Herpes Simplex Virus Cloned DNA Fragments Induce Coumermycin A₁ Resistance in *Escherichia coli*

ROBERT E. PEARSON AND ANTHONY J. CONLEY*

Institute for Molecular Virology, St. Louis University School of Medicine, St. Louis, Missouri 63110

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We have taken a new approach to identify and fine map previously undescribed herpes simplex virus (HSV) functions. In experiments described in this report the antibiotic coumermycin A₁ was used to select two HSV type 1 *Bam*HI fragments cloned in pBR322 that confer partial resistance to drug-susceptible *Escherichia coli*. The genes encoding these HSV functions have been designated *cou*'-1 and *cou*'-2 and have been fine mapped to the HSV sequences. HSV-*cou*'1 is located at the left end of *Bam*HI-F near HSV type 1 genomic map coordinate 0.645. *cou*'-2 maps to *Bam*HI-M', which is a 159-base-pair internal component of the α ICP4-coding sequence located in the reiterated sequences of the S component. In pBR322 both inserts apparently rely on the *tet* promoter for expression. Additionally, *cou*'-2 functions when present as a *Bam*HI insert in pUC7. The analysis of *cou*'-2 "maxi" cell proteins reveals the presence of proteins produced by the fusion of HSV-1 *Bam*HI-M' sequences and the sequences of the vector genes, i.e., the major *tet* product for pBR322 and the modified β -galactosidase for pUC7. These data suggest that the development of bacterial assays for fusion products of eucaryotic DNA open reading frames in plasmid vectors may be a useful technique for initiating gene function studies.

Herpes simplex virus type 1 (HSV-1) and HSV-2 have as their DNA two covalently linked components designated L and S. The complete genomes have approximate molecular weights of 100×10^6 . The ends of the molecules have terminally redundant sequences that are probably required for the mechanism of DNA replication. In vitro studies of HSV-1 DNA have shown that the terminal reiterated sequences allow for the formation of circular molecules (30), which are necessary intermediates for a rolling circle mode of viral DNA replication. Such a model for HSV DNA synthesis has been proposed (23), and it superficially resembles mechanisms proposed for DNA replication in procaryotes (12). One approach to unraveling the complexities of HSV DNA replication would be to probe the effects of viral gene products on known parameters of bacterial DNA replication by supplementing the bacterial replicative machinery with HSV genes and to screen for effects. Our first approach in this regard has been to investigate the alteration of bacterial DNA topology.

Escherichia coli maintains its DNA under constraint in a series of supercoiled loops (20), which are required for replication and transcription. The enzyme complex, DNA gyrase, is responsible for creating supercoils (5) and has a role in segregating the DNA into domains of supercoiling (4). Gyrase is a multimeric complex containing dimers of the *gyrA* and *gyrB* gene products (17). Gellert et al. (6) have found that the antibiotic coumermycin A₁ inhibits supercoiling of DNA by interacting with DNA gyrase. This drug has been specifically shown to inhibit the DNA-dependent ATPase gyrase B subunit (27). The drug also blocks DNA synthesis and induces nucleosome structural changes in cultured mammalian cells (14), perhaps by suppressing a eucaryotic DNA gyrase. In HSV-1-infected cells the drug inhibits viral DNA synthesis approximately 1 order of magnitude more than its effect on cellular DNA synthesis (1). We decided to use this drug for selection of HSV genes that, when present in *E. coli*, may supplement the bacterial gyrase

or have another measurable effect in the presence of the drug.

Certain evidence suggested that this approach would succeed by using HSV-1 DNA fragments cloned in commonly used plasmid vectors. First, the expression of eucaryotic genes in procaryotic plasmid or phage vectors is known to occur (26). Thus extensive and time-consuming plasmid constructions may not always be necessary during screening experiments to identify a functional HSV gene. Second, from analyses to date, it appears that most HSV genes lack introns. Spliced RNA transcripts appear to be the exception rather than a common component of HSV RNA processing. Even in some cases where gene transcripts are spliced (32, 33) the processed portions are upstream of the translated sequences. Thus, genomic DNA clones may be used in lieu of preparing cDNA clones. Finally, the cloned HSV thymidine kinase gene is expressed in *E. coli* (3, 11), and its expression can be selected for in *E. coli* strains lacking thymidine kinase (11). With this background, we have screened a partial HSV genomic library, and we report on the identification of two HSV sequences that contain the function of conferring partial resistance to coumermycin A₁ in drug-susceptible *E. coli*.

MATERIALS AND METHODS

Viruses, plasmids, DNA, and bacteria. The properties of HSV-1 (mP) have been described previously (10). Intact viral DNA was purified by sodium iodide density gradient centrifugation (31) of infected Vero cell cytoplasmic nucleocapsids or whole cell lysates. The HSV-1 (mP) genome was cloned as *Bam*HI fragments in pBR322 by methods previously described (21). All major fragments were present, with the exception of *Bam*HI-A-A' and *Bam*HI-D-H (HSV-1 approximate map coordinates 0.14 to 0.22 and 0.45 to 0.56, respectively). Most clones containing HSV-1 (mP) DNA fragments with molecular weight of less than 0.25×10^6 were not characterized. The *Eco*RI EK fragment from HSV-1 (EMP) pAC205E was cloned in pBR325. The cloned *Bam*HI F fragment from HSV-1 (F), pRB128 (21), was obtained from

* Corresponding author.

B. Roizman, University of Chicago. The vectors pUC8 and pUC9 and *E. coli* JM103 were obtained from P-L Biochemicals, Milwaukee, Wis. The vector pUC7 and *E. coli* JM83 (29) were graciously provided by J. Messing, University of Minnesota. Plasmids were routinely carried in *E. coli* HB101. Plasmids were purified by the cleared lysate technique and banded in CsCl_2 density gradients as described previously (7). During all of the following procedures involving *cou'* plasmids, a sample of the bacterial culture was examined for plasmid content at the start and at the end of an experiment.

Enzymes, restriction endonucleases, drugs, and other reagents. Restriction endonucleases were purchased from New England Biolabs, Boston, Mass., Bethesda Research Laboratories, Bethesda, Md., or P-L Biochemicals. DNA ligase was purchased from either New England Biolabs or Bethesda Research Laboratories. Coumermycin A_1 was purchased from Sigma Chemical Co., St. Louis, Mo. [^{14}C]leucine, [^{14}C]isoleucine, and [^{14}C]valine (specific activity, 340, 347, and 291 mCi/mmol, respectively) and [^{32}P]ATP were purchased from New England Nuclear Corp., Boston, Mass.

Coumermycin A_1 resistance assay. To determine resistance, a single colony of each bacterial stock containing the hybrid plasmid in question was used to inoculate an overnight L-broth culture. Cultures were then diluted 1/50 and growth to the logarithmic phase, at which time equal samples of the cultures were titered on fresh L-agar plates containing 5.5 μg of coumermycin A_1 per ml, plates containing the indicated coumermycin A_1 concentration and 25 μg of ampicillin per ml, and plates containing only 25 μg of ampicillin per ml. Coumermycin A_1 stocks were prepared in Me_2SO_4 . The final concentration of 0.068% Me_2SO_4 had no effect on the assay.

DNA sequencing, plasmid-encoded proteins, and gel electrophoresis. The HSV-1 (mP) *Bam*HI M' fragment was sequenced by the method of Maxam and Gilbert (15) after insertion in the plasmid pUC8 (29). Both strands were sequenced; from the right starting at the *Sal*I site in the vector and from the left starting at the *Eco*RI site in the vector. Plasmid-encoded proteins labeled with [^{14}C]leucine, [^{14}C]isoleucine, and [^{14}C]valine were prepared by the maxicell method of Sancar et al. (25). Processing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the labeled proteins have been described previously (18).

RESULTS

Plasmid-encoded coumermycin resistance. From the library of HSV-1 *Bam*HI fragments cloned in pBR322, two plas-

TABLE 1. Coumermycin A_1 inhibition in *E. coli* strains containing HSV-1 recombinant plasmids

<i>E. coli</i> strain	Plasmid (insert)	Efficiency of plating ^a
HB101	pBR322	4.20×10^{-4b}
	pAC150 (<i>Bam</i> HI-F)	1.13×10^{-1}
	pAC172 (<i>Bam</i> HI-M')	3.89×10^{-2}
JM103	pBR322	1.05×10^{-3}
	pAC150 (<i>Bam</i> HI-F)	Lethal ^c
	pAC172 (<i>Bam</i> HI-M')	1.33×10^{-2}

^a The efficiency of plating is expressed as the ratio of titers at 5.5 μg of coumermycin A_1 per ml plus 25 μg of ampicillin per ml.

^b Other HSV-1 sequence-containing plasmids (including *Eco*RI-EK in pBR325, which contains *Bam*HI-M') gave results similar to pBR322-containing *E. coli*.

^c *Bam*HI-F in the *cou'* orientation will not transform *E. coli* JM103.

FIG. 1. Electrophoretic profiles of restriction endonuclease digests of *cou'*-inducing plasmids. The HSV-1 (mP) *Bam*HI fragments are designated according to the published HSV-1 (F) and HSV-1 (Justin) maps (13). In HSV-1 (mP), the *Bam*HI fragments D and H and A and A' are fused. (A) DNA fragments separated on a 0.50% agarose gel. Lanes: 1, HSV-1 (mP) DNA digested with *Bam*HI; 2, pAC150 digested with *Bam*HI; 3, pAC251 digested with *Kpn*I and *Bam*HI; 4, pAC252 digested with *Sac*I and *Bam*HI; 5, pAC253 digested with *Sal*I and *Bam*HI; 6, pAC172 digested with *Bam*HI. (B) *Bam*HI-digested DNA fragments separated on a 4.0% polyacrylamide gel. Lanes: 1, pAC172; 2, pAC205E; 3, HSV-1 (mP).

mids, pAC150 and pAC172, conferred partial resistance to the antibiotic-sensitive *E. coli* host. In media containing 5.5 μg of coumermycin A_1 per ml, wild-type *E. coli* HB101 had an efficiency of plating of 4.2×10^{-4} . The plating efficiency was increased (Table 1) only for those organisms containing pAC150 and pAC172. Interestingly, cesium chloride gradient-purified and >95% supercoiled pAC150 from *E. coli* HB101 would not transform *E. coli* JM103. Under the routine conditions, $>2 \times 10^3$ transformants could be obtained by using *E. coli* HB101 as the recipient. This observation suggests that the product of *cou'1* is lethal in *E. coli* JM103. The HSV-1 *Bam*HI fragments in pAC150 and pAC172 plasmids were identified as *Bam*HI-F and *Bam*HI-M' (Fig. 1). By blot hybridization, pAC172 (*Bam*HI-M') was found to hybridize to HSV-1 0.25 M junction fragments and S component termini (data not shown). Since an identical fragment is found in *Bam*HI digests of pAC205E (*Eco*RI-EK) (Fig. 1) and has been previously mapped to this position (22), we conclude that the insert in pAC172 is *Bam*HI-M'. This sequence lies both between *Bam*HI-SP and *Bam*HI-Y and between *Bam*HI-Y and *Bam*HI-S (prototype arrangement) in the reiterated sequences of the S component.

Characterization of resistance-encoded in *Bam*HI-F-containing plasmids. A series of experiments was performed to determine the minimal HSV sequences from *Bam*HI-F that were necessary for antibiotic resistance. The plasmids

FIG. 2. Summary of the HSV-1 genomic origin of *couI-1* and construction of plasmids used to fine map *couI-1* from pAC150 (*BamHI-F*). (A) The top two lines represent the genome sequence components and arrangement and present the map coordinates for the genomic origin of *BamHI-F* in the prototype (P) arrangement. Restriction endonuclease maps of *BglII*, *KpnI*, *XhoI*, *SmaI*, *SalI*, and *SacI* for *BamHI-F* are presented in the next three lines. (B) Sequence arrangements of plasmids derived from pAC150 that were used to fine map coumermycin A₁ resistance from *BamHI-F*. Plasmids that induce coumermycin A₁ resistance are listed on the left (i through v), and plasmids that do not induce coumermycin A₁ resistance are listed on the right (vi through viii): (i) pAC150 (*BamHI-F*), (ii) pAC150d*BglII*, (iii) pAC251, (iv) pAC252, (v) pAC253. Open triangles within the figures of (i) through (v) indicate the deletions of the *BglII* site, internal *KpnI* sequences, internal *SacI* sequences, and internal *SalI* sequences in the respective plasmid. (vi) pAC150' (inverted orientation of *BamHI-F* insert in pBR322), (vii) pAC254, deletion of all internal fragments produced by digestion with *SmaI*, (viii) pAC264, sequences derived from the *XhoI* to *BamHI* sites from the right third of *BamHI-F* ligated into *BamHI*- and *SalI*-digested pBR322. (C) Locations of *couI* sequences within *BamHI-F*. Sequences encoding the activity must extend from the *BamHI* site at map coordinate 0.645 to beyond the first *SmaI* site, but not beyond the indicated *SacI* site.

shown in Fig. 2 were constructed for this purpose. When *BamHI-F* was in an inverted orientation, pAC150', the host, was drug susceptible (Fig. 2). Also, this inverted plasmid transformed *E. coli* JM103 at the normal high efficiency. The central portion of the *BamHI-F* sequences was not required since the *KpnI* and *SacI* deletion plasmids pAC251 and pAC252, respectively, retained the resistance of the parent plasmid. However, *E. coli* containing a plasmid created by deletion of all the internal HSV-1 *SmaI* fragments, pAC254, was drug susceptible. Minimum contiguous HSV-1 sequences necessary for drug resistance were found in the plasmid pAC253, which retains only the sequences from the most proximal *SalI* site to the *BamHI* site at 0.645. Thus, it appears that the *couI* function maps from the *BamHI* site at 0.645, beyond the *SmaI* site at 0.650, and has a maximum at the *SacI* site at 0.654. All *couI* *BamHI-F* deletion plasmids were lethal in JM103. Most likely the sequence at the left end of *BamHI-F* is common in all HSV-1 strains since *BamHI-F* from both HSV-1 (mP) and HSV-1 (F), pRB128, exhibit coumermycin resistance in the proper orientation.

Characterization of coumermycin resistance encoded in

FIG. 3. HSV-1 genomic origin of *couI* plasmid. The top three lines represent the genomic sequence components in the prototype (P) arrangement and give an expansion of the *BamHI* restriction endonuclease map for those fragments within the reiterated sequences of the S component that border *BamHI-M'*. (A) Sequence orientation of *BamHI-M'* that is present in the coumermycin A₁-resistant plasmid pAC172. (B) The noninducing orientation of *BamHI-M'* (pAC172') is presented to the right. The initial and terminal sequences of the *BamHI-M'* sequences are presented for each plasmid in a potential noncoding (message sense) strand. The pBR322 *EcoRI* site is indicated in addition to the direction of transcription of the *tet* gene in pBR322. The noncoding strand for the sequence of *BamHI-M'* is presented.

TABLE 2. Coumermycin A₁ inhibition in titrated *E. coli* cultures containing pUC plasmids and pUC *Bam*HI-M' plasmids

Strain	Medium	Pretreatment ^a	Vector	Insert, orientation	Additions to growth medium		Efficiency of plating ^b
					Coumermycin A ₁ (μg/ml)	IPTG	
JM103	M9	IPTG	pUC7	None	4	—	4.70 × 10 ⁻⁴
		IPTG	pUC7	None	4	+	3.74 × 10 ⁻⁴
		IPTG	pUC7	<i>Bam</i> HI-M', active	4	—	Lethal ^c
		IPTG	pUC7	<i>Bam</i> HI-M', active	4	+	Lethal ^c
		IPTG	pUC7	<i>Bam</i> HI-M', inactive	4	—	2.25 × 10 ⁻⁴
		IPTG	pUC7	<i>Bam</i> HI-M', inactive	4	+	2.75 × 10 ⁻⁴
		IPTG	pUC7	<i>Bam</i> HI-M', <i>dl Sac</i> II	4	—	2.66 × 10 ⁻⁴
		IPTG	pUC7	<i>Bam</i> HI-M', <i>dl Sac</i> II	4	+	2.50 × 10 ⁻⁴
JM103	L	None	pUC7	<i>Bam</i> HI-M', inactive	7	—	8.20 × 10 ⁻⁴
		None	pUC7	<i>Bam</i> HI-M', inactive	7	+	3.4 × 10 ⁻⁴
		IPTG	pUC7	<i>Bam</i> HI-M', inactive	7	—	3.86 × 10 ⁻⁴
		IPTG	pUC7	<i>Bam</i> HI-M', inactive	7	+	7.05 × 10 ⁻⁴
		None	pUC7	<i>Bam</i> HI-M', active	7	—	1.61 × 10 ⁻³
		None	pUC7	<i>Bam</i> HI-M', active	7	+	1.88 × 10 ⁻⁴
		IPTG	pUC7	<i>Bam</i> HI-M', active	7	—	Lethal ^d
		IPTG	pUC7	<i>Bam</i> HI-M', active	7	+	Lethal ^d
		Continuous IPTG	pUC7	None	7	—	4.35 × 10 ⁻⁴
		Continuous IPTG	pUC7	None	7	+	5.65 × 10 ⁻⁴
		Continuous IPTG	pUC7	<i>Bam</i> HI-M', active	7	—	3.29 × 10 ⁻³
		Continuous IPTG	pUC7	<i>Bam</i> HI-M', active	7	+	7.9 × 10 ⁻⁴
HB101	L ^e	None	pUC7	None	5	—	9.55 × 10 ⁻⁴
		None	pUC7	None	5.25	—	5.99 × 10 ⁻⁴
		None	pUC7	None	5.25	+	8.45 × 10 ⁻⁴
		None	pUC7	<i>Bam</i> HI-M', active	2	—	1.50 × 10 ⁻¹
		None	pUC7	<i>Bam</i> HI-M', active	2	+	1.20 × 10 ⁻¹
		None	pUC7	<i>Bam</i> HI-M', active	5	—	2.10 × 10 ⁻³
		None	pUC7	<i>Bam</i> HI-M', active	5.25	—	3.5 × 10 ⁻³
		None	pUC7	<i>Bam</i> HI-M', active	5.25	+	1.58 × 10 ⁻³
		None	pUC7	<i>Bam</i> HI-M', inactive	2	—	3.55 × 10 ⁻³
		None	pUC7	<i>Bam</i> HI-M', inactive	2	+	4.02 × 10 ⁻³
		None	pUC7	<i>Bam</i> HI-M', inactive	5.25	—	3.16 × 10 ⁻⁴
		None	pUC7	<i>Bam</i> HI-M', inactive	5.25	+	3.95 × 10 ⁻⁴
		None	pUC9 ^f	<i>Bam</i> HI-M', active	5.25	—	7.94 × 10 ⁻⁴
		None	pUC9	<i>Bam</i> HI-M', active	5.25	+	9.71 × 10 ⁻⁴
		None	pUC7	<i>Bam</i> HI-M', <i>dl Sac</i> II	5.25	—	7.14 × 10 ⁻⁴
		None	pUC7	<i>Bam</i> HI-M', <i>dl Sac</i> II	5.25	+	4.05 × 10 ⁻⁴

^a Pretreatment with isopropyl thio-β-galactoside (IPTG) 1 h before titration.^b Efficiency of plating is expressed as the ratio of titers on the indicated coumermycin A₁ concentration plus 25 μg of ampicillin per ml and that on 25 μg of ampicillin per ml.^c IPTG induction of JM103 containing an active pUC7 *Bam*HI-M' was considered lethal when the efficiency of plating was less than 10⁻⁶. A fivefold reduction in stock titer was consistently observed when active *Bam*HI-M' was induced compared with the other plasmids listed for *E. coli* JM103 in M9 medium.^d In L-medium, IPTG induction reduced stock titer minimally 35-fold. Although undetermined by titration, the lethal effect of the induced pUC7 *Bam*HI-M' product must be greater than that grown in M9 medium.^e Consistent survival of HB101 containing pUC7 *Bam*HI-M' cultures was observed when grown in M9 medium.^f On 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside indicator plates, pUC7 *Bam*HI-M'-containing bacterial colonies are blue, whereas pUC8 and pUC9 *Bam*HI-M'-containing colonies are white. The pUC8 results were consistent with pUC9 *Bam*HI-M' in the active orientation.

***Bam*HI-M'-containing plasmids.** The purpose of characterizing coumermycin resistance in plasmids containing *Bam*HI-M' was to determine whether a specific orientation of *Bam*HI-M' was necessary for resistance. Since M' was estimated by polyacrylamide gel analysis to be approximately 150 base pairs, we determined its sequence. The sequence beginning GATCCCGCG (Fig. 3) must be closest to the *tet* promoter for induced resistance, whereas the opposite orientation has no effect. Most notably this 159-bp sequence is devoid of any known transcriptional or stop or start translational signals and is 85% G+C.

Since *Bam*HI-M' contains part of the sequences coding for ICP4, we determined whether the observed plasmid-induced activity of the product in a hybrid pBR322 plasmid could be transferred as an active domain to a different hybrid plasmid.

Two series of experiments were performed. First, we cloned *Bam*HI-M' in the plasmid series pUC7, pUC8, and pUC9. If the *couI* activity resided in the transferred domain, it might be expected to be transferred to another protein, i.e., the modified β-galactosidase. Also if it were product specific and not simply sequence specific, only one plasmid from the series should exhibit the drug resistance when present in the active orientation, since as a series the pUC plasmids can contain *Bam*HI site inserts fused to the vector gene sequences in each of the three translational reading frames. The results presented in Table 2 show that this is the case. pUC7 containing *Bam*HI-M' exhibits coumermycin A₁ resistance, whereas pUC8 and pUC9 containing *Bam*HI-M' do not. The pUC7 translational frame for the *Bam*HI-M' sequences is the same as in pBR322. Further, pUC7 contain-

FIG. 4. Autoradiographic images of polypeptides from labeled *E. coli* maxicell preparations separated on 14% sodium dodecyl sulfate-polyacrylamide gels. The plasmids contained in each *E. coli* maxicell preparation are as follows: 1. pBR322; 2. pAC150 (*Bam*HI-F); 3. pAC150' (*Bam*HI-F in inverted orientation); 4. pAC172 (*Bam*HI-M' in inverted orientation); 5. pAC172 (*Bam*HI-M' in inverted orientation); 6. pUC7; 7. pUC7 containing *Bam*HI-M' in an orientation giving the coumermycin A₁-sensitive phenotype; 8. pUC7 containing *Bam*HI-M' in orientation giving the coumermycin A₁-resistant phenotype.

ing *Bam*HI-M' JM103 cells are blue on 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside indicator plates, whereas bacteria containing the equivalent pUC8 or pUC9 plasmids are white.

Second, it seemed likely that deletion mutations could be obtained after *in vitro* mutagenesis of the *Bam*HI-M' sequences and selection for the appropriate changes in the plasmid phenotype. The isolation and assay of such mutants is presented in Table 2. pUC7 *Bam*HI-M' *dl* *Sac*II was isolated following digestion of pUC *Bam*HI-M' with *Sac*II and BAL 31, religation, and transformation of *E. coli* HB101. This plasmid, which has lost the internal *Sac*II site, does not induce partial coumermycin A₁ resistance (Table 2).

Characterization of proteins encoded in coumermycin-resistant recombinant plasmids. To determine whether the recombinant plasmids specify observable proteins, "maxicell" cultures were prepared and labeled with ¹⁴C-amino acids (Fig. 4). The most obvious feature of pBR322 maxicells with *Bam*HI inserts is that the major *tet* gene protein, which migrates with an apparent molecular weight of 37,000, is absent. An additional pBR322-specific protein with a molecular weight of 35,000 is also seen in the analysis of these maxicell preparations. This pBR322-specific protein and other hybrid proteins (Fig. 4, lanes 4 and 5) were observed

only when the protein lysis buffer contained 0.400 M NaCl. Maxicells from *Bam*HI-F-containing plasmids do not appear to synthesize a novel product that can easily be identified in sodium dodecyl sulfate-polyacrylamide gels. Faint bands appear in the molecular weight range of 13,000 to 16,500 (Fig. 4, lane 3); however, these proteins cannot unambiguously be assigned to be directed by the *Bam*HI-F-specific sequences contained in the plasmid. Resolution was also not possible in 15% sodium dodecyl sulfate-polyacrylamide gels.

In contrast, *Bam*HI-M' sequence-directed products are observed. In the pBR322 *Bam*HI-M' maxicells, novel M' proteins are seen regardless of the orientation of the insert (Fig. 4, lanes 4 and 5). In the *cou*^r orientation the protein produced has a molecular weight of approximately 45,000, whereas in the *cou*^s orientation the product has a molecular weight of 43,000. Based on the lack of translational stop signals in *Bam*HI-M' in either orientation, this result is expected. pUC7 *Bam*HI-M' maxicells (Fig. 4, lane 8) also reveal novel proteins in addition to the absence of the modified β -galactosidase produced by pUC7 maxicells (Fig. 4, lane 6). The presence of multiple novel products with molecular weights of 16,000, 17,500, 18,000, 24,000, and 48,000 does not allow us to assign one of them solely as the β -galactosidase *Bam*HI-M' product. The observable products of lower molecular weights could be degradation products of the larger product at approximately 48,000 (Fig. 4, lane 8). Another problem was that *E. coli* containing pUC7 *Bam*HI-M' in the *cou*^r orientation consistently exhibited greater UV resistance than *E. coli* containing other pUC7 plasmids. Nevertheless, the indicated bands of lane 8 of Fig.

FIG. 5. Inhibition of growth in *E. coli* containing *cou*^r plasmids pAC150 and pAC172. *cou*^r indicates proximity of the active end of each plasmid to the *tet* gene promoter in pBR322. *cou*^s indicates the inverted orientation of the *Bam*HI insert and sensitivity to the drug.

4 do not appear to correspond to any of the faint residual chromosome-directed proteins in lane 6 or in other analyses of pUC7 maxicells (data not shown).

HSV-1 *Bam*HI-F and *Bam*HI-M' coumermycin-resistant plasmids inhibit *E. coli* in the absence of antibiotic. Since the recombinant sequences specified products that induced coumermycin A₁ resistance and since (Table 1) *cou*'1 plasmids would not transform *E. coli* JM103, it seemed likely that the same products might have an observable effect on *E. coli* replication in the absence of coumermycin A₁. Thus, we examined the pattern of growth of *E. coli* HB101 containing pBR322 *cou*' plasmids in the absence of the drug. The growth of cultures containing *Bam*HI-F in the *cou*' orientation were significantly retarded (Fig. 5). The opposite orientation had no effect. The growth of cultures containing *Bam*HI-M' in the *cou*' orientation were retarded to a lesser extent than were cultures containing *Bam*HI-F. Again the opposite orientation had no effect.

Growth inhibition by pUC7 *Bam*HI-M' plasmids is presented in detail in Table 2. In *E. coli* JM103, titration results were comparable to the inhibition seen in HB101 when *Bam*HI-M' was present in pBR322. In most cases (Table 2) the induction of pUC7 *Bam*HI-M' bacterial cultures with isopropyl thio- β -galactoside resulted in a dramatic decrease in plating efficiency. However, by continual induction it was possible to obtain plating efficiencies that were comparable to that of uninduced culture (Table 2).

We have observed that *cou*' *Bam*HI-F plasmids undergo predictable deletions in greater than 1% but less than 2% of *E. coli* clones containing the plasmid in the active orientation. In all cases observed to date, the deletions resulted in the elimination of the active component of *Bam*HI-F.

DISCUSSION

In this report we describe the results of a screen for previously unknown HSV-1 functions by hybrid plasmid induced resistance to coumermycin A₁ in *E. coli*. Coumermycin A₁ and related antibiotics inhibit *E. coli* replication by release of *E. coli* DNA from constraint or by an arrest of DNA synthesis at the replication fork by an antibiotic-gyrase complex (6). The data presented here suggest that two HSV-1 functions are antagonists of drug inhibition, yet in the absence of drug are themselves inhibitory to *E. coli* replicative processes. *E. coli* H protein has been postulated to balance or counteract the necessary activity of gyrase in replicating forks (8, 24). It is possible that one of the *cou*' products functions in a similar manner. Additionally one of these products may overcome the H protein-gyrase interactions. If this general mechanism is operating, then cells would be partially resistant to the action of coumermycin A₁, since the new polypeptide may partially maintain the superhelicity of the bacterial chromosome. Alternatively, a new product may directly antagonize the inhibitory mode of coumermycin A₁ by preventing the drug from binding to the gyrase B subunit. Aspects of both alternatives are not mutually exclusive. A most intriguing possibility is that the new polypeptide may supplement an inhibited gyrase complex and partially replace the activity required by the B-subunit proteins. In the absence of the drug, the new polypeptide could hinder a normal gyrase complex. Current work (Pearson and Conley, manuscript in preparation) suggests that changes in the superhelicity of *E. coli* DNA can be seen in bacterial cultures containing coumermycin A₁ resistance-inducing plasmids from *Bam*HI-F.

The identification of the HSV-1 gene encoding resistance

from *Bam*HI-F is not known. This position, map coordinate ~0.645, lies to the right of the gC family of transcripts and gC3'-coterminal transcripts (2). Four transcripts have been reported to span position ca. 0.645 (9). Two of these that are abundant are apparently transcribed in the leftward direction in the prototype arrangement, i.e., in the opposite direction proposed for *cou*'1 sequences. However, two additional, less abundant messages of 2 and 1.5 kilobases map at or near position 0.645 (9). Perhaps one of these messages encodes the complete HSV-1 product that was observed to be active in *E. coli*.

The mode of expression of *cou*' from *Bam*HI-M'-containing plasmids most probably involves the synthesis of a hybrid protein with the *Bam*HI-M'-encoded product positioned in the central portion of the molecule. The insertion of *Bam*HI-M' into pBR322 interrupts, but does not create translational stop signals in a potential hybrid transcript. A translational stop would not be found until pBR322 position 1273 (28). The mechanism of resistance of the *Bam*HI-M'-*tet* gene fusion product is unknown. The additional internal residue may modify the action of the protein such that a comparable coumermycin A₁ efflux similar to the tetracycline efflux (16) is established. However the same mechanism would be unlikely for the pUC7 fusion product. Alternatively the resistance may indicate some unknown intrinsic property of the *Bam*HI-M'-coding sequence. There are approximately 523 codons from the potential ICP4 start codon to the *Bam*HI-M'-Y site. At this junction it appears that the pUC7 and ICP4 (19) frames differ. Thus if coding sequences of ICP4 are not involved, further work will be necessary to determine why these novel products have such functions.

Finally, the approach presented in this paper suggests that the alignment of opening reading frames of eucaryotic DNA in common vectors such that fusion proteins are made may be an excellent means to locate and assay a variety of genes and to prepare, select, and assay mutants in these genes outside their normal genomic location. In addition to HSV-1 *Bam*HI-F and *Bam*HI-M', our current studies indicate that other properly aligned HSV-1 cloned DNA fragments alter *E. coli* replicative processes.

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