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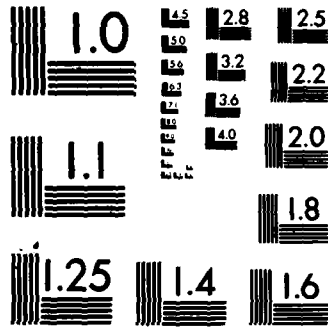
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ANNUAL PROGRESS REPORT

Genetics of Novel Hybrid Bacteriophage and Development  
of a Mutator Phage System for Salmonella

Annual Progress Report

Nobuto Yamamoto, Ph.D.

January, 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,  
Fort Detrick, Frederick, Maryland 21701

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) ✓ <u>E. coli-S. typhimurium</u> hybrid provides excellent systems to isolate bacteriophage hybrids between <u>Salmonella</u> phage P22 and <u>E. coli</u> phages such as $\lambda$ , $\phi$ 80 and Mu-1. A variety of hybrid types such as $\lambda$ -P22imm $\lambda$ c, $\phi$ 80immP22, P22imm $\phi$ 80, MuimmP22 and P22immMu have been isolated and characterized. Studies of these hybrid phages provide invaluable information for the genetic evolution, mutation, transduction and immunity controls of bacteriophages.		

20. ABSTRACT (Continued)

LAMBDA

The  $\lambda$ -P22Im $\lambda$ c hybrid carries the Im region of P22 but retains the c region of  $\lambda$ . This hybrid type is able to replicate in  $\lambda$  lysogens, although the hybrid carries the  $\lambda$ c region, suggesting that one of the Im gene functions (i.e., antirepressor) neutralizes the  $\lambda$ c repressor.

The  $\phi$ 80immP22dis hybrid carries a large P22 segment containing the Im-att-erf-c-h21 genes. Some phages spontaneously induced from their lysogens have lost the Im function (i.e., dis- mutation). This is due to the replacement of a bacterial segment for the phage segment containing the att through Im genes of P22. Such mutants are high transducing phages for proline but not for tryptophan genes.

In crosses between P22 and Mu-1 phages two hybrid classes, P22immMu and MuimmP22, were isolated. Hybrid P22immMu class carries at least the c region of Mu-1 phage and retains the protein coat of P22, whereas MuimmP22 hybrid class carries at least the c region of P22 but conserves the protein coat of Mu-1 phage. The latter hybrid class efficiently lysogenizes the hosts but has lost the mutator activity of Mu-1 phage.

In addition, a new and rapid isolation procedure for P22imm $\lambda$  hybrid is established using WR4028 lysogenic for P22Sie as an indicator host. These new P22imm $\lambda$  isolates provide a system to study the role of the Im gene in the P22 immunity.

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## SUMMARY

The  $\lambda$ -P22 $\underline{Imm}\lambda_c$  hybrid carries the  $\underline{Imm}$  region of P22 but retains the  $\underline{c}$  region of  $\lambda$ . This hybrid type is able to replicate in  $\lambda$  lysogens though it carries the  $\lambda_c$  region, implying that one of the P22 $\underline{Imm}$  gene products (i.e., antirepressor) inactivates the  $\lambda_c$  repressor.

In crosses between P22 and  $\phi 80$  phages we have isolated two hybrid phage classes, P22 $\underline{imm}\phi 80$  and  $\phi 80\underline{imm}$ P22. The P22 $\underline{imm}\phi 80$  hybrid class carries at least the  $\underline{c}$  region of  $\phi 80$  and retains the protein coat of P22, whereas  $\phi 80\underline{imm}$ P22 carries a large P22 segment containing at least the  $\underline{att-erf-c-h21}$  genes and the protein coat of  $\phi 80$ . One type of  $\phi 80\underline{imm}$ P22 class,  $\phi 80\underline{imm}$ P22 $\underline{dis}$  type, carries the  $\underline{Imm-att-erf-c-h21}$  segment of P22. Some phages released from their lysogens have lost the  $\underline{Imm}$  function (i.e.,  $\underline{dis}^-$  mutation). This is due to the replacement of a bacterial segment for the phage segment containing the  $\underline{att}$  through  $\underline{Imm}$  genes of P22. Such mutants were found to be high transducing phages for proline but not for tryptophan regions.

In similar crosses between P22 and Mu-1 phages, two hybrid classes, P22 $\underline{imm}$ Mu and Mu $\underline{imm}$ P22, have been isolated. The P22 $\underline{imm}$ Mu hybrid type carries at least the  $\underline{c}$  region of Mu-1 and the protein coat of P22, whereas the Mu $\underline{imm}$ P22 hybrid class carries at least the  $\underline{c}$  region of P22 and retains the protein coat of Mu-1 phage. The latter hybrid class efficiently lysogenizes the hosts but has lost the mutator function.

Recently, a rapid isolation procedure for P22 $\underline{imm}\lambda$  hybrid was established using WR4028 lysogenic for P22 $\underline{Sie}$  as an indicator host. These new P22 $\underline{imm}\lambda$  isolates provide a system to study the role of the  $\underline{Imm}$  gene in the P22 immunity.

## FOREWORD

Fundamental studies of bacterial and viral genetics not only play an important role in increasing our knowledge of the action of viruses in disease processes, but also contribute 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 phages 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, morphogenesis 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 interobacteriace. 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.

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## PROGRESS

1. Studies of an Unusual  $\lambda$ -P22 Phage Hybrid with the  $\lambda c^+$  Region and the  $I_m$  Region of P22, Designated as  $\lambda$ -P22 $I_m\lambda c$ .

Hybrid phages of the  $\lambda$ -P22 class have the  $\lambda$  protein coat and contain at least the  $c$  region of P22. Such hybrids are isolated from P22 lysates, previously grown on a  $\lambda$  lysogen of a smooth Escherichia coli-Salmonella typhimurium hybrid, WR4028( $\lambda$ ). In phage crosses between the clear plaque mutant P22 $c_2$  and wild-type  $\lambda c^+$ , a new hybrid  $\lambda$ -P22 type which forms turbid ( $c^+$ ) plaques on  $\lambda c^+$  lysogens of WR4027 was isolated. P22- $\lambda c^+$  hybrids, composed of the P22 coat with at least the  $c$  region of  $\lambda$ , are able to form plaques on the smooth host WR4028( $\lambda$ ) because they also have the second immunity ( $I_m$ ) region of P22. Thus, any  $\lambda$ -P22 hybrid with the  $\lambda c^+$  region must also contain the  $I_m$  region of P22 to be able to form plaques on  $\lambda$  lysogens. Lysogens of WR4028 carrying the new hybrid phage  $\lambda$ -P22 $I_m\lambda c$  are immune to both  $\lambda$  and P22. This new phage hybrid contains the erf through  $I_m$  region of P22 and inserts at the P22 att site near the pro region of the bacterial chromosome. In addition, it confers the ability to produce Salmonella 0-1 somatic antigen on appropriate host bacteria. During its replication, the hybrid phage also produces free P22 tails.

Spontaneous induction of  $\lambda$ -P22 $I_m\lambda c$  lysogens occasionally yields phage mutants unable to grow in  $\lambda$  lysogens. Such mutants acquired a bacterial segment substituting for the att-a1-9-I<sub>m</sub> segment of P22 in the  $\lambda$ -P22 $I_m\lambda c$  hybrid.

2. Isolation and Characterization of Hybrid Phages Between E. coli Phage  $\phi 80$  and Salmonella Phage P22

a. Isolation of  $\phi 80$ -P22 Hybrid Phage

E. coli-S. typhimurium hybrid strain WR4027 is a rough bacterium and sensitive to coliphage  $\phi 80$  for its replication but insensitive to P22 phage because of lack of P22 phage adsorption. Therefore, WR4027 lysogenic for phage  $\phi 80$ , WR4027( $\phi 80$ ), is insensitive to P22 phage. By infecting WR4027( $\phi 80$ )

with a mixture of high titer stocks of rough specific Salmonella phages (designated R phages), we were able to isolate R-phage resistant derivatives of WR4027( $\phi$ 80), designated WR4027( $\phi$ 80)/R, which are smooth and fully sensitive to P22 phage. Phage P22 stocks grown on this smooth derivative of the  $\phi$ 80 lysogen give rise to recombinants between P22 and  $\phi$ 80. Such recombinants were recovered by plating on a P22-resistant host which is immune to  $\phi$ 80, namely WR4027( $\phi$ 80). They retain the protein coat of  $\phi$ 80 but have acquired the c region of P22. In addition, these  $\phi$ 80immP22 recombinants carry the h21 marker and P22 DNA replication genes 12 and 18 as well as the x and erf genes of P22. Some  $\phi$ 80immP22 recombinants, designated  $\phi$ 80immP22dis, contain the P22Im region as well as the P22c region, the two widely separated loci involved in the bipartite immunity system of P22.

Although the 0-1 antigen conversion gene a1 and tail gene 9 of P22 are located between the c and Im genes, no  $\phi$ 80immP22dis hybrids carry both the a1 and 9 genes. Some hybrids carry the gene a1 and others carry the gene 9.

Although  $\lambda$ immP22dis hybrids carry both genes 9 and a1,  $\phi$ 80immP22dis hybrids carry only one of these genes (i.e., gene 9 or a1). Since the  $\phi$ 80 protein coat is smaller than that of  $\lambda$ , the  $\phi$ 80immP22dis hybrid phages are unable to accommodate both genes 9 and a1 simultaneously.

#### b. Physico-chemical Analysis of $\phi$ 80-P22 Hybrids

Hybrid  $\phi$ 80-P22 phages, which retain the protein coat of  $\phi$ 80, have been divided into two types with respect to the extent of homology with P22. One hybrid type,  $\phi$ 80immP22, has a large P22 early gene segment containing the att-erf-c-h21 region. The second type,  $\phi$ 80immP22dis, has a larger P22 segment which includes both immunity (c and Im) regions of P22, i.e., Im-att-erf-c-h21. CsCl density centrifugation analyses revealed that the total genome size of

these hybrids increased as the size of the inherited P22 segment increased. The hybrids express the P22 att region and insert at the P22 site near the pro chromosomal genes of the host. Some of the hybrid phages recovered from lysogens were found to contain reductions in the size of the P22 DNA segment. In some cases, the total genome length increased despite a reduction in the size of the P22 segment. This increase could represent replacement of a portion of the P22 DNA segment either by host chromosomal genes or a duplication of phage genes.

c. Transduction with  $\phi$ 80-P22dis Phage

Mutation losing the dis function of  $\phi$ 80-P22dis (i.e.,  $\phi$ 80-P22dis-mutation) is due to the replacement of a bacterial segment for the phage segment containing the att through Im genes of P22. As a consequence, the hybrid phage became a high transducing phage for the proline gene but not tryptophan. Since the size of the bacterial segment substituting for the att-Im segment of the  $\phi$ 80-P22dis hybrid is about equal to that of the  $\phi$ 80 inert segment which is about 10% of the  $\phi$ 80 genome, the mutant phage should be able to carry a few bacterial genes. Thus, it is of great interest to analyze co-transduction of bacterial genes adjacent to the proline gene.

d. Isolation of P22- $\phi$ 80 Hybrid Phages

Since we found both reciprocal recombinants between  $\lambda$  and P22 (i.e.,  $\lambda$ immP22 and P22imm $\lambda$ ), we anticipated finding P22imm $\phi$ 80 hybrids. By employing a technique similar to P22imm $\lambda$  isolation, we were able to isolate P22imm $\phi$ 80 hybrids, which carry the early regions, at least the c region, of  $\phi$ 80 and the entire late regions of P22.

3. Isolation and Characterization of Hybrids between a Mutator Coliphage Mu-1 and Salmonella Phage P22.

a. Isolation of Mu-P22 Hybrid Phages

We have isolated various hybrid phages between Salmonella phage P22 and E. coli phage  $\lambda$  or  $\phi 80$ . These hybrids were found in the lysates of P22 grown on coliphage lysogens. Employing this approach, numerous attempts to isolate hybrids between P22 and a coli mutator phage Mu-1 were unsuccessful. This was thought to be due to lack of induction of Mu-1, because P22 superinfection of  $\lambda$  or  $\phi 80$  lysogens results in induction of their prophages. Dr. Martha Howe supplied us with temperature inducible (ts) mutants of Mu-1 phage. Effort to isolate hybrids by P22 superinfection of such ts mutant lysogens were also unsuccessful. After extensive efforts for about the past three years, we were able to isolate Mu-P22 hybrids on a smooth but P22-resistant derivative of a Mu-1 lysogen.

Phage P22 was grown on various Mu-1 lysogens, and more than  $3 \times 10^{11}$  PFU of such lysate were plated on a Mu-1 lysogenic derivative of WR4027 (rough), which is immune to Mu-1 and resistant to phage P22. No hybrid plaques have been found. However, when a smooth but P22 resistant Mu-1 lysogen was used as a plating host, a few plaques from only two P22 lysates out of several hundred P22 lysates were detected. These plaques were cloned and tested to determine whether they were antigenically identical to coliphage Mu-1. Antiserum against coliphage Mu-1 was found to neutralize the new phage isolates. No inactivation of these phages was detected with anti-P22 serum. These data indicated that the tail antigens responsible for adsorption of this new phage isolate were serologically identical to those of Mu-1. Due to their antigenic structure and capacity to plate on a P22 resistant Mu-1 lysogen of the S. typhimurium hybrid,

we considered these clones to represent hybrids between Mu-1 and P22, henceforth designated as the Mu-P22 class. The Mu-P22 hybrid class forms plaques on a smooth bacterial strain while Mu-1 phage forms plaques on rough bacterial strains. This type of change in host range suggests that the G region of Mu-1 in Mu-P22 hybrid must be inverted. Although the Mu-P22 plaques appear small, it was evident that the c markers of Mu-P22 hybrids mimic those of the P22 strains employed in preparing lysates on Mu-1 lysogens. For example, when a clear plaque P22c1 mutant was used for preparation of lysates, the resulting Mu-P22 hybrid exhibited clear plaques. These findings thus indicate that the Mu-P22 hybrid class contains at least the c locus of phage P22 and conserves the protein coat of Mu-1 (Mu-P22 is also designated as MuimmP22). Although the frequency of lysogeny with Mu-1 phage is rather low, the Mu-P22 phage efficiently lysogenizes smooth hosts, including WR4028. In addition, no mutagenic activity was detected. These observations suggest that the Mu-P22 hybrid carries a specific chromosomal attachment site probably near the proline gene of the hosts.

b. Isolation of P22-Mu Hybrid Phages

Since we were able to find Mu-P22 hybrid phages, we anticipated finding P22-Mu hybrids carrying at least the c marker of Mu and the protein coat of P22 phage. This class of hybrids could be detected infrequently by examining for distinct superimposed turbid (c+) plaques on confluent lysis plates of Mu-1 lysogen of WR4028 infected with P22c2 stocks previously propagated on this lysogen. After purification by plating on a Mu-1 resistant host WR4028, we found that the P22-Mu hybrid class expresses the P22 protein coat and the c marker of the Mu-1 lysogen employed, regardless of the c marker of the P22 strain used. This conclusion was based on our finding that the antiserum against P22 inactivated P22 and representatives of the P22-Mu class at about the same rate. Mutagenic activity of this hybrid is under investigation.

#### 4. A New Isolation Procedure for P22- $\lambda$ Hybrids

When P22<sub>c2</sub> phage stocks previously grown on WR4028 lysogenic for  $\lambda_{c+}$  were plated on WR4028 lysogenic P22 Sie, turbid (c+) plaques were found. These plaque formers were found to be P22- $\lambda_{c+}$  hybrids. Since these P22- $\lambda$  isolates grow in P22Sie lysogens, it is desirable to test whether these P22- $\lambda$  isolates carry the Im gene of P22. This type of genetic study provides a basic information for understanding the immunity role of the P22Im gene.

Publications

1. Yamamoto, N., Yamamoto, S., Gemski, P., and Baron, L.S., 1981. An unusual  $\lambda$ -P22 phage hybrid with the  $\lambda$   $c^+$  region and the immI region of P22. Abs. Am. Soc. Microbiol. In press.
2. Yamamoto, N., Droffner, M.L., Yamamoto, S., Konzelman, J., Gemski, P., and Baron, L.S., 1981. Characterization of hybrids between coliphage  $\phi$ 80 and Salmonella phage P22. Abs. Am. Soc. Microbiol. In press.
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5. Yamamoto, N., Gemski, P. and Baron, L.S. Genetic Studies of new hybrid phage species between coliphage  $\phi$ 80 and Salmonella phage P22. In preparation.



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