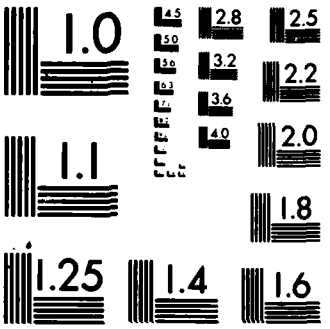
AD-A140 144 GENETIC AND PHYSICAL STRUCTURES OF SALMONELLA-COLI PHAGE HYBRIDS AND DEVE..(U) HAHNEMANN MEDICAL COLL AND HOSPITAL PHILADELPHIA PA N YAMAMOTO JAN 83 1/1

UNCLASSIFIED DAMD17-79-C-9134 F/G 6/13 NL



AD_____

ANNUAL PROGRESS REPORT

Genetic and Physical Structures of Salmonella-coli Phage Hybrids and Development of New Generalized Transducing Hybrid Phages for E. coli.

Annual Progress Report

Nobuto Yamamoto, Ph.D.

January, 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,

Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9134

Hahnemann Medical College
Philadelphia, Pennsylvania 19102

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A140 144	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Genetic and Physical Structures of Salmonella-coli Phage Hybrids and Development of New Generalized Transducing Hybrid Phages for E. coli		5. TYPE OF REPORT & PERIOD COVERED Annual Progress Report (From 2/1/82 to 1/31/83)
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Nobuto Yamamoto		8. CONTRACT OR GRANT NUMBER(s) DAMD17-79-C-9134
9. PERFORMING ORGANIZATION NAME AND ADDRESS Hahnemann Medical College 230 North Broad Street Philadelphia, Pa 19102		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A 3M161102BS10.AE.062
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD 21701		12. REPORT DATE January 1983
		13. NUMBER OF PAGES 14 pages
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

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Bacteriophage, P22, Coli-mutator phage Mu-1, Hybrid bacteriophage, Salmonella typhimurium, Hybrid bacteria, E. coli-S. typhimurium hybrid, Genetic homology, Genetic recombination, Antigenicity, Bacteriophage tail fiber, Lysogeny, Induction, Invertible segment, Flagella phase variation, and Host range

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

E. coli-S. typhimurium provided excellent systems to isolate bacteriophage hybrids between Salmonella phage P22 and E. coli phages such as λ , Ø80 and Mu-1. During the last research support period we focused on isolation of hybrid phages between P22 and Mu and studies on the mechanism of their hybrid formation. Isolation of hybrids betweeen these phages is difficult due to the extremely low frequency of hybrid formation. These rare hybrid phages, however, provide unusually exciting information for genetic evolution, inversion mechanism of phage gene(s) and variation in

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

antigenicity and host range of phage.

We exerted an enormous effort to isolate MuimmP22 hybrid phages carrying the entire late genes of Mu phage and the early genes, at least the c genes, of P22 phage. MuimmP22 hybrid readily lysogenize the host cells whereas the frequency of lysogeny formation with Mu is very low. This observation suggests that MuimmP22 carries the prophage attachment (att) region and integration (int) function of P22.

MuimmP22 lysogens are sensitive to P22 infection though MuimmP22 hybrid carries the c region of P22. P22 lysogens, however, are immune to MuimmP22 infection. This is because P22 has in addition to the c region the 2nd immunity (Im) region carrying the anti-repressor (ant) gene which can inactivate the c repressor. When P22 high-titer stocks (more than 10^{10} PFU/ml) previously grown on MuimmP22 lysogens of WR4028 were plated on WR4028 (MuimmP22)/22S a few plaques were found. These plaque formers were found to be a hybrid class, designated as MuimmP22dis which is dismune over MuimmP22 lysogens. MuimmP22dis lysogenic derivatives of WR4028 are immune to P22 infection, indicating that MuimmP22dis carries both the immunity regions (the c and the Im genes) of P22.

MuimmP22 hybrids infect a smooth host WR4028 but not a rough host WR4027 which is a Mu specific host. Thus the host range of MuimmP22 hybrid differs from that of Mu phage. This seems to be due to inversion of the G segment containing the tail fiber gene S during the process of hybrid formation. Transcription and translation in reverse sequence of the S gene should produce a new polypeptide sequence of the tail fiber to develop a new host range. In addition, we found that anti-Mu serum neutralizes MuimmP22 hybrid at a rate 10-fold slower than Mu phage. This observation suggests that another late protein is a secondary antigenic site responsible for neutralization of plaque-forming ability.

Mu lysogens are not inducible whereas MuimmP22 lysogens are inducible supporting the notion that MuimmP22 hybrid carries the att and int regions of P22. When phage particles spontaneously released from MuimmP22 lysogens were tested for their sensitivity to anti-Mu, more than 30% of the phage particles are insensitive to anti-Mu suggesting that the late gene for a secondary antigenic site for plaque neutralization is inverted. These inversions may occur during hybrid formation or induction process by the aid of functions of the gin gene of Mu phage and the hin gene for the flagellar phase variation of Salmonella strains. Since the gin gene function of Mu phage is known to transact with the hin gene function of Salmonella of flagellar phase variation, the hin gene function should also act on inversion of Mu phage DNA. We suggested that variations in antigenicity and host range of phages are due to transcription and translation in reversed direction of genes such as the tail fiber gene S producing a new polypeptide sequence for tail fibers. To confirm this hypothesis, we isolated MuimmP22 hybrids with selective hosts after crossing P22 with Mu phage carrying an amber mutation in the S gene. These hybrids are able to grow in suppressor negative hosts indicating that the amber phenotype is not expressed, thus supporting our hypothesis that the G segment is inverted in these MuimmP22 hybrids.

Genetic and Physical Structures of Salmonella-coli Phage Hybrids and Development of New Generalized Transducing Hybrid Phages for E. coli.

Annual Progress Report

Nobuto Yamamoto, Ph.D.

January, 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,

FORT DETRICK, FREDRICK, MARYLAND 21701

Hahnemann Medical College
Philadelphia, Pennsylvania 19102

CONTENTS

SUMMARY

The frequency of hybrid formation between Salmonella phage P22 and coli mutator phage Mu is extremely low. Therefore we had to exert enormous effort to isolate hybrids. The hybrid MuimmP22 carries the entire late genes of Mu phage and the early genes, at least the c to att region of P22 phage. MuimmP22 lysogens are sensitive to P22 infection whereas P22 lysogens are immune to MuimmP22 infection because P22 has in addition to the c region the 2nd immunity (Im) region carrying the anti-repressor (ant) gene which can inactivate the c repressor. We also isolated MuimmP22dis hybrid which carries both the c and Im regions of P22 and is dismune over MuimmP22 lysogen.

Although the entire late genes of MuimmP22 hybrid class are derived from Mu phage, MuimmP22 hybrid class infects a smooth P22-sensitive host but not a rough Mu-sensitive host WR4027. Since the tail fiber gene S of Mu phage is situated in the invertable G segment, the S gene appears to be inverted during the process of hybrid formation and thus is translated in reverse direction producing a new peptide sequence of the tail fiber. This mechanism should develop not only a new host range but also a new antigenicity which differs from those of Mu phage. Indeed anti-Mu serum neutralized the plaque-forming activity of MuimmP22 at a rate 10-fold slower than Mu phage, suggesting that another late protein is a secondary antigenic site responsible for neutralization of plaque-forming ability.

Mu lysogens are not inducible whereas Mu*imm*P22 lysogens are inducible supporting the notion that Mu*imm*P22 hybrid carries the *att* and *int* regions of P22. More than 30% of the phage particles released from Mu*imm*P22 lysogens are insensitive to anti-Mu serum suggesting that the late gene for a secondary antigenic site for plaque neutralization is inverted. These inversions may occur during hybrid formation or induction process by the aid of functions of the *gin* gene of Mu phage and the *hin* gene of *Salmonella* for the flagellar phase variation.

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 variation, host specificity, morphogenesis and replication of bacteriophages. 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. Since the hybrid phage genomes are composed of gene clusters from evolutionary diverse phages, hybrid phages often carry dispensable genes. Such hybrid phages should serve as excellent gene cloning vectors in the genetic engineering by replacing the dispensable genes with foreign genes.

PROGRESS

1. Isolation of hybrids between Salmonella phage P22 and coli mutator phage Mu-1

E. coli-S. typhimurium hybrid strain WR4028 served as a host for P22 and various P22 mutants. Strain WR4028, however, is resistant to Mu-1 because it does not adsorb this phage as determined by adsorption experiments. Strain WR4027, which is rough and thereby resistant to P22 is sensitive to Mu-1. It was therefore used for isolation of Mu-1 lysogens, WR4027(Mu-1), which were considered as a possible selective indicator host for isolation of hybrids. A smooth P22 sensitive WR4027(Mu-1) derivative designated NY4027(Mu-1) was recovered by selecting for resistance to a series of rough specific phages (R phages). Growth of P22 phage in such a lysogen should give rise to hybrid phages carrying the protein coat of Mu-1 phage and c region of P22. We exerted an enormous effort to isolate hybrid phages by plating P22 phage stocks previously grown on NY4027(Mu-1) on WR4027(Mu-1) but were unsuccessful. However after multiple extensive efforts for about the past three years we were able to isolate a hybrid phage type forming plaques on a smooth but P22-resistant derivative of a Mu-1 lysogen, NY4027(Mu-1)/22S. These plaque formers were cloned on WR4028/22S. These new clones are neutralized by anti-Mu-1 serum but insensitive to anti-P22 serum. Due to their antigenic structure and capacity to plate on a P22 resistant Mu-1 lysogen, we considered these clones to represent hybrids between Mu-1 and P22, henceforth designated as the MuimmP22 class.

2. Characterization of MuimmP22 hybrids

Since MuimmP22 hybrid forms plaques on a smooth P22-resistant host, we examined its host range. The MuimmP22 hybrid is able to form plaques on a P22-sensitive smooth host WR4028 but not on a Mu-1 phage sensitive rough host WR4027. Since it is known that the G segment containing the tail fiber gene S can be readily inverted, the segment G may be inverted during the process of hybrid formation. Consequently the tail fiber gene S may be transcribed and translated in reverse sequence thus developing a new host range.

Although the late genes of MuimmP22 are derived from those of Mu-1, anti-Mu-1 serum neutralizes plaque-forming activity of MuimmP22 at a rate 10-fold slower than that of Mu-1. Since it is well accepted that the tail fiber of phage is the major antigen responible for neutralization of plaque-forming activity, another protein within the tail structure, particularly one adjacent to the tail fiber, may be a secondary antigenic site for neutralization of plaque-forming activity by anti-Mu-1 serum.

3. Genetic homology between MuimmP22 hybrid and P22

MuimmP22 hybrid carries the late function of coli phage Mu-1 and the early genes, at least c genes of P22. MuimmP22 hybrid readily lysogenize the host cells whereas the frequency of lysogeny formation with Mu-1 is very low. This observation suggests the MuimmP22 carries the prophage attachment (att) region and the integration (int) function of P22.

4. Immunity pattern of MuimmP22 hybrid and isolation of MuimmP22dis hybrid

MuimmP22 lysogens are sensitive to P22 infection though MuimmP22 carries the c region of P22. P22 lysogens, however, are immune to Muimm-P22 infection. This is because P22 has in addition to the c region the 2nd immunity (Im) region carrying the anti-repressor (ant) gene which can inactivate the c repressor.

When P22 high-titer stocks (more than 10^{10} PFU/ml) previously grown on MuimmP22 lysogens of WR4028 were plated on WR4028(MuimmP22)/22S 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, MuimmP22dislysogenic derivatives of WR4028 are immune to P22 infection suggesting that MuimmP22dis carries both the c and Im regions of P22.

5. Antigenic variation in MuimmP22 hybrid phage released from the lysogens

Though Mu-1 prophage is a non-inducible phage, MuimmP22 hybrid phage is inducible supporting the notion that MuimmP22 carries the att and int regions of P22. When spontaneously released phage particles of cells lysogenic for MuimmP22 were tested for their antigenicity by neutralization of plaque-forming activity more than 30 percent of the phage particles were not neutralized by anti-Mu-1 serum. This antigenic variation suggests that during induction process a gene responsible for the secondary antigenic site for for neutralization of plaque-forming activity is inverted.

6. Mechanism of antigenic variation during the process of hybrid formation and prophage induction

Although MuimmP22 hybrid carries the late genes of Mu-1 phage the host range and antigenicity of MuimmP22 are distinguishable from those of Mu-1 phage. We explained that the G segment containing the tail fiber gene S of Mu-1 phage can be inverted by the function of the gin gene which is situated immediately to the right of the G segment and the control gene of inversion of the G segment. Since the gin gene of Mu-1 phage is known to transact with the control gene hin of Salmonella on flagellar phase variation, the hin gene of the host should act on inversion of the G segment of Mu-1 phage. Thus the hin gene accelerates antigenic variation of these phages.

We suggested that this antigenic variation by inversion of the tail fiber gene S is due to transcription and translation in reverse producing a new polypeptide sequence of the tail fiber. In order to confirm this finding, we isolated MuimmP22 with the selective host after crossing P22 with Mu-1 phage carrying an amber mutation in the S gene. These hybrids were able to grow in an suppressor negative host, indicating that the amber phenotype is not expressed. Therefore, we concluded that during formation of hybrids between P22 and Mu-1 the G segment of the Mu-1 portion of the MuimmP22 hybrids is inverted.

Furthermore we suggested that both the hin function of the host and the gin function of the phage act on inversion of another late gene(s) for the secondary antigenic site for neutralization of plaque forming-activity.

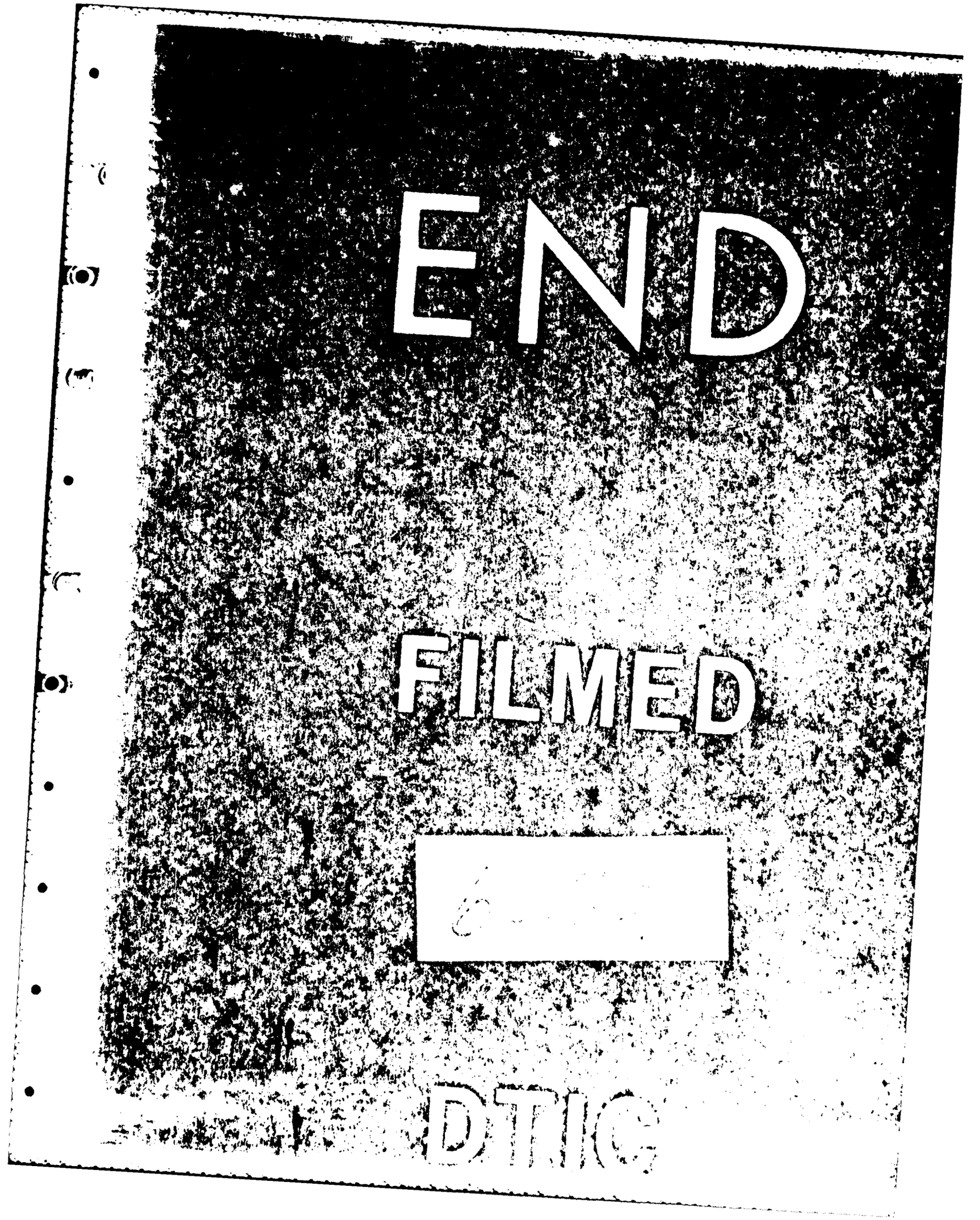
Publications

1. Yamamoto, N., Gemski., and Baron, L.S., 1983 Genetic studies of hybrid phages between coliphage ϕ80 and Salmonella phage P22. J. Gen. Virol. in press

2. Yamamoto, N., Droffner, M.L., Yamamoto, S., Gemski, P., and Baron, L.S., Specialized transducing derivatives of ϕ80immP22 phage. In preparation. To be submitted to Gene.

3. Yamamoto, N., Gemski, P., and Baron, L.S. Isolation and Characterization of hybrids between Salmonella Phage P22 and coli mutator phage Mu-1. In preparation. To be submitted to Gene.

12 Copies	Director (ATTN: SGRD-UWZ-AG) Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D.C. 20012
4 Copies	HQDA (SGRD-AJ) Fort Detrick Frederick, Maryland 21701
12 Copies	Defense Documentation Center ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 Copy	Commandant Academy of Health Sciences, U.S. Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 Copy	Dean School of Medicine Uniformed Services University of Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20014


END
FILMED
DTIC