protect λ DNA from restriction in *trans*. The P1 phages used were P1Cm1 Dar$^+$ and P1CmTc1 Dar$^-$. The *E. coli* hosts were WA921 (nonrestricting) and W3110 (*Eco*K restricting). Cells were grown in tryptone broth containing 0.4% maltose to 2 × $10^8$ cells/ml, $CaCl_2$ was added to 10 m*M*, and the cells were incubated for a further 30 min. The cells were then infected with the P1 phages at the indicated m.o.i. After 15 min at 30° the cells were superinfected with P1-modified λ*vir* and further incubated for 15 min. To measure λ*vir* infective centers the cells were then plated with WA921 as indicator and the plates were incubated at 30°. Filled symbols refer to infections with WA921 as host, and open symbols to infections with W3110. ○, P1Cm1 Dar$^+$.0; □, P1Cm1 Dar$^+$.K; △, P1CmTc1 Dar$^-$.0; ▽, P1CmTc1 Dar$^-$.K.

### DNA modification is stimulated by Dar during vegetative phage growth

A single cycle of growth of a nonmodified phage on nonrestricting but modifying bacteria (r$^-$m$^+$ phenotype) can reveal the efficiency of modification of the phage DNA. Such a test has been used by others to show that the antirestriction function of phage λ operates by stimulating the modification reaction (Zabeau *et al.*, 1980) rather than by directly antagonizing restriction. The experiment shown in Table 7 indicates that Dar also stimulates the modification of the phage DNA. Dar$^-$ phages have less than 1% of the modification of Dar$^+$ phages after a single cycle of growth on a r$^-$m$^+$ host.

### Identification of the Dar proteins

All of the experiments described above are consistent with the idea that the *dar* gene products are phage structural proteins that are injected into host cells along with the DNA. Other support for this idea comes from the finding that all the type B P1Cm derivatives tested have a higher density in CsCl gradients than wild-type P1 phages (Mise and Arber, 1976; Iida and Arber, 1977). These P1Cm derivatives, which included P1CmTc1 and P1Cm2, are all Dar$^-$. Because P1 packages its DNA by a "headful" mechanism (Ozeki and Ikeda, 1968), these results indicate that Dar$^-$ phage particles have either less protein or more DNA than wild-type phage particles.

We therefore examined the proteins of CsCl gradient purified Dar$^+$ and Dar$^-$ phage particles by SDS–polyacrylamide gel electrophoresis with the results shown in Fig. 4. The Dar$^-$ phages (*darA*$^-$) used in this work lack two proteins present in wild-type phages. Both proteins are head proteins because they are found in purified heads isolated from a lysate of P1Cm0 *sus*50, a phage which produces large amounts of free DNA-filled heads (Yamamoto, 1982). The more abundant of the two proteins has a mol wt of 68,000 and is the product of the *darA* gene (Streiff *et al.*, 1987, accompanying paper). The second protein has a mol wt close to 200,000 and, judging from the intensity of the stained band on gels, is present in only a few copies per phage head. All three *darB* mutants carrying IS*30* lack this protein but still contain the *darA* gene product (data not shown). Thus, we have tentatively concluded that the *darB* gene encodes the 200,000 $M_r$ protein. The *darB* mutants fail to protect against the classical type I enzymes, *Eco*B, *Eco*K, *Sty*SB, *Sty*SP, and *Sty*SQ. The *darA* mutants besides being sensitive to these classical type I systems are also sensitive to the *Eco*A and *Sty*SA systems. Thus, the *darA* gene product seems to suffice for protection against *Eco*A and *Sty*SA. We do not know whether protection against the *Eco*K family of enzymes is a property of gp*darB* alone or whether it requires gp*darA* and gp*darB*, because we have no mutants that make phage particles containing gp*darB* but which lack the *darA* gene products.

## DISCUSSION

The phage P1 antirestriction mechanism described in this paper is different from that of any other bacteriophage so far investigated. We had initially expected that it might show some similarity to the phage Mu-coded antirestriction function *mom* (Toussaint, 1976). This was because the *mom* gene maps in the segment of the Mu genome, adjacent to the *gin* gene and the invertible G segment (Kahmann, 1983; Fig. 1). This part of the Mu genome has structural and functional homology with the P1 genome and the P1 *darA* gene maps in a position analogous to Mu *mom* with respect to this region. However, *mom*-mediated antirestriction is based on an acetimidation of about 15% of the adenosyl residues in DNA (Hattman, 1979; Swinton *et al.*, 1983), whereas the Dar proteins do not chemically modify the DNA. Our results indicate that the Dar pro-

TABLE 7
MODIFICATION OF P1 Dar$^+$ AND P1 Dar$^-$ PHAGES AFTER GROWTH ON $r^-m^+$ HOSTS[a]

| Phage and history | On *Eco*K host | | On *Eco*B host | |
|---|---|---|---|---|
| | e.o.p. | e.o.t. | e.o.p. | e.o.t. |
| P1Cm1 Dar$^+$.0 | 0.12 | 0.09 | 0.03 | 0.02 |
| P1Cm1.K | 1.1 | 1 | | |
| P1Cm1.B | | | 0.9 | 1.4 |
| P1CmTc1 Dar$^-$.0 | $2.6 \times 10^{-4}$ | $8.2 \times 10^{-4}$ | | |
| P1CmTc1.K | 1 | 0.9 | $2 \times 10^{-4}$ | $7 \times 10^{-4}$ |
| P1CmTc1.B | | | 1 | 1.1 |
| P1Cm1.0 grown on SI1065 ($r_K^- m_K^+$) | 1 | 0.9 | | |
| P1CmTc1.0 grown on SI1065 | $9 \times 10^{-3}$ | $9 \times 10^{-3}$ | | |
| P1Cm1.0 grown on WA837 ($r_B^- m_B^+$) | | | 0.6 | 0.9 |
| P1CmTc1.0 grown on WA837 | | | $7 \times 10^{-3}$ | $7 \times 10^{-3}$ |

[a] P1.0, P1.K, and P1.B were prepared from lysogenic derivatives of WA921, W3110, and WA960, respectively. For growth on SI1065 or WA837 the cells were infected at a m.o.i. of 0.7–0.9 and grown in broth for 120 min before the surviving bacteria were killed with $CHCl_3$. The e.o.p. and e.o.t. of the lysates were then measured with WA921, W3110, and WA960 as nonrestricting, *Eco*K-restricting, and *Eco*B-restricting hosts, respectively.

teins are phage structural proteins that are injected along with infecting DNA and that prevent restriction in the recipient cells.

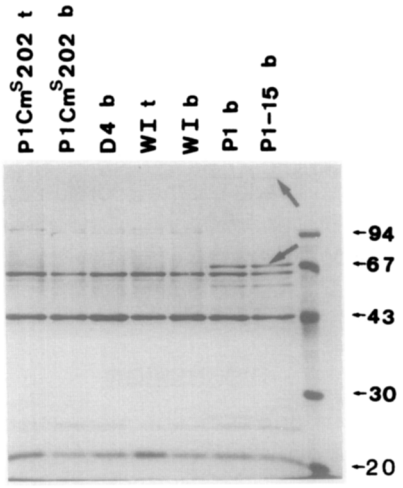


FIG. 4. The proteins of P1 Dar$^+$ and Dar$^-$ phage particles. Phage particles were purified by CsCl step gradient centrifugation and then dialyzed against 10 m*M* Tris–HCl, pH 7.5, 10 m*M* $MgCl_2$. Phage (100 $\mu$l) was diluted to 1 ml with $H_2O$, the sample was sonicated briefly to disrupt the DNA, and the proteins were precipitated with trichloroacetic acid. The pellet was washed three times with ethanol, dried, and suspended in 50 $\mu$l SDS gel sample buffer (Laemmli, 1970). The samples were run on a 10% polyacrylamide–SDS gel which was stained with Coomassie blue. "b" indicates plaque-forming particles and "t" indicates small-headed particles found at lower densities in the CsCl gradients (Iida *et al.*, 1977; Walker *et al.*, 1979). The arrows indicate the position of the Dar proteins. The numbers at the side of the figure are the molecular weights (in kDa) of the marker proteins. Phages P1 and P1-15 are Dar$^+$, whereas P1Cm$^s$202, P1-15::Tn*10* D4 (indicated by D4) and P1-15::Tn*2653* WI1 (indicated by WI) are Dar$^-$.

Several lines of evidence indicate that the *dar* genes are expressed late after expression and that they are associated with P1 head morphogenesis. The *darA* gene lies in an operon which also contains the *lyd* gene(s) involved in the control of lysis. The *darA* gene product first appears about 25 min after induction of lysogens (Streiff *et al.*, 1987, accompanying paper) and all of the *darA* mutants that we have tested also show the Vad phenotype; that is, they produce large amounts of abnormally small heads (Iida and Arber, 1977; Walker *et al.*, 1979). We believe that both the *darA* and *darB* gene products are phage head internal proteins which bind to the phage DNA and remain associated with it after injection. The DNA binding is not specific for the phage genome because any DNA packaged into a P1 head is protected against restriction. Ikeda and Tomizawa (1965) have suggested that proteins may covalently attach to the ends of the bacterial DNA in generalized transducing P1 phage particles. It is unlikely that the *dar* gene products are involved in this because they are also found in phage particles containing phage DNA and because they seem to be removed from DNA by the phenol treatment.

P1 *darA* mutant phage particles lack two polypeptides of $M_r$ 68,000 and 200,000, whereas only the latter protein is absent from *darB*$^-$ phage particles. We have shown that the *darA* gene product is indeed the $M_r$ 68,000 protein which is abundant in phage particles (Streiff *et al.*, 1987, accompanying paper; Fig. 4). Most likely, the *darB* gene product is unable to incorporate into phage head precursors in the absence of gp*darA*. P1 *darA* mutants are sensitive to all of the type I restriction systems tested, while the *darB* mutants are

sensitive only to the subclass of allelic type I systems whose best known member is *Eco*K (Murray *et al.*, 1982). The genes for *Sty*SA lie next to the *Sty*SB genes (which are allelic to the *Eco*K genes) in the *Salmonella* chromosome but are clearly distinct from them (Van Pel and Colson, 1974; Bullas *et al.*, 1976). Since P1 *darA* phages become sensitive to *Eco*A and to *Sty*SA restriction but P1 *darB* phages are resistant to both of them, *Eco*A may be closely related to or perhaps allelic to *Sty*SA.

We do not know how the *dar* gene products act to hinder restriction. One possibility would be that they prevent the restriction enzymes from tracking from the recognition sites to the cleavage sites on DNA (Yuan *et al.*, 1980; Endlich and Linn, 1985). Whatever the exact mechanism may be, it is the *dar* gene products that allow phage P1 to mediate efficient generalized transduction between bacteria carrying different type I restriction systems.

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