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Mechanisms of Recombination and Function of DNA in Bacteria

PROGRESS REPORT

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Progress in the Current Period

Major effort in this period has gone to (i) analysis of the process of plasmid transformation in pneumococcus, (ii) continued study of resistance determinants inserted in the chromosomes of clinical strains of pneumococcus and their transfer by conjugation, a novel phenomenon we discovered last year, and (iii) temperate phages of pneumococcus. Six papers have been published or are in press on the first two topics, as listed below. They constitute the bulk of this report. Two more papers are in active preparation and a third will be shortly.

In addition, papers were presented by Michael Smith and by Charles Saunders, both students, at the 24th Annual Transformation Meeting (now called the Wind River Conference on Genetic Exchange), in June, and the PI will present two papers at the 5th European Meeting on Bacterial Transformation and Transfection in Florence, Italy, in September. The PI also gave an invited talk entitled "Transfer of Resistance in Streptococcus pneumoniae" at the annual meeting of the American Society for Microbiology in Miami in May, 1980, and presented the conjugation work at the Gordon Conference on Extrachromosomal Elements in New Hampshire in June.

A brief outline of the major results is as follows.

Plasmid transformation in pneumococcus. We have found that plasmids as such play no role in the drug resistance now being found in some clinical isolates of pneumococcus, and only one naturally occurring pneumococcal plasmid has been found (Smith and Guild, 1979). However, resistance plasmids can be introduced from other streptococci (Smith et al, 1980) and used to examine the processes by which they transfer by either conjugation or transformation. The latter is analogous to transfection by phage DNA, in that the entering DNA must establish a new replicon in the recipient cell. It provides a further tool for examining the mechanisms of this process, and we have asked whether the results force one to postulate new or unexpected features of the DNA processing. This is particularly relevant to an apparent conflict between our work and results and/or interpretations of experiments in B. subtilis. In this system it is thought that processing of transforming DNA is like that in pneumococcus (both species are gram-positive and naturally competent), in that it involves breakage and entry of single strand segments of donor DNA. In B. subtilis, however, Trautner and his colleagues (a) have interpreted transfection results in terms of a double strand entry process, and (b) find no transforming activity associated with monomer plasmid DNA forms.

Two papers in press by C.W. Saunders and the P.I. show (a) that plasmid transformation in pneumococcus appears to use the same single strand entry pathway as does chromosome transformation; (b) that monomer covalently closed (CC) plasmid DNA transforms readily and with two-hit kinetics that imply cooperation between two single strands that enter separately; and (c) that dimer CC forms have much higher activity and transform with single-hit kinetics. A paper in preparation will describe experiments showing rigorously that non-CC forms, both open circle (OC) and linear, also have detectable transforming activity, though the monomer OC and linear forms are much less active than monomer CC. Dimer OC and dimer linear forms are again much more active than the corresponding monomers and give single-hit kinetics.

The results suggest the possibility that trimer and higher forms may be present and contribute a small part of the total activity. Because the dimer and higher forms represent only a trace of the total DNA present and are detectable only by their transforming activity after various physical fractionation steps (the experiments involve sequential separations by electrophoresis and sedimentation, coupled with dye-buoyancy and kinetic studies), it is not now possible to assign quantitative values to the relative activities. Further, because the monomer forms show second order concentration dependence, the proportions of the total activity contributed by the various forms depends on the concentration used for assay, as well as on the previous treatment of the preparation.

The results are consistent with a simple model for the entry of single strand segments of plasmid DNA followed by their association into partial duplexes that can regenerate intact replicons. Fig. 6 of the paper entitled "Monomer plasmid DNA transforms S. pneumoniae" shows this model, and the further data on non-CC forms reinforce our view that this is the correct interpretation. It is essentially identical to the model for transfection by phage DNA in Porter and Guild (1978). Whether the apparent lack of transforming activity by monomer plasmid DNA in B. subtilis implies a qualitatively different pathway in that species or reflects only a quantitative difference in some aspect of the assay system remains to be seen.

These studies show that plasmid transformation in pneumococcus differs greatly in pathway from that in artificially competent systems such as E. coli. They provide a solid basis for further studies involving plasmids as model systems or as cloning vectors in streptococci.

Conjugation and drug resistance in pneumococcus. In work described briefly last year we found that a number of R-determinants in clinical isolates of pneumococcus represented insertions of foreign DNA into the chromosome. Further, these transfer by a conjugation-like process to other cells independently of the presence of any plasmid. This is the first proven case of conjugative transfer of chromosomal elements in gram-positive eubacteria and appears to represent a novel system of transfer that has aroused considerable interest. Apparently similar examples are being found by workers at the Pasteur Institute and by Clewell et al at Michigan, and we are in close contact with these groups. One paper has been published (Shoemaker et al, 1980), a second is in press (Smith and Guild, 1980) and two more are in preparation on recent work.

The published papers deal with strains carrying only chloramphenicol (Cm) and tetracycline (Tc) resistances. The new work examines other strains carrying multiple resistance. For example, a Paris strain, BM4200, has a block of four linked R-determinants - Cm, Tc, erythromycin (Em), and kanamycin (Km) - that transfer en bloc by conjugation, plus several other resistances due to point mutations in genes normally present. We have several lines of strong direct evidence that all these genes are in the chromosome rather than in plasmids. Another strain of interest is B381, from South Africa. It carries some point mutations along with two inserted blocks of R-determinants, neither of which transfers by conjugation and both of which include a Tc determinant.

In addition to intraspecies conjugation, we have also found several examples of interspecies conjugal transfer of chromosomal determinants among streptococci. A CmTcEm block in the chromosome of a group B streptococcus, isolated by Horodniceanu in Paris, transfers to pneumococcus at relatively high frequency (near 10^{-4} per donor) and from the pneumococcal transconjugant to S. sanguis. Also a Tc transposon present in the chromosomes of several S. faecalis strains characterized by Clewell et al. transfers by conjugation to our pneumococcus recipients.

We are analyzing these phenomena in clinical strains and numerous lab derivatives by a variety of procedures, with two broad objectives at this stage, to describe the sorts of phenomena occurring and to find out the molecular details of how the genes are organized and how they transfer.

Phage studies. The properties of various temperate phages present in some clinical strains of pneumococcus are being examined with the objective of developing one or more systems that should be useful as tools for examining restriction systems and as targets for transposition of some of the R-determinants. These should allow us to ask rigorously whether or not any of the determinants are present as parts of transposons.

Foreign Travel. No foreign travel was performed in this period, but the P.I. plans to go to Italy in early September to attend the 5th European Transformation Meeting and present some of this work. Most expenses will be paid from an NIH grant.

Compliance. The work performed is in close compliance with that proposed in prior applications, and the P.I. has devoted a substantial fraction of his effort (more than 20%) to this work.

Personnel. Shulamith Hazum began work Octoberl, 1979, in effect replacing Nadja Shoemaker, who resigned to take a research position with John W. Drake at the National Institute for Environmental Health Sciences. Michael A. Bleyman, Ph.D., started work November 1, 1979, supported by the NIH grant to work on the bacteriophages. Scott Priebe is a new graduate student in the laboratory, starting in May, 1980. C. W. Saunders and M. D. Smith continue as graduate students and each expects to finish their Ph.D. work in the next year. All three students are now supported as trainees in genetics under a National Research Service Award, 1 T32 GM07754, from the NIH. Versie L. Lee continues as Research Technician, providing valuable support work with preparation of competent cells, making of DNA preparations, and maintaining supplies of media, glassware, etc. We have provided partial support for Gerda Michalsky, who operates the departmental electron microscope laboratory.

Publications.

Smith, M.D., N.B. Shoemaker, and W.R. Guild (1980). Chromosomal location of plasmid-type resistance genes in Streptococcus pneumoniae. in Stuttard and Rozee (Eds.), "Plasmids and Transposons". Academic Press N.Y. (pp 125-128). [DOE/EV/03941-41]

Smith, M.D., N.B. Shoemaker, V. Burdett, and W.R. Guild (1980). Transfer of Plasmids by Conjugation in Streptococcus pneumoniae. Plasmid 3. 70-79. [DOE/EV/03941-42]

Shoemaker, N.B., M.D. Smith, and W.R. Guild (1980). DNase-resistant transfer of chromosomal cat and tet insertions by filter mating in pneumococcus. Plasmid 3, 80-87. [DOE/EV/03941-43]

Saunders, C.W., and W.R. Guild (1980). Properties and transforming activities of two plasmids in Streptococcus pneumoniae. Mol. Gen. Genetics (in press). [DOE/EV/03941-45]

Saunders, C.W. and W.R. Guild (1980). Monomer plasmid DNA transforms Streptococcus pneumoniae. Mol. Gen. Genetics (in press). [DOE/EV/03941-46]

Smith, M.D., AND W.R. Guild (1980). Improved Method for Conjugative Transfer of Chromosomal Genes and Plasmids by Filter Mating in Streptococcus pneumoniae. J. Bacteriol. (in press). [DOE/EV/03941-51]

As noted above, 4 abstracts have been submitted on the above material for papers presented or to be presented at meetings. These have been numbered DOE/EV/03941-47 thru -50. Copies of these Abstracts and of each of the above papers have been submitted previously and should be considered as attachments to this report. They document in some detail the matters described briefly in the text of this report.