

AD-A051 160

TEMPLE UNIV PHILADELPHIA PA SCHOOL OF MEDICINE
GENETICS OF NOVEL HYBRID BACTERIOPHAGE AND DEVELOPMENT OF GENER--ETC(U)
DEC 77 N YAMAMOTO

F/G 6/13

DAMD17-74-C-4051

NL

UNCLASSIFIED

| OF |

AD
A051160



END

DATE

FILMED

4 -78

DDC

ADA 051160

AD _____

12

[Handwritten signature]

Annual Progress Report No. 4

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

9 Annual Progress Report, no. 4, 1 Jan - 31 Dec 77, (From 1/1/77 to 12/31/77)

10 Nobuto Yamamoto Ph.D.

11 34 Dec [redacted] 77

DDC
MAR 14 1978
RESISTIVE
[Handwritten initials]

Supported by 12 11p.

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,
Washington, D.C. 20314

15 Contract No. DAMD 17-74-C-4051

16 3M161102BS01 17 00

Temple University
Philadelphia, Pennsylvania 19140

DDC AVAILABILITY STATEMENT: Approved for public release;
distribution unlimited

The findings of this report are not to be construed as an official
Department of the Army position unless so designated by other
authorized documents.

346 400

[Handwritten signature]

AD No. _____
DDC FILE COPY

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Genetics of Novel Hybrid Bacteriophages and Development of Generalized Transducing System for Salmonella typhosa		5. TYPE OF REPORT & PERIOD COVERED Annual Progress Report (From 1/1/77 to 12/31/77)
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Nobuto Yamamoto		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-74-4051
9. PERFORMING ORGANIZATION NAME AND ADDRESS Temple University School of Medicine 3420 N. Broad Street Philadelphia, Pa. 19140		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A 3M161102BS01.00.010.
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Washington, D.C. 20314		12. REPORT DATE December 30, 1977
		13. NUMBER OF PAGES 12
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES N.B. don't confuse ϕ (= phi) with \emptyset (= zero) λ = lambda ; $\phi 80$ = phi-80		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Bacteriophage, Hybrid bacteriophage, <u>Salmonella typhimurium</u> , Hybrid bacteria, <u>E. coli-S. typhimurium</u> hybrid, <u>Salmonella typhosa</u> , Generalized transduction, Genetic recombination, Antigen conversion, Cell surface, Gene expression, Bacteriophage tail.		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) D Various hybrid phages between Salmonella Phage P22 and coliphages such as lambda and $\phi 80$ have been isolated by using their common bacterial hosts, <u>E. coli-S. typhimurium</u> hybrids. Among those hybrid phages, λ -P22dis and $\phi 80$ -P22 hybrid classes carry a large genetic segment of P22 phage containing the 'Im' → (over)		

20. Abstract (continued)

gene in addition to the 'c' genes. Since the Salmonella somatic antigen conversion gene 'al' and tail component gene 9 of P22 are located between the c and Im genes, these hybrid phages should carry the al and tail gene 9 of P22. Therefore we studied the antigen conversion of various bacterial hosts and the P22 tail gene expression, although these genes are dispensable for these hybrid phages.

Both λ-P22dis and φ80-P22 can convert E. coli-S. typhimurium with somatic antigen O-1. However, E. coli K12 was not converted by these hybrid phage classes. This can be explained by the fact that E. coli does not have O-1 antigen acceptor sites which are [gal-rha-man] repeating units of Salmonella typhimurium cell surface. Moreover, the P22 tail component gene 9 in λ-P22dis is expressed during lytic replication of λ-P22dis hybrid phage.

ACCESSION for	
NTIS	White Section <input checked="" type="checkbox"/>
DDC	Buff Section <input type="checkbox"/>
UNANNOUNCED	<input type="checkbox"/>
JUSTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODES	
A	

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

Annual Progress Report
(From 1/1/77 to 12/31/77)

Nobuto Yamamoto

December 30, 1977

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,
Washington, D.C. 20314

Temple University
Philadelphia, Pennsylvania 19140

Summary

We mapped the phage chromosomes of hybrids between Salmonella phage P22 and coliphages. Since the genomes of hybrid phages consist of clusters of genes derived from evolutionary diverse bacteriophages, we studied the controls of various gene expressions in these hybrid phages. In this progress report we showed the expression of dispensable genes such as the somatic antigen conversion gene al and the P22 tail component gene 9 in these hybrid phages.

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 λ .

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

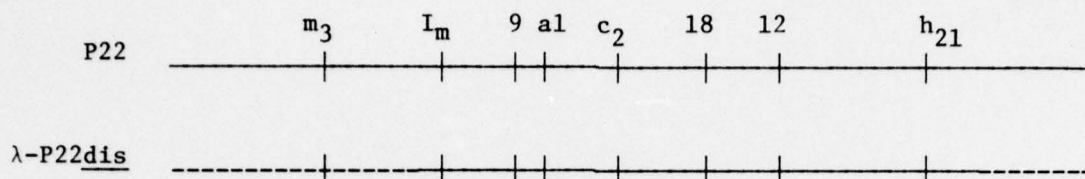
λ genes containing the cI, cII, cIII and N genes.

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. The hybrid phages may be used for intergeneric transduction of chromosomal genes from different genera of the enterobacteriaceae. 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. An unusual hybrid phage between coliphage λ and Salmonella phage P22

As we reported previously, the genetic homology between P22 and λ -P22 has been analyzed by recombination experiments. The length of the homologous region between P22 and λ -P22 varied among many independent λ -P22 isolates. Although P22 and λ -P221 share c regions, most λ -P22 lysogens are not immune to P22 infection. However, one of λ -P22 groups (see Fig. 1) λ -P22dis, conferred immunity to superinfection with P22 and formed plaques on bacterial strains lysogenic for λ -P22. Therefore it is evident that P22 supplies an additional marker Im (immunity), as well as the c, g and h21 markers to form λ -P22dis. It should be mentioned here that λ -P22 lysogens are not only immune to λ -P22 but also immune to another hybrid phage species P221, carrying the c region of P22. Although the c region of P22 is enough to confer immunity to these phages, the c region alone is not enough to establish immunity to P22 and λ -P22dis infection. Thus the Im region of P22 is required for establishing the immunity to P22 and λ -P22dis superinfection. The finding of various λ -P22 types provides a crucial demonstration of the second region responsible for establishing the immunity to phage P22.

The above finding implies that the length of homology between P22 and λ -P22dis group is longer than that between P22 and λ -P22. Thus, it is unequivocally concluded that P22 supplies various lengths of its genetic segment to form a variety of λ -P22 types. From these observations, it may be concluded that λ -P22 arises as a consequence of recombination between P22 and λ though no evidence for genetic homology between these phages was observed. Thus, the genome of λ -P22 consists of parts of P22 and λ . The length of P22 segment inserted into λ -P22 groups varies from strain to strain of λ -P22 groups.

Figure 1. Genetic structure of P22 and λ -P22dis

2. Antigen conversion gene of λ -P22dis hybrids

λ -P22dis hybrids form plaques on λ -P22 lysogens, because the left end of the P22 segment in the λ -P22dis extends to the left of the I_m gene of P22. As shown in Figure 1, the antigen conversion gene a_1 of P22 is located between c_3 and I_m genes of P22. Accordingly, we tested the antigen conversion of various bacterial strains lysogenic for λ -P22dis and found that about 100% of λ -P22dis strains conferred antigen 01 conversion to E. coli-S. typhimurium hybrid WR4027.

3. Antigen conversion of E. coli by a λ -P22 hybrid phage strain.

As we reported previously, we have isolated phage λ -P22dis, an unusual hybrid between P22 and λ . This hybrid carried a large P22 genetic segment containing I_m (the 2nd immunity gene) as well as c , g and h_{21} genes. In addition we found that some λ -P22dis strains also carry the antigen 0-1 conversion gene a_1 of P22. Therefore they can confer antigen 0-1 conversion to E. coli-S. typhimurium strain WR4027. However they do not convert E. coli K12. This can be explained by the fact that E. coli K12 does not have 0-1 antigen acceptor sites which are [gal-rha-man] repeating units of Salmonella typhimurium cell surface. When E. coli K12 derivatives carrying a small Salmonella genetic segment for the [gal-rha-man] repeating units were examined, they were readily converted by the λ -P22dis strains.

4. Antigen conversion of E. coli-S. typhimurium hybrids by ϕ 80-P22 hybrids.

Phage ϕ 80-P22 is a hybrid type between coliphage ϕ 80 and Salmonella

phage P22. All ϕ 80-P22 strains isolated carry the antigen conversion gene as well as Im, c and h21 genes of P22. Therefore these ϕ 80-P22 strains are able to convert E. coli-S. typhimurium strain WR4027 and E. coli K12 carrying the repeating units of Salmonella typhimurium.

5. Expression of the P22 Tail Gene 9 in λ -P22dis hybrids

Since λ -P22dis carries the P22 tail gene 9, it was desirable to see whether the gene 9 is expressed during λ -P22dis replication. In order to test expression of the gene 9, the in vitro self-assembly method of Israel, Anderson and Levine (Proc. Nat. Acad. Sci. 57, 284-291, 1967) was used.

When S. typhimurium Q (2×10^8 cells/ml) was infected with a temperature sensitive mutant of P22 gene 9 and cultured for 1 hour at 39°C, according to Israel, Anderson and Levine the resulting lysate theoretically should contain about 2×10^{10} tail-less P22 head particles which still contain intact whole P22 genome. When this head donor preparation was added to the lysates of λ -P22dis previously grown in E. coli-S.typhimurium hybrid WR4027 and incubated for 1 hour and plated on a P22 specific indicator strain Q or Q/221 at 25° because P22 head preparation carried a genome with temperature sensitive tail gene, about a 10,000 fold increase of P22 plaque forming activity was found. This increased plaque formation was completely inhibited by using λ -P22dis lysate pretreated with anti-P22 but not affected by λ -P22dis lysate pretreated with anti- λ . From these observations it is concluded that the gene 9 of P22 in λ -P22dis was expressed.

DISTRIBUTION LIST

4 copies	HQDA (SGRD-AJ) Wash., D.C. 20314
12 copies	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Dean, School of Medicine Uniformed Services University of the Health Sciences Office of the Secretary of Defense 4301 Jones Bridge Road Bethesda, Maryland 20014

Publications

1. Baron, L.S., Life, C.A., Gernski, P. and Yamamoto, N. Expression of Salmonella somatic antigen 0-1 in Escherichia coli K12. Abt. Am. Soc. Microbiol., p. 76, 1977.
2. Yamamoto, N., Ushijima, N., Gernski, P. and Baron, L.S. Studies of hybrids between coliphage λ and Salmonella phage P22: Genetic analysis of the P22- λ hybrid class. Mol. Gen. Genetics, 155, 117-122, 1977.