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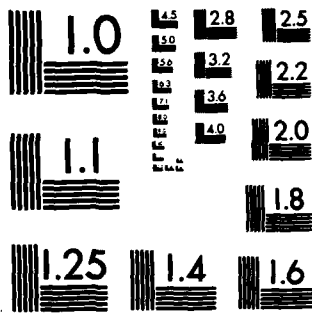
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ANNUAL PROGRESS REPORT

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

Annual Progress Report

Nobuto Yamamoto, Ph.D.

February, 1980

Supported by

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

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Hahnemann Medical College
Philadelphia, Pennsylvania 19102

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The λ immP22 hybrids, which retain the protein coat of λ , were found to contain various lengths of homology with <u>Salmonella</u> phage P22. The length of the entire λ immP22 genome also varies from strain to strain. Density gradient centrifugation revealed that the genome of a λ immP22dis strain is 5% larger than that of a λ immP22 strain. Hybrids λ immP22dis are heat unstable (at 55 C) whereas λ immP22 are rather stable. Because the λ immP22dis genome is tightly packed in λ protein coat, λ immP22dis particles are heat unstable. Survivors		

20. Abstract (Continued)

After heat treatment are often found to be deletion mutants lacking the a1 and 9 genes of P22.

Hybrids phages between Salmonella Phage P22 and coliphage ϕ 80 have been isolated by using E. coli-S. typhimurium hybrids. Among those hybrid phages, ϕ 80immP22dis hybrids carrying both immunity related genes, immC and immI regions of P22 were isolated. Since P22 tail component gene 9 and somatic antigen conversion gene a1 are located between the immC and immI regions of P22, we examined whether ϕ 80immP22dis hybrids carry these genes. Some ϕ 80immP22dis hybrids carry gene a1 but not gene 9 whereas the remaining ϕ 80immP22dis hybrids carry gene 9 only. No ϕ 80immP22 hybrid phages containing both the P22 genes 9 and a1 were found. These observations suggest that ϕ 80immP22dis hybrid is formed as a consequence of multiple crossovers.

Although λ immP22dis hybrid phages carry both genes 9 and a1, ϕ 80immP22dis hybrids carry only one of these genes 9 or a1. Since the size of ϕ 80 phage genome is about 92% of the λ genome, we concluded that the ϕ 80immP22 genome is unable to contain both genes 9 and a1.

Numerous attempts to isolate hybrids between P22 and coli mutator phage M_{μ} -1 were unsuccessful. This may be due to lack of induction of M_{μ} -1 prophage by P22 superinfection although we found that P22 infection of λ or ϕ 80 lysogens results in induction of their prophages. Accordingly we prepared lysogens with temperature inducible (cts) mutants of M_{μ} -1 phage. When P22 phage stocks were prepared by P22 infection of temperature induced lysogens and plated on M_{μ} -1 lysogenic WR4027, pin point plaques were found at a frequency of about 10^{-10} . We consider these pin point plaques formers are M_{μ} immP22 hybrids.

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SUMMARY

Length of λ immP22 hybrid genome varies from strain to strain. The genome of λ immP22dis is 5% larger than that of λ immP22 and is tightly packed in the λ protein coat. Therefore λ immP22dis particles are heat unstable. Survivors after heat treatment were often found to be deletion mutants lacking the a1 and 9 genes of P22. By employing an approach similar to that previously used to isolate λ immP22 hybrids, we have been able to isolate hybrids between P22 and coliphage ϕ 80. We showed the origin of genetic segments in the hybrid phage genomes and suggested that the hybrids are formed as a consequence of multiple crossovers. Moreover, we have been trying to isolate hybrids between P22 and coli mutator phage Mu-1

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 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, 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 interbacteriace. 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 the 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-K12 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 immP22 hybrid class, which has the protein coat of λ , was found to contain at least the c region of P22. The other class, termed P22imm λ , has the protein coat of Phage P22 and has inherited at least the c marker of λ . Length of P22 genetic segment in these hybrids varies from strain to strain.

By employing an approach similar to that previously used to isolate λ immP22 hybrids, we have been able to isolate hybrids between P22 and coliphage ϕ 80. These newly isolated hybrid phages ϕ 80immP22 were found to be extremely valuable phages for understanding formation mechanism of hybrids between unrelated phages. Success of isolation of ϕ 80immP22 provides an approach to isolate hybrids between P22 and coli phage Mu-1.

A. Genomic Structure of λ imm P22 Hybrids

1. Variation in Genome Size of λ immP22 Hybrids.

A strain of λ immP22dis hybrid was rapidly inactivated in nutrient broth at 55 C whereas λ immP22 was rather stable. Density gradient centrifugation with CsCl revealed that the genome of the λ immP22dis strain contain about 5% larger than that of the λ immP22 strain. These observations suggest that the genome of λ immP22dis hybrid is tightly packed in the λ protein coat, resulting in heat instability of λ immP22dis particles.

2. Deletion Mutations of λ immP22dis Hybrid

Hybrid λ immP22dis strains carry the gene 9 and a1 of P22. Their deletion mutants lacking these genes were isolated. Although λ immP22dis hybrid is heat unstable (at 55 C) the deletion mutants are quite heat stable. After heat treatment of λ immP22dis hybrids, survivors were often found to be deletion mutants.

B. Studies of Hybrid Phages between E. coli Phage ϕ 80 and Salmonella Phage P22.

1. Isolation and Characterization of Hybrid Phages between E. coli Phage ϕ 80 and Salmonella Phage P22.

E. coli-S. typhimurium hybrid strain WR4027 is a rough bacterium and sensitive to coliphage ϕ 80 for its replication but insensitive to P22 phage because of lack of P22 phage adsorption. Therefore WR4027 lysogenic for phage ϕ 80, WR4027(ϕ 80), is insensitive to P22 phage. By infecting WR4027(ϕ 80) with a mixture of high titer stocks of rough specific Salmonella phage (designated R phages), we were able to isolate R-phage resistant derivatives of WR4027(ϕ 80), designated WR4027(ϕ 80)/R, which are smooth and fully sensitive to P22 phage. Phage P22 stocks grown on this smooth derivative of the ϕ 80 lysogen give rise to recombinants between P22 and ϕ 80. Such recombinants were recovered by plating on a P22 resistant host and immune to ϕ 80, namely WR4027(ϕ 80). They retain the protein coat of ϕ 80 but have acquired the immC region of P22. In addition these ϕ 80immP22 recombinant carries P22 DNA replication genes 12 and 18 as well as the x and erf genes of P22. Some ϕ 80immP22 recombinants, designate ϕ 80immP22dis, contain the immI region as well as the immC region, the two widely separated loci involved in the bipartite immunity system of P22.

2. Characterization of Unusual Hybrid Phages between E. coli phage ϕ 80 and Salmonella phage P22.

Although the O-1 antigen conversion gene a1 and tail gene 9 of P22 are located between immC and immI genes, no ϕ 80immP22dis hybrids carry both the a1 and 9 genes. Some hybrids carry the gene a1 and others carry the gene 9. (Fig. 1). As shown in Fig.2, both λ and ϕ 80 phage genomes contain physically corresponding and functionary similar genes. These phage genomes also carry genetically inert DNA segments which are located

between their respective att and tail (J) genes. However, the entire physical length of the $\phi 80$ phage genome is about 92% of the size of λ phage genome. This seems to be reflection of difference in sizes of their inert segments: $\phi 80$ carries an inert DNA segment smaller than that of λ (Fig. 2). since the inert segments can be replaced by genes 9 and a1 to form dis hybrid phages, we concluded that the $\phi 80$ immP22dis hybrid phages are unable to accomodate both genes 9 and a1 simutaneously.

C. Attempts to Isolate Hybrid Phages between P22 and Mutator Phage Mu-1

E. coli - S. typhimurium hybrid strain WR4028 is also insensitive to coliphage Mu-1 for its replication. Therefore we isolated Mu-1 lysogenic strains by infecting WR4027 with phage Mu-1. P22 sensitive derivatives of the above lysogen were also isolated by infecting Mu-1 lysogenic WR4027 with a mixture of high titer stocks of rough specific Salmonella phages (designated R). To isolate MuimmP22 (abbr. Mu-P22) hybrid phage, high titer stocks of P22 grown on WR4027(Mu-1)/R should be plated on WR4027(Mu-1). Numerous attempts to isolate such hybrids were unsuccessful. This may be due to lack of induction of Mu-1 prophage by P22 superinfection because we found that P22 infection of λ or $\phi 80$ lysogens results in induction of their prophages. Accordingly we prepared lysogens with temperature inducible (cts) mutants of Mu-1 phage. When P22 phage stocks were prepared by P22 infection of temperature (39°) induced lysogens and plated on Mu-1 lysogenic WR4027, pin point plaques were found at a frequency of about 10^{-10} . We consider these pin point plaque formers are Mu-P22 hybrids.

Publications

Yamamoto, N. Wohlhieter, J.A., Gemski, P. and Baron, L.S. λ immP22dis: A hybrid coliphage λ with both immunity region of Salmonella phage P22, Molecular General Genetics, 166, 233-243, 1978.

Yamamoto, K., Numa, S. Whlhieter, J.A., Gemski, P. and Baron, L.S. Isolation of hybrids between Salmonella phage P22 and coliphage $\phi 80$. Abt. Am. Soc. Microbiol. p. 247, 1979.

Yamamoto, N., Gemski, P. and Baron, L.S. 1980. Hybrid phages between Salmonella phage P22 and coliphages: Expression of Salmonella somatic O-1 antigen conversion gene a1 of hybrids phages in E. coli and Shigella. Manuscript in preparation to be submitted to J. Gen. Virol.

Yamamoto, N., Gemski, P. and Baron, L.S. 1980. Variation in genome lengths of λ immP22 hybrids. Manuscript in preparation.

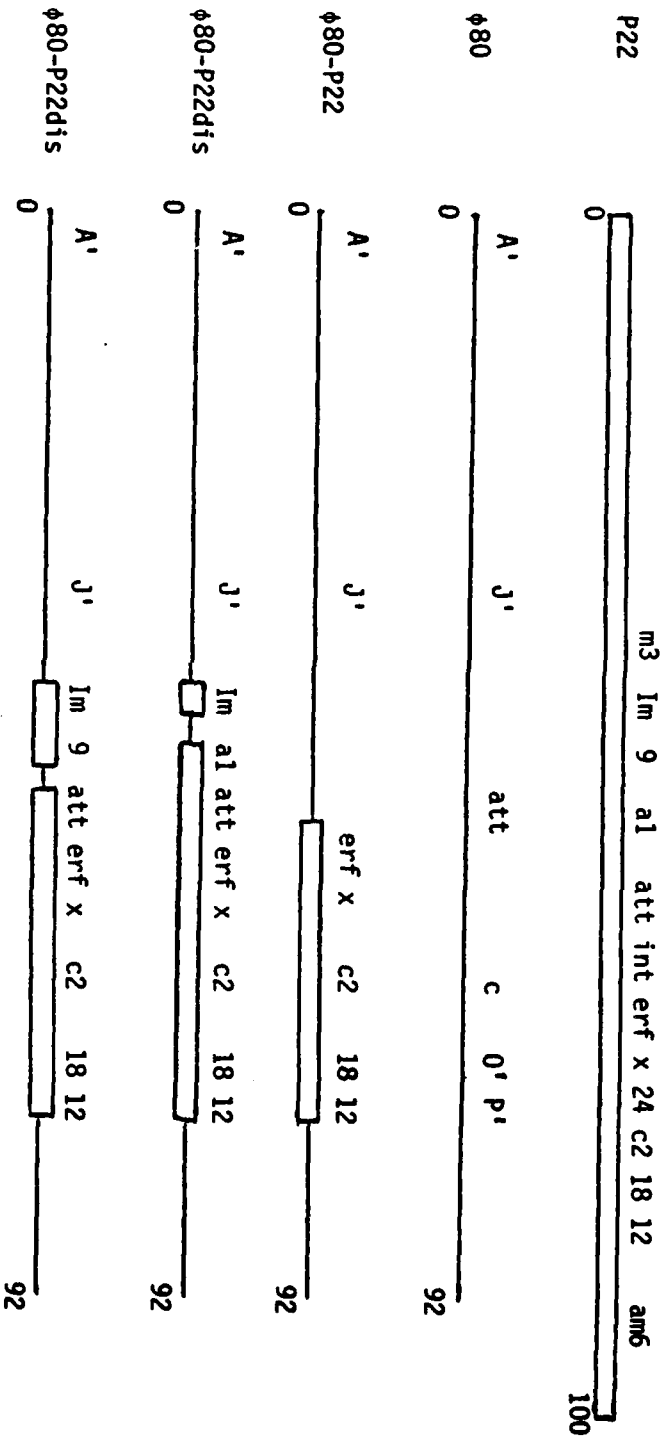


Figure 1. Genome structures of hybrids between Salmonella phage P22 and coliphage phi80.

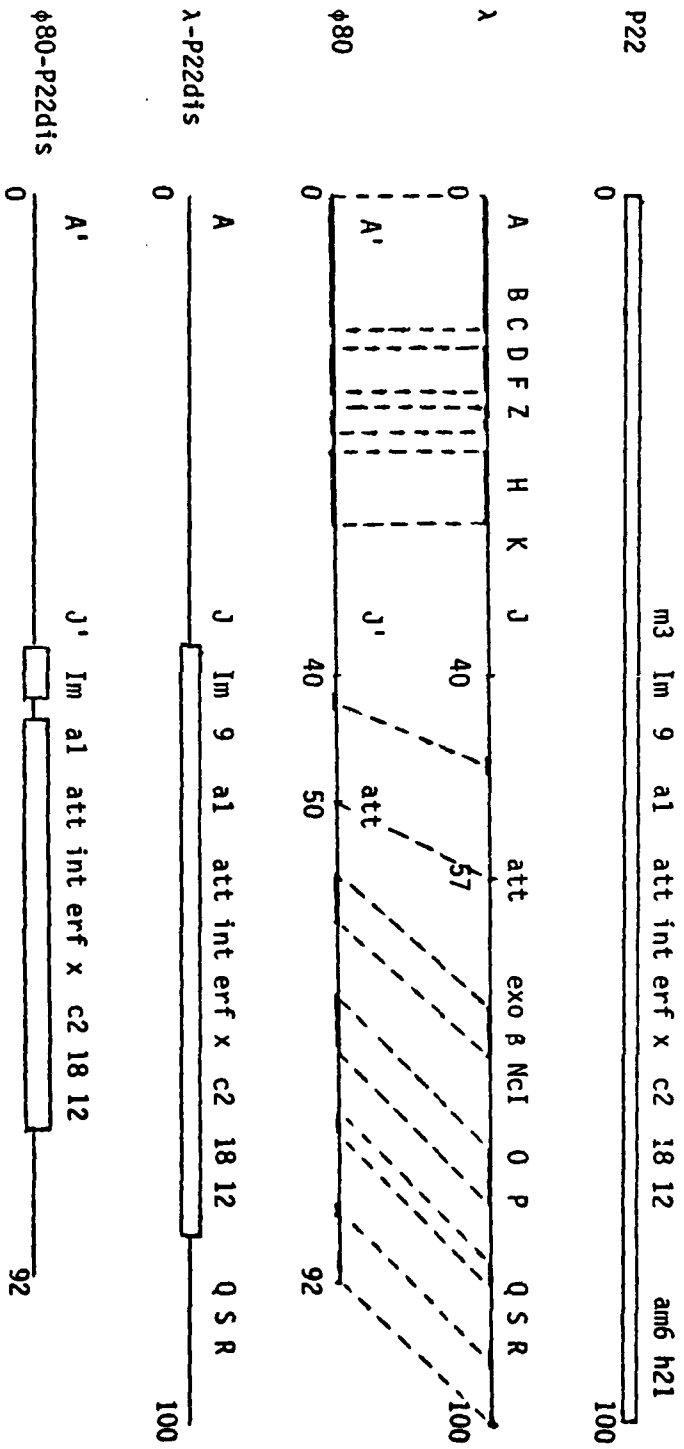


Figure 2. Genome structures of P22, λ , $\phi 80$, λ impP22dis and $\phi 80$ impP22dis phages.

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