An Autonomously Replicating DNA Fragment in the PBSX Region. L. M. Anderson, M. A. Hollis, and K. F. Bott, Bacteriology Department, UNC Medical School, Chapel Hill, North Carolina 27514.

A B. subtilis 168 EcoRI fragment was cloned using the chimeric plasmid pCS540 of S. Chang, which contains a chloramphenicol resistance determinant from pC194. In rec+ recipients this plasmid becomes integrated into the chromosome and confers Cm resistance to progeny, but remains stable even in the absence of Cm selection. Upon integration, the chromosomal restriction enzyme fragments to which this DNA hybridizes are altered. The site of plasmid integration maps near the MetC locus. In fact, several recipients thought to be defective in PBSX are repaired by this insertion. Strains not believed to carry PBSX are altered in hybridization profiles, obtained by probing EcoRI digests with this cloned fragment. Some transformants which receive this plasmid DNA are altered in their ability to sporulate; others display altered tolerance to Nalidixic acid or change in their response to mitomycin C induction. RecE derivatives are capable of maintaining this DNA as an autonomously replicating plasmid. We believe that, like E. coli, B. subtilis contains defective phage components capable of autonomous replication when liberated from their normally integrated chromosomal site. Although it is not certain whether the effects of this insertion process on sporulation result from alteration of a structural or regulatory component, it is clear that a functional origin of replication exists as part of this defective phage region.


We have identified several tetracycline resistance plasmids in clinical isolates of group B streptococcus. Two of these are small, non-conjugative, multicopy plasmids capable of transforming S. sanquis to tetracycline resistance. Using the small plasmids as probes for homology in Southern hybridization experiments, we have shown that several small tetracycline resistance plasmids share a common sequence of about 2,000 bp in size. Chimeric plasmids constructed in E. coli between ColEl-amp and two of the small tetracycline resistance plasmids from group B streptococcus have been mapped and the tetracycline resistance region further defined by deletion analysis. In addition, several resistance determinants which are located on the chromosome have been cloned from Streptococcus.

Conjugal Transfer of Tetracycline Resistance from Streptococcus mutans to other Streptococci. J. A. Donkersloot, R. J. Harr, L. N. Lee, and D. J. Loblanc, National Institute of Dental Research, Bethesda, Maryland 20205.

A porcine isolate of Streptococcus mutans (DL5) that is highly resistant to streptomycin, erythromycin, lincomycin, and tetracycline (Tc) was found to transfer its Tc resistance trait (Tcr) to S. faecalis JH2-2 (a human isolate) and S. mutans 6715-10 by a conjugation-like transfer mechanism.
mechanism. Transfer of streptomycin or erythromycin resistance was never observed (frequency < 10^{-9} per donor colony forming unit). The highest Tc\(^{-}\) transfer frequencies were observed in aerobic interspecies matings between DL5 and JH2-2, both on filters (2 \times 10^{-4}) and in shake cultures (10^{-9}). Transfer of Tc\(^{-}\) between S. mutans strains DL5 and 6715-10 was observed only after anaerobic matings on filters and at a 1000-fold lower frequency (2 \times 10^{-8}). Transconjugants from the latter mating donated their Tc\(^{-}\) to JH2-2, but at a much lower frequency than that of the DL5 \times JH2-2 mating (2 \times 10^{-8}). So far, we have been unable to demonstrate a plasmid in the S. mutans DL5 donor strain or the S. mutans transconjugants. However, all S. faecalis transconjugants had acquired an 8-megadalton plasmid. Thus, although our interspecies data are consistent with the concept of chromosomal transfer of antibiotic resistance markers, our interspecies results suggest that the observed transfers were plasmid-mediated.

**Cloning and Characterization of Escherichia coli Transfer RNA Genes.** G. L. Duester, R. K. Campen, and W. M. Holmes, Medical College of Virginia, Richmond, Virginia 23298.

In an attempt to study the regulatory DNA sequences for specific transfer RNA genes, the EcoRI fragments of the E. coli chromosome were cloned using the plasmid pBR322. Clones harboring chimeric plasmids bearing transfer RNA genes were located by hybridization of plasmid DNA to specific radiolabelled transfer RNAs. Many chimeric plasmids hybridized to both transfer RNA and ribosomal RNA. One particular plasmid pGD4 carries a 3.9-kilobase pair EcoRI fragment corresponding most likely to the distal end of the ribosomal operon rRN. The nucleotide sequence of part of this region was determined and consists of the last 133 bases of a 23 S rRNA gene, a spacer of 92 bases, a 5 S rRNA gene, a spacer of 12 bases, the tRNAThr gene, a spacer of 37 bases, another 5 S rRNA gene, and a transcription termination site for rRN. Thus, it appears that tRNA\(^{Thr}\) is cotranscribed with ribosomal RNA. Another chimeric plasmid designated LA4 hybridized to tRNA\(^{Ile}\), but not ribosomal RNA. The nucleotide sequence upstream of the tRNA\(^{Ile}\) gene was determined and a putative promoter was located about 40 bases from its 5' end. Thus, tRNA\(^{Ile}\) appears to be controlled independent of ribosomal RNA. In addition, this promoter has a G-C rich region near the site of initiation which is a common feature of all promoters for genes regulated by the stringent response.

**Membrane Fluidity and Proton Motive Force in Escherichia coli Conjugation.** J. O. Falkingham, III, and R. T. Smith, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

We have investigated the roles of membrane fluidity and proton motive force in recipient cells during conjugal transmission of Ri100-1. These studies involved the use of: (1) an unsaturated fatty acid auxotroph (fabB\(^{-}\)) in which membrane lipid phase (fluidity) can be manipulated by growth supplementation and temperature or, (2) a hemA mutant, unable to synthesize cytochromes, in which proton motive force (pmf) can be changed. Initial studies led to the development of conditions for growth and mating in which fluidity and pmf could be changed without losses in viability. Temperature-induced membrane lipid phase transitions resulted in alteration of the temperature coefficient for conjugation as demonstrated by an Arrhenius plot. The decrease in conjugation proficiency brought about by the non-fluid membrane state was reversible and was not due to decreases in either pair formation or plasmid maintenance. Conjugal transmission of Ri100-1 was dependent upon the presence of a recipient pmf, and failure to form drug-resistant transconjugants was not due to a requirement of pmf for either pair formation or plasmid maintenance. Expression of Ri100-1 encoded tetracycline resistance, though not streptomycin resistance, required a proton motive force. Most probably, the dependence of conjugation upon membrane fluidity and proton motive force involves DNA transfer.

**Physical and Genetic Characterization of R Plasmids Specifying Trimethoprim Resistance.** Mary E. Fling, Leslie M. Walton, Sallie Eure, and Lynn Elwell, Department of Microbiology, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709.

R plasmids encode dihydrofolate reductases which have a much lower affinity for trimethoprim than their chromosomal counterpart, thus conferring upon their hosts trimethoprim resistance. Two enzymatically distinct classes of R-plasmid-specified dihydrofolate reductases (types I and II) have been described. Discrete DNA fragments containing the structural genes for both types of resistant enzymes have been cloned and the resultant gene products analyzed in minicells. Type I dihydrofolate reductase, originating in R483 (and Tn7) was a dimer composed of 18,000 molecular weight subunits. The type II dihydrofolate reductase originating in R67, was a tetramer composed of 8500 molecular weight subunits. These enzymes were antigenically distinct in that anti-type II dihydrofolate reductase (R67) antibody did not cross-react with type I (R483) enzyme. Antiserum to purified E. coli chromosomal enzyme showed no antigenic cross-react with any plasmid-specific dihydrofolate reductase tested. Restriction endonuclease maps of hybrid plasmids harboring the type II enzyme genes originating in plasmids R388, R67, and R751 were compared. According to the mapping analysis these unrelated, naturally occurring R plasmids appeared to have a region (0.8 Mdal) in common which included the type II dihydrofolate reductase structural gene. This result supported the finding that the amino acid sequences of the type II reductases encoded by
plasmids R67 and R388 are about 80% homologous. No obvious sequence homology exists between the type II enzyme subunit and any other characterized bacterial or vertebrate dihydrofolate reductase. The origin(s) of these interesting enzymes is, to date, unknown.


We have localised the replication terminus of the plasmid R6K in a 215 base pair DNA fragment. The replication terminus has been cloned into the vectors pBR 322 and M13 mp5 and the nucleotide sequence has been determined using the chain termination method of Sanger. The DNA of the clone pJG2 which consists of pBR322 with the replication terminus cloned into the Pvu II site has been replicated in cell extracts of plasmidless E. coli. The major conclusions from the in vitro replication experiments are: (a) the replication terminus of R6K is active in vitro and manifests its activity by arresting the progression of the replication fork at the cloned terminus sequence, (b) membrane attachment is not necessary for the activity of the replication terminus and (c) any trans-acting proteins which may be necessary for the termination of replication must come from the host.

Chromosomal Location of Conjugative R Determinants in a Multiply Resistant Strain of Streptococcus pneumoniae. W. R. Guild, S. Hazum, and M. D. Smith, Biochemistry Department, Duke University, Durham, North Carolina 27710.

Linkage to a chromosomal gene and cosedimentation of cat with chromosomal DNA were used to show that cat and tet are in the chromosomes of BM6001, N77, and derivatives (Shoemaker et al., 1979, J. Bacteriol. 139, 432–441). We have used cosedimentation data to draw the same conclusion for a block of four genes (cat tet erm aphA) found in strain BM4200. Sulfonamide resistance in this strain transforms wild type cells with the efficiency of a point marker, is linked to str in the transformants, and is due to a mutation in a gene normally present. Neither it nor resistances to trimethoprim or penicillin transfer by conjugation, but the cat tet erm aphA block transfers as a unit, and the transconjugants behave as donors similar to BM4200. This entire block rarely transfers by transformation, but cat can separate from it and transform wild type cells about 10^-2 as often as does a point marker. When the recipient carries a tet region from BM6001, cat, erm, and aphA transform it at frequencies about 5% of that for point markers. Cotransfer of erm and aphA is high. In lysates of BM4200 each of these transforming activities cosedimented with the total chromosomal DNA, as marked by the sul transformants, both when the lysate contained very large DNA and after it had been sheared to a mean size of 5 to 7 Mdal by passage through a needle. The shear only slightly reduced the level of the transforming activities but shifted their velocity distributions drastically, implying that the genes were carried initially on a very large DNA particle but could transform almost as well from much smaller fragments. Because these results differ strongly from those for plasmid transformation or phage DNA transfection, they are incompatible with the hypothesis that the transformants arose by reestablishing a new replicon in the recipients. An alternative is that they were on a very large plasmid in the donor but transformed by inserting into the normal genome of the recipient. If so, the result is that the determinants are inserted into the chromosomes of the transformants, which also transfer them by either conjugation or transformation with properties not distinguishable from those of the original donor. One has to conclude that inserted R determinants can transfer from one chromosome to another by a process that looks like conjugation. The absence of detectable plasmid DNA is consistent with this conclusion but is not the basis for it.

A New IS Element in Escherichia coli. Brigitte Hauser and Marc Kahn, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

The genes encoding the HhaII restriction endonuclease (r) and modification methylase (m) have been cloned in pBR322 (Mann et al., Gene 3, 97–112, 1978), creating the recombinant plasmid pD110. We have isolated derivatives of pD110 which have temperature-sensitive mutations in the HhaII methylase gene. Cells bearing these plasmids can grow at 32°C, but not at 42°C because the inactivation of the methylase allows the host DNA to be degraded by the restriction endonuclease. Revertants that can grow at 42°C occur at a frequency of about 10^-4; all of 30 colonies examined were r^-m^-.

Nuclei of Thymidine-kinase- (TK-) mouse cells were co-micro-injected with two chimeric pBR322 plasmids, one containing the TK gene of herpes simplex virus (plasmid $X$) and the second containing a human globin gene (plasmid $K$). A resulting TK+ cell, shown also to contain human globin DNA, was grown for 60 generations and its DNA extracted and used in bacterial transformation experiments with selection for tetracycline resistant colonies (that is, for cells containing $K$). A total of 42 tetracycline resistant colonies were isolated, indicating 0.5 extrachromosomal plasmids per mouse nucleus. Characterization of plasmid DNA extracted from each of these colonies revealed that 75% of the plasmids were identical to the originally injected $K$ molecule. The remaining 25% of the plasmid molecules were grouped into five different classes of recombinant molecules, all of which appear to contain a common deletion end-point appearing at a similar region of the pBR322 segment of $K$. These studies establish an alternative prokaryote-eukaryote-prokaryote DNA transfer and recovery system which should be of use in future experiments on the regulation of gene expression in higher eukaryotes.


The 17-MD plasmid pAMB1, first isolated by Clewell et al. from Streptococcus faecalis has recently been transferred by a conjugal mechanism to Streptococcal strains of Lancefield groups A, B, D, F, H, K and N, to Lactobacillus casei, and to Staphylococci. Cell mixtures containing about 10^7 group F Streptococci containing pAMB1, and 10^9 B. subtilis were impinged on membrane filters, incubated on DNase-containing expression media and resuspended and plated 0, 4, 8, and 20 h later on selective media containing DNase. Transfer of resistance and of plasmid DNA was observed in the 20-h samples. The transfer frequency was 10^{-4} to 10^{-5}. Using a similar technique, transfer of plasmid DNA and of antibiotic resistance from a quadruply auxotrophic donor to a triply auxotrophic recipient B. subtilis was obtained with frequencies as high as 10^{-3} per recipient after 20 h and at lower frequencies after 4, 8, and 12 h. Unselected chromosomal donor markers were found in the large majority of antibiotic resistant recombinants. In a small sampling, 35% of erythromycin resistant recombinant recipients had received all three of the unselected donor auxotrophic markers although these markers occupy sites 1/3 of a chromosome length apart. In recombinants cured of antibiotic resistance and of plasmid DNA newly acquired chromosomal markers were retained.

Transduction of a cos Plasmid in Bacillus subtilis. R. MARRERO, F. A. CHIAFARI, AND P. S. LOVETT, University of Maryland Baltimore County, Catonsville, Maryland 21228.

pPL1010 (4.6 Md) was constructed by joining to pUB110 an EcoRI generated fragment of SPO2 DNA which spans the cohesive termini. The plasmid is transduced by SPO2 at a frequency of 10^{-2} transductants per plaque-forming unit and the particles exhibit a lower buoyant density than infectious particles. Ultraviolet irradiation of transducing lysates did not rapidly inactivate the transducing activity when the recipient was recombination proficient. Sedimentation of DNA extracted from transducing particles demonstrated that the predominant species co-sediments with the linear form of lambda DNA. A shoulder of faster sedimenting material was also observed. The data suggest that SPO2 (pPL1010) particles carry pPL1010 predominantly as a linear heptamer whose monomeric subunits are directly repeated. Dye-buoyant density centrifugation of DNA extracted from SPO2 (pPL1010) particles revealed no CCC species.

Characterization of a recombination deficient mutant of Streptococcus sanguis (Challis). I. L. RAINT, F. L. MACRINA, K. R. JONES, AND P. H. WOOD, Department of Biology, University of Chicago, Chicago, Illinois 60637, and Department of Microbiology, Virginia Commonwealth University, Richmond, Virginia 23298.

A mutant of Streptococcus sanguis that is hypersensitive to ultraviolet irradiation was isolated following nitrosoguanidine mutagenesis. Mutant and wild-type cells bind comparable amounts of 3H-chromosomal DNA to thymidine kinase- (TK-) mouse cells. Although the wild-type strain may be genetically transformed with a variety of chromosomal markers (transformation frequencies ~10^{-4} to 10^{-5}/recipient) the mutant fails to yield transformants in such assays (frequency < 10^{-7}/recipient). Both the mutant and the wild-type strain could be transformed with conjugative (pAMβ1, pIP501) and non-conjugative (pVA736) plasmids of streptococcal origin (frequencies of 10^{-2} to 10^{-5}/recipient). Such plasmids were stably maintained in mutant and wild-type cells grown at 37°C. Transfer of conjugative plasmids from the mutant by the filter mating method occurred at frequencies of 10^{-3} to 10^{-4}/input donor cell. In addition, the mutant behaved normally as a conjugative recipient in matings with pIP501 and pAMβ1 donors. Ultraviolet-resistant revertants of the mutant strain have never been detected not have we been able to introduce the lesion for UV sensitivity into a wild-type strain by transformation (>10,000 clones screened). Unlike many recombination deficient strains of other systems, this S. sanguis mutant does not degrade its DNA following ultraviolet irradiation. Preliminary analysis of this recombination deficient mutant revealed that the mutational lesion has occurred in the
DNA segment encoding the information for inducible functions associated with the differentiated cellular state called competence.


Virulent Shigella sonnei strains synthesize a cell surface antigen, termed Form I, which is required for epithelial cell invasion. Upon restreaking on solid agar medium, smooth Form I colonies generate at high frequency rough Form II colonies that have irreversibly lost the Form I antigen and are avirulent. Plasmid DNA comparison showed that a 120-megadalton plasmid was consistently lost during this Form I to II transition. None of 14 antibiotic resistances or 150 metabolic traits could be associated with this Form I plasmid. Following the insertion of transposons Tn3 or Tn5 into the Form I plasmid, conjugal transfer was undetectable. However, mobilization of the Form I::Tn3 plasmid by recombination with F' lac::Tn3 was obtained. Upon receipt of the Form I plasmid, Form II cells reacquired Form I antigen synthesis but were avirulent, apparently due to some property expressed by F' lac. However, a second system utilizing R386 as the mobilizing plasmid allowed both transfer of Form I antigen synthesis and virulence expression. In addition, plasmid transfer to and expression of the Form I antigen in other enterobacterial species suggest that this large plasmid carries the structural genes for the Form I antigen.


We have examined transformation of pneumococcus by pMV158, a 3.6-Mdal plasmid conferring tetracycline resistance that was isolated from a group B streptococcus by V. Burdett. Most of the plasmid transformants arose from forms that sedimented more rapidly than monomer CC in sucrose gradients, even though multimere forms constitute only a minor fraction of the total plasmid DNA. We fractionated pMV158 DNA by preparative gel electrophoresis. The transforming activity in the fastest moving peak (i) co-eluted with monomer CC as detected on analytical gels of the fractions, (ii) banded as CC in EtBr-CsCl buoyancy gradients, (iii) sedimented with the velocity of monomer CC in sucrose gradients, and (iv) gave two-hit kinetic responses as functions of the DNA concentration and the time of exposure of the cells to DNA. These results show that the monomer CC form is active in transforming pneumococcus and that cooperation of two donor molecules is required. Another active form of pMV158 co-eluted with monomer OC on the preparative gel, but the activity was shown to be largely due to dimer CC DNA, because it (i) banded as CC in dye-buoyancy gradients, and (ii) sedimented with the velocity expected for dimer CC. This material gave linear kinetic responses, implying that a single donor particle was sufficient to transform the recipient.

In Vivo Recombination between Plasmids Carrying Virulence Factor and Antibiotic Resistance Genes in E. coli. P. L. Shipley, A. D. Allen, and T. N. Swanson, Medical College of Virginia/Virginia Commonwealth University, Richmond, Virginia 23298.

Enterotoxigenic Escherichia coli (ETEC) strains which cause neonatal diarrhea in pigs often possess the proteinaceous surface antigen, K88, which enables the bacterium to adhere to the mucosal epithelium of the anterior small intestine of the pig. The genetic determinants for K88 antigen production and utilization of the trisaccharide raffinose (Raf) are located on a 52-Mdal nonconjugative plasmid. Mobilization of the K88/Raf plasmid by conjugative plasmids in the ETEC strains frequently involves covariant linkage of the two plasmid genomes. We have examined some parameters of the recombination between a K88/Raf plasmid, pPS900, and pPS030, a conjugative R factor carrying the determinants for resistance to tetracycline and streptomycin. The K88/Raf plasmid was mobilized with equal efficiency from RecA+ and RecA- donor strains. Recombinant plasmids were observed in up to 50% of the Raf+ transconjugants from matings with either RecA+ or RecA- donors. Comparison of the restriction endonuclease fragment patterns of recombinant plasmids isolated from different matings suggests that the regions of the two plasmids involved in recombination are the same in each recombination event. In seven of the eight recombinants examined, recombination has been accompanied by deletion of all or part of an 8.2-Mdal HindIII fragment containing the K88 determinant.


Shoemake et al. (Plasmid 3, 80-87) found that chromosomal insertions of cat and tet genes found in clinical isolates of pneumococcus cotransferred by a DNase-resistant filter mating process. The results excluded transformation, generalized transduction, and cell fusion, and fit the working definition of conjugation. Further experiments have shown that other chromosomal insertions from pneumococcus (cat tet erm aphA), group B streptococcus (cat tet erm), and Streptococcus faecalis (Tn916 tet) also transferred to pneumococcal recipients. No plasmids were found in the donors or in the transconjugants. Pneumococcus strain B381 from South Africa apparently contained
separate cat tet and tet erm insertions, but was unable to transfer either one by conjugation. The presence of introduced plasmids had no influence on the transfer of the chromosomal R determinants. The nonconjugative insertion tet erm was not mobilized by the conjugative plasmid pIP501 or by the simultaneous presence of a conjugative cat tet insertion, and the nonconjugative pMV158 was not mobilized during conjugative transfer of a cat tet insertion. The overall results show that R determinants inserted in the chromosomes are widespread among streptococci, that they occur in different structural arrangements, and that some of these can be transferred both within and between species by a process that fits the operational definition of conjugation.

A Termination Signal for RNA Polymerase I Stops Transcription by RNA Polymerase III. BARBARA SOLLNER-WEBB AND DANIEL BOGENHAGEN, The Johns Hopkins Medical School and Carnegie Institute, Baltimore, Maryland 21218.

In eukaryotes, 5 S RNA is transcribed by RNA polymerase III (RNAP III) while the 40 S precursor to 18 + 28 S rRNA is transcribed by RNAP I. The nucleotide sequence surrounding the 3' ends of the 5 S and 40 S genes of Xenopus laevis share many features, including a T₃ cluster within which transcription terminates (Cell 18, 485, 1979). Are these DNA regions similar enough to be functionally interchangeable? To answer this question we constructed recombinant plasmids containing the transcriptional initiation region of the 5 S gene joined next to the transcriptional termination region of the 40 S gene. Plasmids containing these hybrid 5 S–40 S "genes" were transcribed by RNA polymerase III in vitro (Cell 15, 1077, 1978). RNA synthesis begins at the 5 S initiation site and proceeds toward the 40 S termination region. The lengths of the resultant RNAs demonstrate that transcription by RNAP III is efficiently stopped at the sequence where RNAP I terminates in vivo. The termination does not solely result from rU:dA instability. If a protein is involved in this transcriptional termination, it may similarly recognize the termination signals of the 5 S and the 40 S genes.


A physical map of the B. subtilis ribosomal RNA (rRNA) genes was constructed using P-32 labeled rRNA as a hybridization probe against restriction digests of whole B. subtilis DNA. By probing E. coli clone banks carrying fragments of B. subtilis DNA we recovered cloned fragments derived from individual rRNA operons. These clones were used to correlate cloned DNA with the hybridizing restriction fragments of total B. subtilis DNA. Because the spacer sequences adjacent to rRNA operons consist of unique DNA, each operon can also be genetically mapped after integrating a cloned spacer fragment with its plasmid vector into its homologous site on the chromosome. PBS1-mediated transduction then establishes the locus of the inserted antibiotic resistance determinant. At least one ribosomal RNA cluster maps near GuaA, thus outside the ribosomal cluster adjacent to the bacteriophage SPO2 attachment site.

Transformational Analysis of a Streptococcus lactis Lactose Plasmid. E. J. ST. MARTIN, L. N. LEE, AND D. J. LeBLANC, NIH, NIDR, Bethesda, Maryland 20205.

Streptococcus sanguis (7868) and S. lactis (11454) metabolize lactose by the same catabolic pathway. Lactose enters the cells by a phosphoenolpyruvate-dependent phosphotransferase system (PTS) followed by cleavage of intracellular lactose-phosphate by a phospho-B-galactosidase (P-B-gal). Lactose utilization by S. lactis, unlike S. sanguis, is an unstable phenotype that is associated with the presence of a 32-megadalton plasmid. In order to determine which, if any, of the lactose catabolic enzymes in S. lactis are coded for by this plasmid, we have analyzed the plasmid by heterologous transformation into S. sanguis. Purified lactose plasmid was capable of transforming a P-B-gal-negative mutant of S. sanguis to a lactose-positive phenotype. Molecular sizing of the P-B-gal enzyme in the transformant revealed that it was the same size as the P-B-gal of the S. lactis donor strain. Isolated plasmid was also capable of transforming a double mutant of S. sanguis that was missing both lactose PTS and P-B-gal activity. Analysis of these transformants revealed that they had acquired a lactose PTS activity with the same substrate specificity as the S. lactis donor strain. Thus, the 32-megadalton plasmid present in S. lactis codes for the synthesis of both lactose PTS and P-B-gal activity.

Genetic and Physical Characterization of Plasmids Encoding Colonization Antigens of E. coli. T. N. SWANSON, A. ALLEN, AND P. L. SHIPLEY, Virginia Commonwealth University, Richmond, Virginia 23298.

The production of acute diarrheal disease by enterotoxigenic E. coli depends upon the simultaneous elaboration of enterotoxin and colonization factor antigens which mediate specific adherence to gut epithelial cells. Both of these virulence determinants are known to reside on bacterial plasmids. The plasmid encoding the human colonization factor CFA/1 is a 60-megadalton nonself-transmissible plasmid which bears no selectable markers. Colonization factor plasmids from clinically isolated enterotoxigenic strains were isolated by in vivo genetic engineering. The ampicillin transposon Tn3 was introduced into these strains on a plasmid temperature sensitive for replication. After heat-curing of these strains, the Tn3 element was observed to transpose frequently to the CFA plasmid. Insertion of Tn3 appeared
to be random and precise. Tn3 labelled plasmids were then subjected to limit digestion with restriction endonucleases followed by ligation to give a family of deletion mutants affecting various parts of the CFA plasmid; 9 of 14 possible HindIII fragments are not solely due to this phenomenon. Strains which are CFA+ as a result of deletion fall into at least three different classes. From this information it should be possible to identify the set of restriction fragments which contain CFA gene sequences; this will facilitate the isolation of this determinant by recombination in vitro, as well as fine structure genetic mapping.

Plasmids from Streptosporangium and their Prospective Use as Cloning Vehicles. D. P. Taylor, L. R. Pare, and D. Rucigay, Smith Kline & French Laboratories, P. O. Box 7929, Philadelphia, Pennsylvania 19101.

Our objective is to use the recombinant DNA approach to enhance the scope of antibiotic production by actinomycetes and otherwise manipulate the diverse metabolic capabilities of these organisms, particularly the genus *Streptosporangium*. Cryptic plasmids were isolated and will serve as the basis for construction of a cloning vehicle. One plasmid, pSgB-1, was isolated from *S. brasiliense* and shown to have a molecular weight of $9 \times 10^6$ by gel electrophoresis and electron microscopy. Mapping of pSgB-1 revealed unique sites for EcoRI, ClaI, MstII, XbaI, and additional sites for BamHI (5), BglII (3), SacI (2), BclI (3), and other endonucleases. Cleavage by BclI and XbaI indicated that the DNA was not methylated. Fragments of pSgB-1 generated with EcoRI and BamHI were cloned in *E. coli* using pBR322. Numerous changes in the fragments were observed in the cloned fragments indicating that nuclease contamination may be a problem when working with DNA from these microorganisms. Markers such as thioestrepton resistance from *Streptomyces* have been added to pSgB-1 clones in *E. coli*. The chimeric plasmids will be reintroduced in *Streptosporangium* by PEG-mediated transformation of protoplasts to determine whether they can function as vectors.

Helix-Destabilizing Activity Associated with Mitochondrial DNA. G. C. Van Tuyle, P. A. Pacco, and J. Wetstein, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

Our studies of rat liver mitochondrial DNA (mtDNA) packaging have led to the discovery of a nucleoprotein complex containing nascent DNA strands that are protected from parental branch migration by bound proteins. Mitochondria were labeled with $^3$H-TdR in *vitro* such that the label was incorporated into nascent strands only. The labeled nucleoprotein complex was released from the organelles by SDS-lysis and isolated by sedimentation velocity in sucrose density gradients. The material in the 39 S peak was then subjected to either density banding in metrizamide gradients formed *in situ*, or by hydroxylapatite column chromatography. The complexes emerging from these two different procedures appeared identical in composition and exhibited a large DNA to protein ratio of at least 10 to 1. HindIII cleavage of the complexes produced in agarose gels an extra diffuse band that contained the D-loop and which migrated slightly more slowly than the largest 6.03-kb fragment (I) upon which the D-loop is known to form. Furthermore, fragment I exhibited a reduced relative stain intensity. On the basis of electron micrographs, the DNA in the extra band and in fragment I appeared to be identical in length. If, however, the complex was deproteinized by SDS/phenol extraction either before, or after, HindIII digestion, the extra band was converted to fragment I. Analysis of HindIII fragments on sucrose density gradients showed that the deproteinized DNA lost the labeled nascent strands by branch migration upon strand scission. This process did not occur, however, in the case of the non-deproteinized complex.

Analysis of the proteins associated with the DNA in the complex revealed a single polypeptide ($M_r = 16,000$) on SDS–polyacrylamide gels. In a two-dimensional gel system this polypeptide exhibited two charge-altered species with pf's of approximately 7.5 and 7.7. In the absence of a thiol reagent, these species appeared to be able to form dimers and, to a lesser extent, high oligomers.


The K, killer plasmid, [KIL-k,], of *Saccharomyces cerevisiae* is a 1.25 x 10^6-dalton linear double-stranded RNA plasmid coding for a protein toxin and immunity to that toxin. The [KIL-sd,] plasmid is a replication-defective mutant of [KIL-k,] that has become dependent on one of the recessive chromosomal superkiller (ski-) mutations for its maintenance (A. Toh-e and R. B. Wickner, 1979, *Genetics* 91, 673–682). The [KIL-sd,] mutant defines a killer plasmid site or product essential for its own replication. This report concerns an alternate means by which [KIL-sd,] can be stably maintained in a *Ski*+ host. Strains carrying a plasmid we call [HOK] (helper of killer) can stably maintain [KIL-sd,]. [HOK] segregates 4 [HOK]:0 in meiotic crosses and can be transferred by cytoplasmic mixing (heterokaryon formation). Maintenance of [HOK] depends on the products of *PET18, MAK3*, and *MAK10*, three chromosomal genes needed to maintain [KIL-k,], but is independent of 10 other MAK genes and of *MKT1*. [HOK] is not mitochondrial DNA and is unaffected by agents which convert *ψ*+ strains to *ψ*-. [HOK] is also distinct from the previously described plasmids [URE3], 20 S RNA, 2 μ DNA, L ds RNA, [EXL], and [NEX]. [HOK] is found in about 60% of the nonkiller
strains in our collection. We have been unable to detect a ds RNA species whose presence or absence is correlated with the presence or absence of [HOK].

Restriction Analysis of Hybrid Plasmids Containing Bacillus subtilis rRNA and tRNA Sequences. P. A. Zuber, University of Virginia, Charlottesville, Virginia 22903.

Cloned segments of Bacillus subtilis tDNA were examined by restriction endonuclease cleavage and by RNA–DNA hybridization. Two pBR313 plasmids, p14B1 and p14B8, contained BamH₁ fragment inserts of 5.3 and 4.8 Kb, respectively. Restriction analysis demonstrated that the p14B1 contained a region in the center of the insert which was missing in the p14B8 insert. RNA–DNA hybridization using in vivo ³²P-labeled 23s, 16s, 5s, and 4s RNA and Smal/HindIII fragments of the two plasmids suggest that each of the BamH₁ inserts contain parts of two rRNA operons separated by heterologous spacer regions. The proposed arrangement of the rRNA coding sequence in p14B1 and p14B8 is: (BamH₁)–23s–5s–sp–16s–23s–(BamH₁). In the case of p14B1 the spacer contains a sequence complementary to 4s RNA. These experiments lend support to previous studies which indicate that (i) rRNA operons are arranged in the order 16s–23s–5s, (ii) tRNA spacer sequences between 16s and 23s rRNA gene found in E. coli are not present in rRNA operons of B. subtilis, (iii) rRNA operons are clustered in a tandem arrangement, (iv) some tRNA sequences are closely linked to rRNA genes.


Streptococcus faecalis strain JH2-2 was used as a recipient for conjugation with two β-hemolytic (horse blood), tetracycline (Tc)-resistant isolates of S. faecalis, strains DL4 and DS5. Donors, recipient, and transconjugants were examined for plasmid content, expression (constitutive or inducible) and levels of Tc resistance, and hemolytic activity. Recipient strain JH2-2 contained no detectable plasmids, was sensitive to less than 2 μg/ml of Tc, and was nonhemolytic. Strain DL4 harbored two plasmids, a 50-Mdalton molecule associated with resistance to Tc, kanamycin, streptomycin, and MLS antibiotics, and a 35-Mdalton molecule associated with β-hemolysis. This strain was inducible for resistance to Tc at 512 μg/ml, and hemolytic in the presence or absence of Tc. A Tc-resistant DL4 × JH2-2 transconjugant had the same Tc resistance traits as the donor strain, but was sensitive to the other antibiotics, possessed a single 43-Mdalton plasmid, and was hemolytic only in the presence of Tc. Donor strain DS5 (Clewell et al., 1974, J. Bacteriol. 117, 283) contained plasmids of 6 (Tc resistance), 17 (MLS resistance), and 35 (hemolysis) Mdalton, expressed constitutive resistance to Tc at 512 μg/ml, and was hemolytic. A Tc-resistant DS5 × JH2-2 transconjugant harbored two plasmids of 6 and 39 Mdalton, was constitutively resistant to 32 μg/ml of Tc, and nonhemolytic in the presence or absence of Tc. These results suggest that Tc resistance may be either inducible or constitutive among strains of S. faecalis, and that the expression of plasmid-mediated hemolysis can be affected by Tc resistance determinants, possibly as a result of the insertion of such determinants into the hemolysin plasmids.