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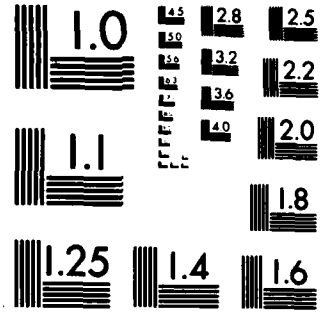

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ANNUAL PROGRESS REPORT

Genetic and Physical Structures of Hybrid Bacteriophage Genomes

Annual Progress Report

Nobuto Yamamoto, Ph.D.

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E. coli-S. typhimurium hybrids provides excellent systems to isolate phi bacteriophage hybrids between Salmonella phage P22 and E. coli phages such as λ, ϕ80 and Mu-1. A variety of hybrid types such as λ-P22Imλc, ϕ80immP22, amd MuimmP22 have been isolated and characterized. Studies of these hybrid phages provide invaluable information for the genetic evolution, mutation, transduction, immunity controls and gene expression of bacteriophages.

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We have isolated ϕ80immP22dis and MuimmP22dis hybrid phages carrying both c and Im regions of P22. Derivatives of ϕ80immP22dis which have lost the dis function were recovered from ϕ80immP22dis lysogens. Such dis⁻ derivatives were formed by replacement of a P22 phage segment containing att through Im regions with bacterial regions adjacent to prophage insertion site. Therefore the dis⁻ hybrid derivatives are high frequency transducing phages for proA, argF and metD but not for tryptophan.

Although homology between ϕ80immP22 hybrids and P22 have been mapped in detail, ϕ80 segments in the hybrids have not been analyzed. Mapping of ϕ80 segments are now feasible since we found WR4027 amber suppressors for ϕ80 amber mutants.

P22-λ hybrid phage carries a segment of the λ early regions and the entire late region of P22. Thus the P22 late genes should be regulated by the λ early genes. This was analyzed by complementation capacity of λ or ϕ80 for P22 amber mutants within P22 early regions. P22 amber mutants: both am24 for early regulatory gene and am23 for late regulatory gene and am7 for endolysin gene were complemented by λ and ϕ80 phages. However am12 for control of P22 DNA replication gene was not complemented.

Salmonella phages P22 and ES18 are serologically and morphologically unrelated. However ES18 has a homology with the entire P22 early regions and the gene for generalized transduction, suggesting that ES18 is a generalized transducing hybrid phage as a consequence of recombination between P22 like group A Salmonella phage and a group B phage.

CONTENTS

SUMMARY

Replication of P22 phage in Mu-1 lysogen gave rise to a hybrid phage designated Mu*imm*P22 carrying the protein coat of Mu-1 and *c* region of P22. Since P22 carries antirepressor gene, P22 can grow in MuimmP22 lysogen and gives rise to another hybrid type disignated Mu*imm*P22*dis* which carries both *c* and *Im* region of P22.

We have also isolated ø80*imm*P22*dis* hybrids which carries both the immunity regions (*c* and *Im*) and prophage attachement region (*att*) of P22. Hybrid derivatives which have lost the *dis* function (i.e. ø80*imm*P22*dis*$^-$) have been recovered from ø80*imm*P22*dis* lysogens. These *dis*$^-$ derivatives were formed by replacement of a P22 phage segment containing *att* through *Im* regions with bacterial segment adjacent to the prophage insertion site. Thus these *dis*$^-$ hybrid derivatives are high frequency transducing phages for proline but not tryptophan. They cotransduce *proA*, *argF* and *metD* genes.

We have mapped homologous segment between ø80immP22 hybrids and P22 in detail. To analyse the ø80 segments in the hybrids, we isolated WR4027 amber suppressors for ø80 amber mutants.

P22-λ hybrid phage carries a segment of the λ early regions and the entire late region of P22. Thus the λ early genes should regulate expression of the P22 late genes in P22-λ hybrid phage. This was analyzed by complementation capacity of λ or ø80 for P22 amber mutants within their early regions. Complementation should be demonstrable by superinfecting λ or ø80 lysogens with P22 amber mutants because the P22 *ant* function induces these prophages. P22 amber mutants; both *am24* for early regulatory gene and *am23* for late regulatory genes, and *am7*

for endolysin gene were complemented by λ and ϕ80 phages. However, am12 for control of DNA replication gene was not complemented.

Although two generalized transducing Salmonella phages P22 and ES18 are serologically and morphologically unrelated. ES18 has a homology with the entire early regions and gene for generalized transduction of P22, suggesting that the generalized transducing phage ES18 is created as a consequence of recombination between a group A phage (P22 like phage) and a group B phage.

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 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 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

1. Isolation of a new hybrid between Salmonella phage P22 and coli mutator phage Mu-1

Phage Mu-1 is unable to grow in a smooth E. coli-S. typhimurium hybrid strain WR4028 but able to grow in a rough strain WR4027. In contrast, P22 phage cannot infect this rough strain and its Mu-1 lysogenic derivative WR4027(Mu-1). When a mixture of Salmonella rough specific phage (designated as R phage) was plated on WR4027(Mu-1), smooth derivatives which are resistant to R phage were isolated. Thus, P22 is now able to grow in this new lysogen, WR4027(Mu-1)/R, and give rise to hybrid phages carrying the protein coat of Mu-1 phage and c region of P22. Such hybrids, designated MuimmP22, were isolated by plating the P22 lysates previously grown on WR4027(Mu-1)/R on a smooth but P22-resistant derivative of Mu-1 lysogen, WR4027(Mu-1)/R/22. These results suggest that the Mu-1 tail fibre component of MuimmP22 phage changed its receptor specificity from rough to smooth hosts, indicating inversion of the G region. Thus, MuimmP22 phage infects WR4028 but not WR4027. When P22 high-titer stocks (more than 10^{10} PFU/ml) previously grown on MuimmP22 lysogens of WR4028 were plated on WR4027/R/22(MuimmP22), a few plaques were found. These plaque formers were found to be a new MuimmP22 hybrid phage class which is dismune over MuimmP22 lysogens. Like λimmP22dis hybrid phage, MuimmP22dis lysogenic derivatives of WR4028 are immune to P22 infection, suggesting that MuimmP22dis carries both c and Im^2 region of P22.

2. Isolation of amber suppressor strains of E. coli - S. typhimurium hybrid WR4028/22 met$^+$

Both smooth and rough strains of E. coli - S. typhimurium hybrids WR4028 and WR4027 require methionine. We have isolated a met$^+$ prototroph of WR4028 by

transduction with P22c2 phage. Because of P22c2 infection, all met$^+$ transductants are rough strains termed WR4028/22 met$^+$. Using this prototroph, we have isolated various amino acid requiring mutants. We then looked for suppressor revertants with the consideration that some of the original auxotrophic mutations would be amber mutations. After large scale screening of these revertants by infecting known ϕ80 amber mutants suppressible by E. coli suppressor I, we found five suppressor mutants. These amber suppressors should be useful for mapping ϕ80immP22 hybrid phages.

3. Isolation and characterization of transducing ϕ80immP22 hybrids

Hybrid ϕ80immP22 phages, which retain the protein coat of ϕ80, have been divided into two types with respect to the extent of homology with P22. One hybrid type has a large P22 early gene segment containing the att-erf-c-h21 region. The second type, ϕ80immP22dis, has a larger P22 segment which includes both immunity (c and immI) regions of P22, i.e., immI-att-erf-c-h21. Since the dis hybrids carry the P22 att region, the prophage is integrated at the P22 insertion site which is near the pro 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 as detected by loss of dis function. 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 by host chromosomal genes.

Derivatives which have lost the dis function of ϕ80immP22dis (i.e., ϕ80immP22dis$^-$) are due to the replacement of the phage segment containing the att through Im genes of P22 with bacterial segment adjacent to the prophage insertion site. As a consequence, the hybrid phage became a high frequency transducing phage for the proline gene but not tryptophan. Since the size of the bacterial segment

substituting for the att-Im segment of the φ80immP22dis hybrid is about equal to that of the φ80 inert segment which is about 10% of the φ80 genome, the derivative phage should be able to carry a few bacterial genes. Indeed we found that all φ80immP22dis⁻ carry proA, and argF and about 20% of φ80immP22dis⁻ isolates also carry metD as demonstrated by cotransduction. Frequency of these transductions are about 10-20% of infected cells for all markers present, indicating that 100% of lysogenized cells are transduced.

4. Complementation of the P22 early gene functions with λ and with φ80

We have previously isolated P22-λ hybrid phage which carries a segment of the λ early region and the entire late region of P22. Thus, the λ early genes should regulate expression of the P22 late genes in P22-λ hybrid phage. In order to test this possibility, we analyzed complementation capacity of λ or φ80 for P22 amber mutants within their early regions. P22am24 mutants spotted on a soft agar overlay containing smooth lysogens carrying either λtltm or φ80 wild type show replication as observed by formation of plaques or lysis zones whereas P22am12 mutants cannot replicate in these lysogens. Moreover, P22 amber mutants in genes 23 and 7 (endolysin) grow on these lysogens. The same concentration of this P22 amber mutant did not produce any sign of phage replication on a soft agar overlay with a non-lysogenic strain.

5. A new isolation procedure for λ-P22Imλc hybrids

P22-λ hybrid phage grows on λ lysogens though they share the c immunity region. This is because P22-λ hybrid carries the P22 anti-repressor which inactivates λ repressor as well as P22 repressor. When P22-λ hybrid phage stocks previously grown on λ lysogens of WR4028 were plated on a λ lysogen of a rough derivative (WR4027), small plaques were found at a frequency of

about 10^{-5}. These plaque formers should be λ-P22Imλc hybrid type because λimmP22 hybrid type carrying λc and the P22 antirepressor gene can replicate in a rough derivative of λ lysogen, WR4027(λ).

6. Homology between two generalized Salmonella transducing phages ES18 and P22

Salmonella phages have been divided into two subgroups by Boyd: Group A phages are heat stable (resistant to high temperature) and have generalized transducing ability while group B phages are heat labile and do not carry generalized transducing activity with one exception. One of the group B phages, ES18, is a generalized transducing phage. Since we previously found that ES18 is homologous in the c regions, homology analyses were extended to various other genes of P22. Our recent studies show ES18 has homology with the entire early regions of P22. In addition, ES18 and P22 share the generalized transducing gene as observed by complementation of P22am3 mutants with ES18. Thus, we consider that phage ES18 is a generalized transducing hybrid phage as a consequence of recombination between a group A phage and a group B phage.

Publications

1. Yamamoto, N., Yamamoto, S., Gemski, P., and Baron, L.S., 1981. An unusual λ-P22 phage hybrid with the λ c^+ region and the immI region of P22. Abs. Am. Soc. Microbiol. p. 114.

2. Yamamoto, N., Droffner, M.L., Yamamoto, S., Konzelman, J., Gemski, P., and Baron, L.S., 1981. Characterization of hybrids between coliphage ϕ80 and Salmonella phage P22. Abs. Am. Soc. Microbiol. p. 114.

3. Yamamoto, N., Gemski, P., and Baron, L.S., 1981. Isolation of a hybrid between Salmonella phage P22 and coli mutator phage Mu-1. Abs. Am. Soc. Microbiol. p. 115.

4. Yamamoto, N., Gemski, P., and Baron, L.S., 1982. Genetic studies of new hybrid phage species between coliphage ϕ80 and Salmonella phage P22. Submitted to J. Gen. Virol.

5. Yamamoto, N., Droffner, M.L., Yamamoto, S., Gemski, P., and Baron, L.S., 1982. Specialized transducing derivatives of ϕ80immP22 phage. In preparation.

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