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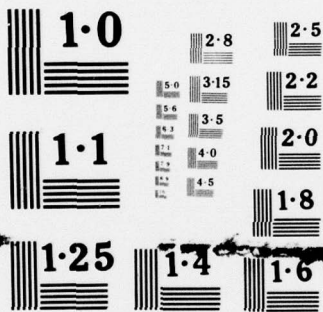
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Annual Progress Report No. 3

Genetics of Novel Hybrid Bacteriophage and Development of Generalized Transducing System for Salmonella typhosa

Annual Progress Report
(From 1/1/76 to 12/31/76)

Nobuto Yamamoto, Ph.D.

December 16, 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Various hybrid phages between coliphage and <i>Salmonella</i> phage were isolated using bacterial hybrids between <i>E. coli</i> and <i>Salmonella typhimurium</i> . These hybrid phage species were genetically mapped for homology with their parental phages. The hybrid phages $\phi 80$ -P22 and Mu-P22 carry the P22 early genes (at least <i>c</i> genes) and conserve the protein coats of $\phi 80$ and Mu-1 phage respectively. Moreover, another hybrid class P22- $\phi 80$ carries the protein coat of P22 and has			

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at least the c region of $\phi 80$. Preliminary experiments suggest that the hybrid phage P22- $\phi 80$ is a generalized transducing phage. These hybrid phages provide valuable tools with their intergeneric transduction for investigating the genetic character of host bacteria and the pathological role of their somatic antigens.

$\phi 80$

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Summary

By employing an approach similar to that previously used to isolate λ -P22 hybrid class, hybrids between Salmonella phage P22 and coli-phage $\phi 80$ have been identified and characterized. The $\phi 80$ -P22 carries the c (c1, c2 and c3) genes and the DNA synthesis genes (gene 18 and 12) of P22. However, $\phi 80$ -P22 seems to carry $\phi 80$ att gene. Similarly a hybrid phage (designated Mu-P22) between P22 and a mutator coliphage Mu-1 was isolated. The Mu-P22 carries the P22 genetic segment containing the entire regions att, int, Xis and c genes.

In addition we have isolated a new hybrid species between $\phi 80$ and P22. This hybrid species, designated P22- $\phi 80$, conserves the protein coats of P22 and at least the c genes of $\phi 80$. Preliminary studies suggested that P22- $\phi 80$ is a generalized transducing phage.

Foreword

Fundamental studies of viral genetics not only play an important role in increasing our knowledge of the action of viruses in disease processes, but have contributed greatly to our knowledge of the whole problem of cell replication, genetic transfer, gene control, morphogenesis, and antigen conversion. The significance of the study of bacterial hybrids between E. coli and Salmonella has greatly broadened with the recent discoveries of hybrid phage between coliphage and Salmonella phage. The study supported by this contract will bring many important answers for mechanisms of genetic evolution, transduction, recombination, gene expression, antigen conversion and viral replication. In addition, such newly constructed hybrids may prove useful in achieving intergeneric transduction via a hybrid phage vector, of chromosomal genes from different genera of enterobacteriace. Therefore such hybrid phages may serve as useful vectors in the genetic engineering of a polyvalent oral attenuated vaccine which expresses immunogenic determinants for antigens of

Shigella, Salmonella and perhaps even cholera.

Progress

Present Status of This Project

We have previously reported the isolation of an unusual Salmonella typhimurium hybrid sensitive to coliphage λ and Salmonella phage P22 (Gemski, Baron and Yamamoto, PNAS 69, 3110, 1972). This hybrid, constructed by mating an Escherichia coli K-12 Hfr donor with an S. typhimurium recipient, was characterized as an excellent host for achieving genetic recombination between λ and P22. Two broad hybrid phage classes, each with representative types differing presumably in the extent of gene exchange, have been isolated and described in our previous reports. The λ -P22 hybrid class, which has the protein coat of λ , was found to contain at least the c region of P22. The other class, termed P22- λ , has the protein coat of phage P22, and has inherited at least the c marker of .

By employing an approach similar to that previously used to map homologous chromosomal regions of P22 and P221 (Virology 28, 168, 1966), we have studied representatives of the λ -P22 class and determined the extent of their genetic recombination. λ -P22 type 1 hybrids have replaced the int through Q chromosomal segment of λ with functionally related P22 genes, this region representing approximately 25% of the λ genome. In λ -P22 type 2 hybrids, however, a shorter segment containing int through P of λ have been replaced by P22 genes. Similarly, we have studied representatives of the P22- λ class and determined the extent of their genetic recombination. Representatives of the P22- λ phage class, selected for inheritance of the c region of λ during recombination between genetically marked λ and P22 derivatives have been characterized by genetic procedures. P22- λ type 1 hybrids have replaced the c through gene 12 chromosomal segment of P22 with functionally related λ genes carrying the c through P genes. P22- λ type 2 hybrid, however, have replaced the c gene segment of P22 with the corresponding λ genes containing the cI, cII, cIII and N genes.

We have also isolated hybrids between Salmonella phage P22 and coliphage $\phi 80$ or Mu-1. These hybrid phages provide excellent models for studying a mechanism of genetic evolution, control of gene expression within gene clusters derived from diverse phages, phage morphogenesis, chromosome structure and nature of transduction. These hybrid phages may be used for intergeneric transduction of chromosomal genes from different genera of the enterobacteriace. Consequently, a new system for investigating, from a genetic point of view, the pathogenesis of distinct enteric infections (for example, salmonellosis vs colibacillosis) is now feasible. Such hybrid phages, besides being transductional vectors of chromosomal genes, could also achieve antigenic conversion of various Salmonella determinants on an intergeneric level.

1. Genetic Homology between ϕ 80-P22 and P22.

As described in the 1975 annual report, we found a hybrid species, ϕ 80-P22, between ϕ 80 and P22. ϕ 80-P22 carries the protein coat of ϕ 80 and at least the c region of P22. By employing an approach similar to that previously used to map homologous regions between λ -P22 and P22. We have determined the extent of genetic homology between ϕ 80-P22 and P22. The ϕ 80-P22 carries the c (c1, c2 and c3) genes and the DNA synthesis genes (gene 18 and 12) of P22. Experiments to determine the ϕ 80-P22 prophage attachment site suggest that the ϕ 80-P22 prophage is located near the tryptophan gene.

2. Isolation of a New Hybrid Phage Species P22- ϕ 80 between Coliphage ϕ 80 and Salmonella Phage P22.

Using a technique similar to that previously used to isolate P22- λ hybrid class, we recently isolated a new hybrid species between ϕ 80 and P22. This hybrid species, designated P22- ϕ 80 hereafter, conserves the protein coats of P22 and at least the c genes of ϕ 80. Thus P22- ϕ 80 is a recombinant reciprocal to the hybrid ϕ 80-P22. Since ϕ 80 is a specialized transducing phage and P22 is a generalized transducing phage, we are currently characterizing the transducing nature of P22- ϕ 80 hybrid. Preliminary experiments suggested that P22- ϕ 80 carries a generalized transducing capacity.

3. Preparation of *E. coli* - *S. typhimurium* Hybrid Bacteria Lysogenic for *E. coli* Phages P2 and Mu-1.

E. coli - *S. typhimurium* hybrid strains WR4028 and WR4027 are insensitive to coliphages P2 and Mu-1. Therefore we prepared their lysogenic strains by transferring the prophage along with neighboring genes in *E. coli* through bacterial mating between WR4028 or WR4027 and *E. coli* Hfr strains lysogenic for P2 or Mu-1. These newly established lysogens of *E. coli* - *S. typhimurium* provide excellent host systems for isolation and understanding formation mechanism of various hybrid phages between P22 and P2 or Mu-1.

4. Finding of Phage Hybrids between Salmonella Phage P22 and a Mutator Coliphage Mu-1.

When some 10^{11} P22 particles grown on WR4028 lysogenic for Mu-1 were plated on WR4027 lysogenic for Mu-1, about 10 faint plaques were observed. Since WR4027 (Mu-1) strain is a P22 resistant rough strain lysogenic for Mu-1 we suggested that these plaque-formers were hybrid phage between P22 and Mu-1. A number of suspected hybrid phages were cloned and tested to determine whether they are antigenically identical to coliphage Mu-1. We found that these clones conserve the protein coat of Mu-1 phage and carries at least the c genes of P22. Thus, these hybrid clones were henceforth designated as Mu-P22.

5. Determination of Chromosome Insertion Site of Mu-P22 in E. coli.

The mutator phage Mu-1 has no specific prophage insertion site in E. coli chromosome and will enter anywhere in the chromosome, resulting in various mutations. This may be an explanation for the mutator capacity of Mu-1. Tumor virologists believe this type of mechanism for viral oncogenesis, simply because they could not find specific insertion sites in mammalian cell chromosomes. We will locate the prophage attachment site of Mu-P22. Preliminary experiments suggest that the Mu-P22 prophage is located at the P22 attachment site near the pro gene. This observation might indicate that Mu-P22 lacks the mutator function. In addition, in the lysate of a M-P22c+ lysogen after superinfection with P22c2, P22c+ recombinants were found at a high frequency of about 1%. These observations suggest that Mu-P22 phage carries the att, int and xis genes of P22 because these P22 genes are situated between the att and c genes in the P22 genome.

Publications

Yamamoto, N., Gemski, P., Jr., Wohlhieter, J.A. and Baron, L.S. Genetic characteristics and structure of phage hybrid λ -P22 derived from recombination between coliphage λ and Salmonella phage P22. Submitted to Virology.

Yamamoto, N. A generalized transducing Salmonella phage ES18 recombines with a serologically unrelated phage Fels 1. Submitted to Journal of General Virology.

Yamamoto, N., Ushijima, N., Gemski, P. and Baron, L.S. Genetic map of P22-hybrids. Manuscript in preparation. To be submitted to the Journal of Molecular Genetics.

Baron, L.S., Gemski, P. and Yamamoto, N. Sensitivity of Salmonella hybrids to phage recombinants between λ and P22. Manuscript in preparation. To be submitted to the Journal of Molecular Genetics

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