JOURNAL OF BACTERIOLOGY, Mar. 1980, p. 1366-1374
0021-9193/80/03-1366/09$02.00/0

Vol. 141, No. 3

Properties of Erythromycin-Inducible Transposon Tn*917* in *Streptococcus faecalis*

PAUL K. TOMICH, F. Y. AN, AND DON B. CLEWELL*

Dental Research Institute and Departments of Oral Biology and Microbiology, Schools of Dentistry and Medicine, The University of Michigan, Ann Arbor, Michigan 48109

Streptococcus faecalis strain DS16 harbors two plasmids, a conjugative plasmid, pAD1, which encodes hemolysin and bacteriocin activities, and a nonconjugative plasmid, pAD2, encoding resistance to streptomycin, kanamycin, and erythromycin, the latter of which is inducible. The erythromycin resistance determinant is located on a 3.3-megadalton transposable element designated Tn*917*, which could be transposed to pAD1 as well as to two other plasmids, pAmγ1 and pAMα1. When strain DS16 was exposed to low (inducing) concentrations of erythromycin for a few hours, the frequency of Tn*917* transposition from pAD2 to pAD1 increased by an order of magnitude. This induction paralleled induction of erythromycin resistance and was prevented by exposing the cells to inhibitors of deoxyribonucleic acid, ribonucleic acid or protein synthesis. The exposure of strain DS16 to inducing concentrations of erythromycin also enhanced the frequency of erythromycin-resistant transconjugants appearing during mating. Initially, cointegrate molecules, whose molecular weights were approximately the sum of pAD1 and pAD2, accounted for these transconjugants; however, as the induction time increased, pAD1::Tn*917* became increasingly prominent.

Over the last 5 to 6 years, extensive studies on transposable elements have significantly advanced our understanding of recombinational processes resulting in deletion formation, inversion, and translocation (8, 11, 14). Most of the reported transposons code for drug resistance determinants and are flanked by homologous sequences of DNA in either the same or opposite orientation (8).

We recently reported evidence suggesting that an erythromycin (Em) resistance transposon is located on nonconjugative R-plasmid pAD2 (15 megadaltons [Mdal]) of *Streptococcus faecalis* DS16 (16). The erythromycin resistance is inducible and reflects the MLS phenotype (i.e., resistance to macrolides, lincosamides, and streptogramin type B antibiotics) (17). The pAD2 plasmid also determines resistance to streptomycin (Sm) and kanamycin (Km). Transposition of the erythromycin resistance determinant from pAD2 to coresident hemolysin plasmid pAD1 (35 Mdal) was first detected by mating DS16 with a plasmid-free recipient strain and screening for hemolytic, Em^r transconjugants that were sensitive to streptomycin and kanamycin. Such transconjugants were found to harbor a single plasmid about 3 Mdal larger than the 35-Mdal pAD1 plasmid (16).

The study presented here involves a characterization of the transposon (designated Tn*917*) and a demonstration that transposition can be induced by exposure of DS16 cells to a subinhibitory (inducing) concentration of erythromycin. In addition, we provide evidence that transposition occurs via a cointegrate intermediate and that DNA synthesis is required. (A preliminary account of these findings has been reported elsewhere [15].)

MATERIALS AND METHODS

Most of the chemicals, materials, radioisotopes, and procedures used in this paper have been reported elsewhere (2, 4). The DNA inhibitor 6-(*p*-hydroxyphenylazo)-uracil was a generous gift of B. W. Langley. Electron microscopic heteroduplex analysis of DNA has also been described (13, 18).

The strains utilized in this study are listed in Table 1. For the labeling of DNA, cells were grown in an M9-Casamino Acids-yeast extract medium (3). Transposition experiments made use of Penassay (AB3) broth (Difco). Cells were cultivated at 37°C and monitored by using a Klett-Summerson colorimeter (no. 54 filter).

To perform the transposition enhancement experiments, a culture of DS16 was grown in the presence or absence of 0.5 μg of erythromycin per ml (unless indicated otherwise), and fractions were removed at various times. The cells were washed free of drug by centrifugation and suspended in fresh media at the original volume. The cells were then mated for 4 h with plasmid-free strain JH2-2, hemolytic transconjugants were selected on blood agar plates containing fusidic acid (25 μg/ml), rifampin (25 μg/ml), and erythromycin (50 μg/ml), and the transconjugants were

TABLE 1. *S. faecalis strains employed in this study*

Strain	Chromosomal resistance[a]				Plasmid resistance and other markers					Comments (reference)
	Rif[b]	Fus[b]	Sm	Sp	Tc	Em	Sm	Km	Hemolysis[c]	
DS16	S	S	S	S	S	R	R	R	Yes	Carries plasmids pAD1 and pAD2 (16)
JH2-2	R	R	S	S	S	S	S	S	No	Plasmid-free strain (7)
DS-5C1	S	S	S	S	R	S	S	S	Yes	Derived by curing Em^r plasmid pAMβ1 from DS-5 (2)
JH2SS	S	S	R	R	S	S	S	S	No	Spontaneous mutant of JH2 (7) resistant to streptomycin and spectinomycin
PT300	R	R	S	S	R	R	R	R	Yes	Derived from JH2-2 harboring plasmids pAMγ1, pAD2, and pAMα1
PT350	R	R	S	S	S	R	S	S	Yes	JH2-2 strain harboring pAD1 carrying Em^r marker of pAD2
PT400	S	S	R	R	S	R	S	S	Yes	JH2SS derivative harboring pAMγ1 which carries Em^r determinant of pAD2
PT410	S	S	R	R	R	R	S	S	Yes	JH2SS strain which contains plasmids pAMγ1 and pAMα1, the latter plasmid carrying Em^r marker of pAD2

[a] R, Resistant; S, sensitive.
[b] These resistances were described by Jacob and Hobbs (7). Rif, rifampin; Fus, fusidic acid.
[c] Hemolysin production is always accompanied by bacteriocin production.

subsequently screened for sensitivity to streptomycin and kanamycin. The extent of transposition is expressed as a percentage based on the number of Em^r, Sm^s, and Km^s transconjugants divided by the total number of Em^r transconjugants. Thus, variations which result from fluctuation in donor and recipient viabilities (determined at the time of plating) are eliminated when data from different experiments are compared.

RESULTS

In 4-hour broth matings between *S. faecalis* strains DS16 (contains pAD1 and pAD2) and JH2-2 (plasmid free), various types of transconjugants have been observed (16). The conjugative hemolysin plasmid pAD1 transfers at a frequency per donor of about 10^{-3} to 10^{-2}; however, hemolytic transconjugants resistant to erythromycin, streptomycin, and kanamycin appear at a frequency of only 10^{-8} to 10^{-6}. The latter transconjugants (referred to as class I) generally contain a single plasmid with a molecular weight of about 50×10^6, approximately the sum of the molecular weights of pAD1 and pAD2 (based on a 70S sedimentation through neutral sucrose density gradients). From such transconjugants the resistances can be transferred to new recipients at a frequency similar to that of pAD1, and a linkage between resistance and hemolysin production is maintained (i.e., the cointegrate appears stable).

Another type of transconjugant (referred to as class II) is also detected in DS16 × JH2-2 crosses; these are hemolytic, Em^r transconjugants sensitive to streptomycin and kanamycin. Such transconjugants appear at a frequency of 10^{-8} to 10^{-7} and contain a single plasmid with a mass of about 38 Mdal (based on a 60S sedimentation through neutral sucrose density gradients, using internal markers consisting of pAD1 [58S] and pAD2). Figure 1A shows an alkaline sucrose density gradient of such a plasmid (from strain PT350) clearly observed sedimenting slightly ahead of pAD1. (Alkaline, as opposed to neutral, sucrose gradients are more clearly demonstrative of this phenomenon; the latter result in nicked [relaxed] forms of the larger plasmid sedimenting in nearly the same position as that of the supercoiled forms of pAD2. In alkali, all nicked forms appear near the top of the gradients, whereas the rapidly sedimenting covalently closed structures are clearly resolved.) Strains harboring such plasmids donate erythromycin resistance and the hemolysin trait at high frequency (about 10^{-2}) without a change in plasmid structure. The generation of such molecules is believed to reflect a transposition of the

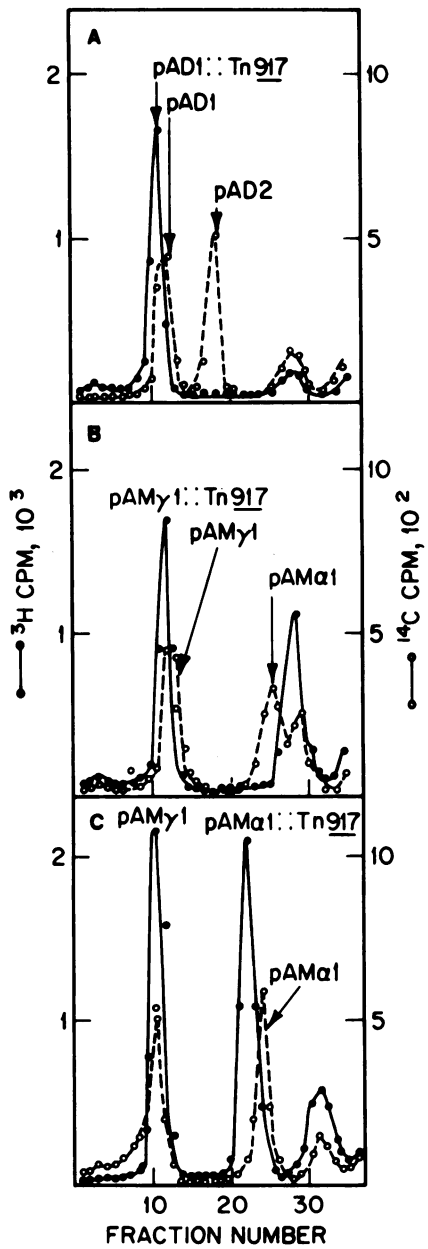

FIG. 1. *Sedimentation profiles of plasmids carrying the erythromycin transposon Tn917. Log cultures (15 ml) were labeled with 0.01 mCi of either [^{3}H]-thymidine or [^{14}C]thymidine, and lysozyme-Sarkosyl lysates were run in ethidium bromide-CsCl buoyant density gradients (2). Satellite (plasmid) DNA was dialyzed and sedimented (SW50.1 rotor at 45,000 rpm for 30 min at 15°C) through a 5 to 20% alkaline sucrose gradient (1). Sedimentation is from right to left. The ^{3}H-labeled material represents plasmid DNA from strains PT350 (A), PT400 (B), and PT410 (C). The ^{14}C-labeled marker represents plasmid DNA from strain DS16 for (A) and plasmid DNA from strain DS-5C1 for (B) and (C). In all cases the marker was mixed with the ^{3}H-labeled samples before centrifugation.*

erythromycin resistance determinant from pAD2 to pAD1. Data presented below support this view. The frequency of transposition is approximately 10^{-6} (based on the frequency of transfer of pAD1 and the frequency of appearance of class II transconjugants), a frequency within the range of known transposons (8).

It is worth noting that still another type of transconjugant can be obtained in DS16 × JH2-2 crosses (16); these transconjugants represent nonhemolytic strains resistant to all three drugs and appear at a very low frequency (10^{-9} to 10^{-8}). Such strains contain pAD2 alone (presumably mobilized intact by pAD1) and are completely incapable of further transfer (even if matings are carried out overnight on filter membranes).

Transposition to other replicons. As additional support for the notion that the Em^r determinant on pAD2 is located on a transposon, we sought to detect its transposition to replicons other than pAD1. A strain was constructed which harbors pAD2 in addition to pAMγ1 and pAMα1. pAMγ1 is a 35-Mdal conjugative plasmid which also encodes synthesis of a hemolysin-bacteriocin; pAMα1 is a nonconjugative 6-Mdal tetracycline (Tc) resistance plasmid that can be mobilized by pAMγ1 at a relatively high frequency (about 10^{-3} to 10^{-2}) (4). This strain, designated PT300, was obtained from a mating using JH2-2(pAD2) as a recipient and DS-5C1 (harbors pAMα1 and pAMγ1) as a donor and selecting for Tc^r, hemolytic transconjugants. PT300 was then mated with the isogenic plasmid-free recipient JH2SS, and Em^r transconjugants were selected on plates containing spectinomycin (500 μg/ml) and 50 μg of erythromycin per ml. Thirteen Em^r transconjugants were observed (frequency about 10^{-8} per donor), of which four were Km^s. Of these four, one transconjugant was Tc^r. All of the transconjugants were hemolytic. The absence of kanamycin resistance with the simultaneous acquisition of erythromycin resistance suggested that the erythromycin resistance determinant had been transposed from pAD2 to another replicon. (Loss of the streptomycin resistance determinant could not be monitored because of the chromosomal Sm^r mutation in the host.) We therefore looked at the plasmid DNA of two Em^r Km^s transconjugants, one of which was Tc^s, and the other of which was Tc^r.

Figure 1B shows an alkaline sucrose gradient of plasmid DNA from a transconjugant PT400,

which is hemolytic, Em^r, and Tc^s. A single plasmid is observed sedimenting slightly faster than the internal pAMγ1 marker. From neutral gradients (not shown), we estimated the sedimentation rate as 60S, compared with a rate of 58S for pAMγ1. This is consistent with an insertion of about 3 Mdal of DNA. The peak sedimenting behind the pAMα1 marker represents open circular forms in the preparation which migrate near the top of the alkaline sucrose density gradient. The separation between pAMγ1 and pAMγ1 carrying the erythromycin resistance determinant has been consistently observed and essentially exhibits the same difference between pAD1 and pAD1 with the erythromycin resistance marker (16). In addition, subsequent matings with this strain as a donor showed high-frequency transfer of the Em^r trait and hemolysin production.

Figure 1C represents the Tc^r transconjugant strain PT410 and shows a new plasmid sedimentating well ahead of pAMα1, whereas pAMγ1 appears normal. On a neutral gradient (not shown) this plasmid sediments at 33S compared with pAMα1, which sediments at 28S. Subsequent mobilization of tetracycline resistance showed that the tetracycline and erythromycin resistance determinants were now linked. These data indicate that the erythromycin resistance determinant is capable of transposition to pAMγ1 and pAMα1. The transposon will subsequently be referred to as Tn*917*.

Heteroduplex analyses. Transposons consist of DNA flanked by homologous DNA segments frequently oriented in opposite directions. Consequently, we looked for the classical stem-loop structure within self-annealed pAD2 molecules. Figure 2A (and B) shows an electron micrograph of a representative molecule. One can readily observe two stem-loop structures. The short-stem (280 bases) and large single-stranded loop (4.5 kilobase [kb]) structures have an approximate mass for double-stranded DNA of 3.3 ± 0.10 (standard deviation) Mdal. Five circular molecules and four broken (i.e., linear) molecules were photographed and measured. The other structure is more complex; it has a longer stem (1,100 bases) and two loops attached to it (one of 470 bases and the other of 1,700 bases). The total structure would have an approximate mass of 2.9 Mdal as double-stranded DNA.

In addition, heteroduplex molecules of pAD1 annealed to pAD1::Tn*917* (data not shown) and pAMα1 annealed to pAMα1::Tn*917* were analyzed. In both cases the smaller stem structure seen on pAD2 (280 base stem) associated with a 4.5-kb loop was observed. Figure 2C shows pAMα1 heteroduplexed with pAMα1::Tn*917*. This structure, therefore, represents Tn*917*. (We wish to point out that the 280-base pair length of the inverted repeats should be viewed as a maximum value owing to the lack of precision in measuring shorter lengths by this technique.)

The second stem-loop structure on pAD2 remains functionally unknown. We were unable to observe any transposition from pAD2 to pAD1 of either the streptomycin or the kanamycin determinant. If this process does occur, the frequency must be at least 100-fold lower than that of Tn*917*.

Inducibility of erythromycin resistance and transposition. Erythromycin resistance was demonstrated to be inducible in strain DS16 (16). Since genetic determinants on some transposons have been shown to be involved in the transposition process (5, 6), the possibility existed that transposition of Tn*917* might be linked to the expression of resistance and might be inducible by growth in low (inducing) concentrations of erythromycin. Preliminary experiments indeed suggested that this was the case. As a result, the following experiment was performed. DS16 was exposed to 0.5 μg of erythromycin per ml; portions of the culture were removed and challenged with 500 μg of erythromycin per ml (Fig. 3). The initial growth rates (Fig. 3A) were faster for the cultures induced for the longer times.

Figure 3B shows the result of an analogous experiment, but in this case pAD1::Tn*917* transconjugants were plotted as a percentage of the total Em^r, hemolytic transconjugants. When DS16 was grown in the absence of erythromycin, the level of transposition remained at a low, constant level. However, growth of DS16 in inducing concentrations of erythromycin yielded an increasing transposition frequency with increasing induction time.

A comparison of induction kinetics for both transposition and erythromycin resistance as a function of induction time shows a striking parallel, even though two entirely different phenomena are being measured (Fig. 3A and B). In an additional experiment, a culture of DS16 was "induced" for 4 h by decreasing the concentrations of erythromycin from 0.5 to 0.0001 μg/ml. Transposition frequency and growth after challenge (500 μg/ml) were measured as described above. A reduction to 0.001 μg of erythromycin per ml still gave enhanced transposition, and the cells were inhibited only partially upon challenge with 500 μg of erythromycin per ml. However, when the cells were exposed to only 0.0001 μg of erythromycin per ml, neither process occurred. These data strongly suggest that the two processes have a common regulatory step.

Inhibitors of macromolecular synthetic processes: their effect on transposition. If

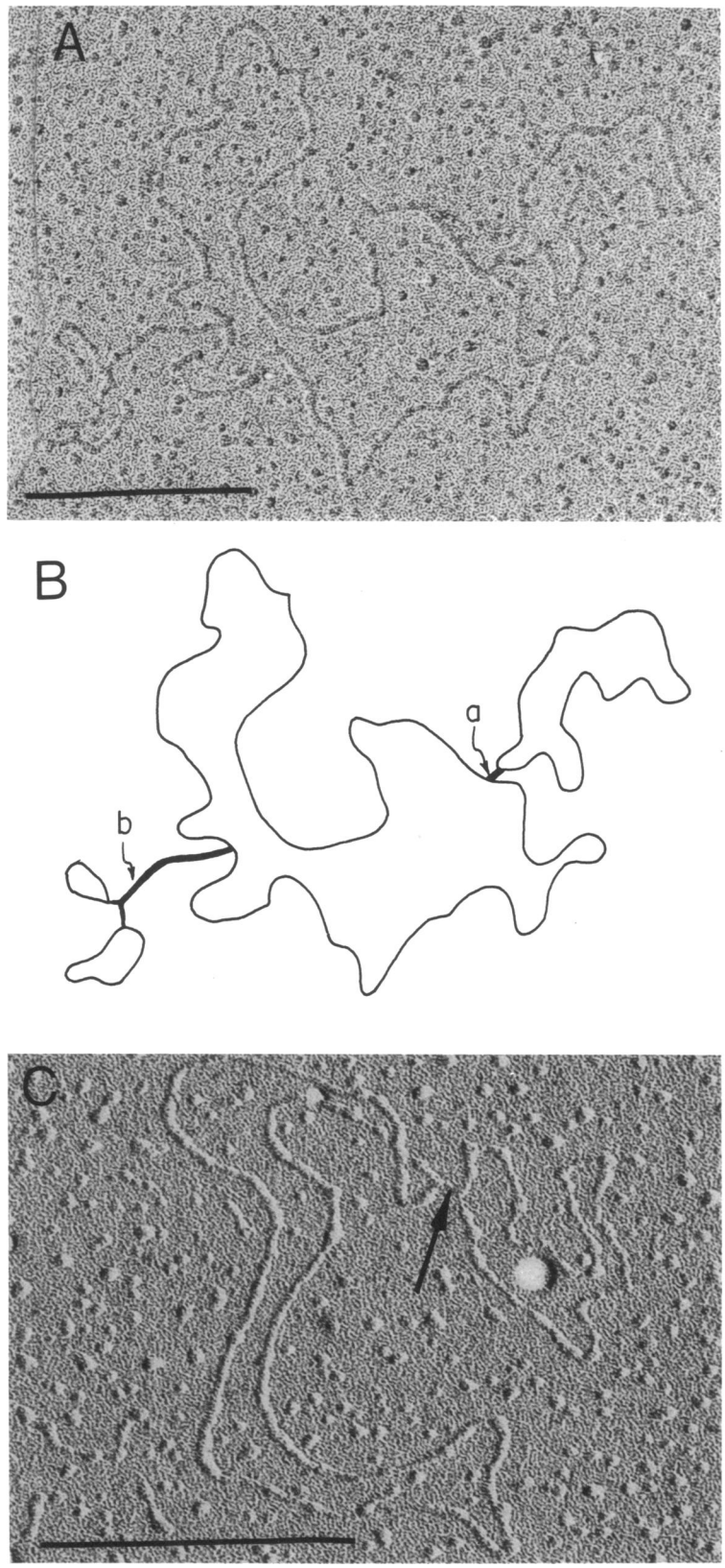

FIG. 2. *Heteroduplex analysis. (A) Self-annealed pAD2 molecule. These were observed at a frequency of about 1%. (B) Drawing of the molecule represented in (A). The stem-loop structure (arrow marked "a") represents the Tn917 transposon. The other stem-loop structure (arrow marked "b") contains two loops on a 1,100-base stem. (C) Heteroduplex molecule of pAMα1 and pAMα1::Tn917. These heteroduplex molecules were found at a frequency of about 5%. Arrow points to the 280-base stem of the transposon. Bar represents 0.5 μm.*

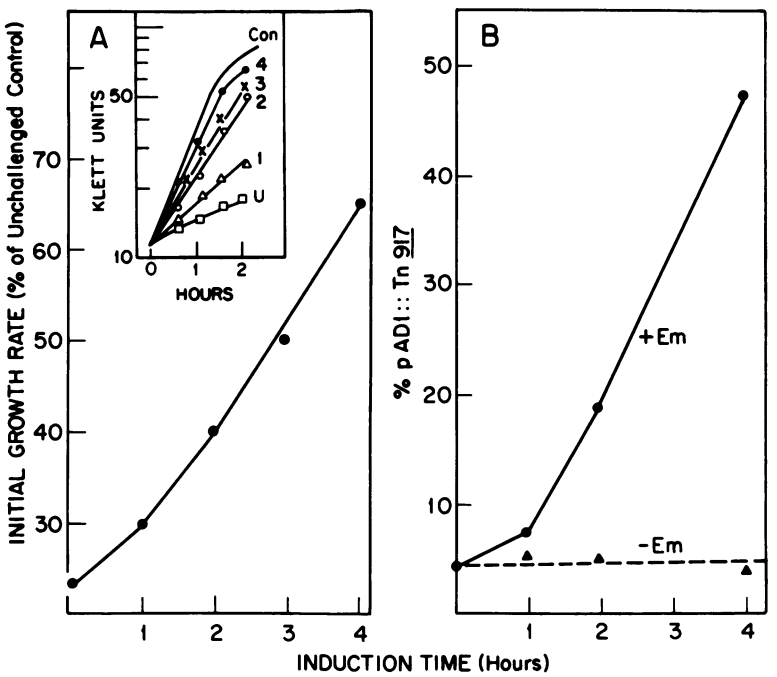

FIG. 3. *Kinetics of erythromycin resistance and transposition. (A) Induction of erythromycin resistance. DS16 cells were induced by the addition of 0.5 μg of erythromycin per ml at zero time, and portions were removed after 1, 2, 3, or 4 h and then challenged with 500 μg of erythromycin per ml. The induced culture was maintained in logarithmic growth by diluting it into fresh inducing medium when necessary. Just before challenge, all fractions were diluted to an optical density of 12 Klett units. The inset shows initial growth from which data points of the curve were derived. Controls were an uninduced, challenged culture (labeled U) and an unchallenged, induced culture (labeled Con). The kinetics are presented as the initial growth rates after challenge, expressed as a percentage of the unchallenged control. (B) Induction of transposition of Tn917 from pAD2 to pAD1. Transposition is expressed as a percentage of the total number of Em^r transconjugants (see Materials and Methods).*

inducibility of transposition is a property of Tn*917*, any agent which inhibits resistance-induction should prevent elevation of the transposition rate. Similarly, if protein(s) encoded by the transposon is involved in the transposition process (5), inhibitors of protein synthesis would be expected to block transposition. Furthermore, transfer of the transposon from one replicon to another could occur by one of two methods. In one case, a copy of the transposon on the donor replicon could be made on the recipient replicon. In the other case, DNA within the inverted repeats of the transposon could be cleaved from the donor replicon, and the entire transposon could be inserted into the recipient replicon. The former process would require extensive DNA synthesis; the latter would not.

Inhibitors of RNA (rifampin), protein (chloramphenicol), and DNA [6-(*p*-hydroxyphenylazo)-uracil] were tested for their effect on transposition (Fig. 4). In the absence of inhibitor we observed the expected increase in the transposition frequency as the induction time increased. However, rifampin, chloramphenicol, and 6-(*p*-hydroxyphenylazo)-uracil all brought about an immediate cessation of transposition. It is noteworthy that, in the case of chloramphenicol and 6-(*p*-hydroxyphenylazo)-uracil, cell viability remained essentially constant during drug exposure, and the frequency of conjugal transfer of erythromycin resistance after drug exposure was unaltered. In the case of rifampin, viability decreased by 10- to 100-fold (in different experiments) over the course of 3 h; yet, interestingly, the absolute number of transconjugants after drug exposure did not decrease accordingly.

Evidence for the formation of cointegrate structures. During experiments involving the transposition of Tn*917* from pAD2 to pAD1, we consistently noted an increase in the frequency of Em^r transconjugants in the case where the donor cells were exposed to inducing concentrations of erythromycin. As shown in Table 2 a six- to sevenfold increase in the number of transconjugants per donor appeared after exposure of the donor cells to erythromycin for 30 and 60 min. The frequency did not increase beyond

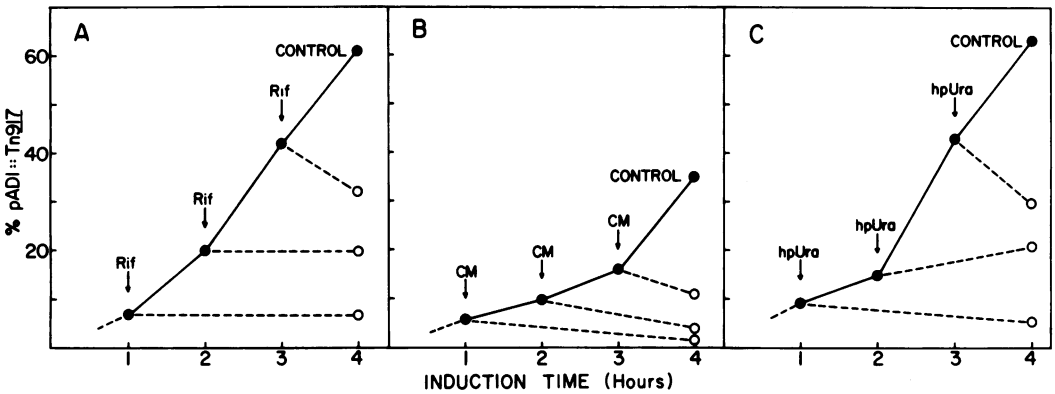

FIG. 4. *Inhibition of transposition by metabolic inhibitors. Effects of (A) 10 μg of rifampin per ml, (B) 50 μg of chloramphenicol per ml, and (C) 500 μg of 6-(p-hydroxyphenylazo)-uracil per ml on enhanced transposition are plotted as described in Fig. 3B. The arrows indicate time of addition of the drugs after addition of erythromycin (zero time). The optical density of DS16 was monitored at these times before harvesting (centrifugation of cells), and, upon suspension, donor cells were adjusted to the same optical density to maintain approximately the same input of donor cells (DS16) in the mating mixes. Symbols: (○) level of transposition in drug-exposed cells; (●) controls of induced DS16 for the times indicated.*

these values on further exposure to erythromycin (up to 4 h). Since we normally do not observe a significant increase in the appearance of pAD1::Tn*917* structures until after 1 h of donor exposure to erythromycin (Table 2 and Fig. 3), it was of interest to determine the nature of the plasmid content in transconjugants obtained in relation to the shorter-term erythromycin exposure. Four transconjugants resistant to erythromycin, streptomycin, and kanamycin, involving a 60-min exposure of DS16 donors, were analyzed for plasmid content, and all were found to contain a single plasmid which sedimented at 70S through sucrose density gradients (data not shown). This is consistent with a mass approximately corresponding to the sum of pAD1 and pAD2. These apparent cointegrate structures, which must be generated in the donors during the early stages of induction, appeared stable and could be transferred at a high frequency ($\sim 10^{-3}$ to 10^{-2} per donor) in secondary matings (showing genetic linkage of the hemolysin and resistance determinants). This transfer occurred without dissociation of the cointegrate even if these donors were exposed to erythromycin for 4 h. As we have pointed out, and as is evident in Fig. 3, Em^r transconjugants from matings not involving donor erythromycin exposure also contain a single 70S structure (class I transconjugants). Whether or not these structures are identical to those appearing after a short exposure of donors to erythromycin remains to be determined.

DISCUSSION

The data presented here describe an erythromycin resistance transposon, designated Tn*917*, located on the pAD2 plasmid of *S. faecalis* strain DS16. Tn*917* has a size of 3.3 Mdal, including inverted repeat segments (280 base pairs), and has been shown to transpose to pAD1, pAMγ1, and pAMα1. The most interesting feature of Tn*917* is that it can be induced to transpose to pAD1 by exposing the cells to a low concentration of erythromycin. This induction could be inhibited by exposure of the cells to inhibitors of RNA, protein, or DNA synthesis. In addition, pAD1-pAD2 cointegrate formation occurs during the first 30 min of induction.

Tn*917* is one of only a few transposons recently identified in gram-positive bacteria (9, 10). Yagi and Clewell (18, 19) have previously reported an amplifiable tetracycline resistance determinant flanked by "direct repeats" on pAMα1 in *S. faecalis*; however, transposition of this element to other replicons has not yet been demonstrated. Interestingly, Phillips and Novick (10) have reported that Tn*554*, which encodes erythromycin resistance in *Staphylococcus aureus*, has a repressor controlling transposition and, thus, is under negative control. In *Escherichia coli*, Heffron et al. have generated in vitro deletions in Tn*3* and, in turn, have revealed that the transposon encodes a protein(s) involved in the transposition process (6). An extension of these studies (5) has also implicated a regulatory gene product affecting the frequency of transposition. Heffron et al. also provide evidence that transposition occurs via a cointegrate intermediate in which the donor and recipient replicons are fused and contain a duplication of the Tn*3* element (5).

Shapiro (12) has proposed a general model which includes cointegrate intermediates having

TABLE 2. *Transfer of erythromycin (Em) resistance from strain DS16 to JH2-2 after exposure to an inducing concentration of erythromycin*[a]

Min of induction	Frequency of Em^r transconjugants		pAD1-pAD2 cointegrate[b] (%)		pAD1::Tn*917* (%)	
	−Em	+Em	−Em	+Em	−Em	+Em
0	$2.5 \pm 0.2 \times 10^{-8}$	$2.5 \pm 0.2 \times 10^{-8}$	100	100	0	0
30	$2.5 \pm 1.7 \times 10^{-8}$	$1.8 \pm 0.7 \times 10^{-7}$	100	100	0	0
60	$3.7 \pm 0.7 \times 10^{-8}$	$1.6 \pm 0.6 \times 10^{-7}$	99.3 ± 0.47	93 ± 3.3	0.67 ± 0.47	7 ± 3.3

[a] Hemolytic, Em^r transconjugants were picked and tested for their sensitivity to kanamycin and streptomycin. Experimental protocol is as described in the legend to Fig. 3. Frequencies are expressed as the number of transconjugants per donor at the end of the 4-hour mating. All values are the average of three experiments and are represented with their standard deviations.

[b] Represents the percentage of Em^r transconjugants which are also resistant to kanamycin and streptomycin. When such transconjugants were analyzed, they contained a single ~50-Mdal plasmid. (For the 60-min case, the DNAs of four isolates were examined.)

duplications of the transposable element located at the junction points of the two replicons. The generation of a second transposition element in the intermediate implies a requirement for DNA synthesis. Our observation that inhibition of DNA synthesis prevents transposition of Tn*917* supports this assumption. Whereas inhibitors of RNA and protein synthesis also blocked transposition, it is not conclusive whether these drugs might be active by indirectly inhibiting DNA synthesis. (Initiation of pAD1 and pAD2 plasmid replication requires protein synthesis [unpublished data] and possibly RNA primer.) According to the Shapiro model, however, replication from an origin outside the transposable element is not necessary for transposition to occur (12). Thus, the inhibition of transposition by these agents may be due directly to the blockage of induced synthesis of a product on the putative erythromycin operon needed for transposition.

The appearance of pAD1-pAD2 cointegrate structures during the early stages (i.e., the first 30 min) of induction is also consistent with the Shapiro model, although the presence of two copies of Tn*917* remains to be determined. That such cointegrate structures appear stable after transfer to strain JH2-2, even after exposure of the latter to erythromycin for several hours (unpublished data), suggests that certain host factors not present in JH2-2 may facilitate the next step in the transposition process. (Extensive efforts have never succeeded in demonstrating transposition of Tn*917* from pAD2 to pAD1 when both plasmids are harbored by JH2-2 [unpublished data]; transposition from pAD2 to pAMγ1 or pAMα1, however, could be demonstrated in the JH2-2 background.) Alternatively, the process of conjugal transfer may alter the molecule in such a way as to preclude its dissociation into separate replicons.

To our knowledge, Tn*917* is the first example of a drug resistance transposon where the related drug can induce transposition. That some transposons are so highly evolved is particularly disquieting from a clinical standpoint. Large-scale exposure of bacteria to drugs (e.g., in animal feeds and clinical environments) not only selects for organisms with specific R-plasmids but may also enhance the proliferation and spread of some transposons to different replicons. If the Shapiro model is correct, every transposition event actually results in generating a new copy of the transposon. It will be of significant interest to see if additional transposons are found to be induced to transpose by the drug to which resistance is conferred.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grants DE02731 from the National Institute of Dental Research and AI10318 from the National Institute of Allergy and Infectious Diseases, and a grant from the Upjohn Co. D.B.C. is the recipient of Public Health Service Research Career Development award K04 A10061 from the National Institute of Allergy and Infectious Diseases.

We thank Yoshihiko Yagi and Arthur Franke for helpful discussions and for their assistance in some of the electron microscopy.

LITERATURE CITED

1. **Clewell, D. B., and D. R. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9**:4428–4440.
2. **Clewell, D. B., Y. Yagi, G. M. Dunny, and S. K. Schultz.** 1974. Characterization of three plasmid deoxyribonucleic acid molecules in a strain of *Streptococcus faecalis*: identification of a plasmid determining erythromycin resistance. J. Bacteriol. **117**:283–289.
3. **Clowes, R. C., and W. Hayes.** 1968. Experiments in microbial genetics. John Wiley & Sons, Inc., New York.
4. **Dunny, G. M., and D. B. Clewell.** 1975. Transmissible toxin (hemolysin) plasmid in *Streptococcus faecalis* and its mobilization of a noninfectious drug resistance plasmid. J. Bacteriol. **124**:784–790.
5. **Gill, R., F. Heffron, G. Dougan, and S. Falkow.** 1978. Analysis of sequences transposed by complementation of two classes of transposition-deficient mutants of Tn*3*.

J. Bacteriol. **136**:742–756.

6. **Heffron, F., P. Bendinger, J. J. Champoux, and S. Falkow.** 1977. Deletions affecting the transposition of an antibiotic resistance gene. Proc. Natl. Acad. Sci. U.S.A. **74**:702–706.
7. **Jacob, A. E., and S. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **117**:360–372.
8. **Kleckner, N.** 1977. Translocatable elements in procaryotes. Cell **11**:11–23.
9. **Novick, R. P., I. Edelman, M. D. Schwesinger, A. D. Gruss, E. C. Swanson, and P. A. Pattee.** 1979. Genetic translocation in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U.S.A. **76**:400–404.
10. **Phillips, S., and R. P. Novick.** 1979. Tn*554*—a site specific repressor-controlled transposon in *Staphylococcus aureus*. Nature (London) **278**:476–478.
11. **Schwesinger, M. D.** 1977. Additive recombination in bacteria. Bacteriol. Rev. **41**:872–902.
12. **Shapiro, J.** 1979. Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc. Natl. Acad. Sci.U.S.A. **76**:1933–1937.
13. **Sharp, P. A., M. Hsu, E. Ohtsubo, and N. Davidson.** 1972. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. I. Structure of F prime factors. J. Mol. Biol. **71**:471–497.
14. **Starlinger, P.** 1977. DNA rearrangements in procaryotes. Annu. Rev. Genet. **11**:103–126.
15. **Tomich, P. K., F. Y. An, and D. B. Clewell.** 1978. A transposon (Tn*917*) in *Streptococcus faecalis* which exhibits enhanced transposition during induction of drug resistance. Cold Spring Harbor Symp. Quant. Biol. **43**:1217–1221.
16. **Tomich, P. K., F. Y. An, S. P. Damle, and D. B. Clewell.** 1979. Plasmid-related transmissibility and multiple-drug resistance in *Streptococcus faecalis* subsp. *zymogenes* strain DS16. Antimicrob. Agents Chemother. **15**:828–830.
17. **Weisblum, B.** 1975. Altered methylation of ribosomal ribonucleic acid in erythromycin-resistant *Staphylococcus aureus*, p. 199–206. *In* D. Schlessinger (ed.), Microbiology—1974. American Society for Microbiology, Washington, D.C.
18. **Yagi, Y., and D. B. Clewell.** 1976. Plasmid-determined tetracycline resistance in *Streptococcus faecalis*: tandemly repeated resistance determinants in amplified forms of pAMα1 DNA. J. Mol. Biol. **102**:583–600.
19. **Yagi, Y., and D. B. Clewell.** 1977. Identification and characterization of a small sequence located at two sites on the amplifiable tetracycline resistance plasmid pAMα1 in *Streptococcus faecalis*. J. Bacteriol. **129**: 400–406.