

AD-A170 322

GRADUATE FELLOWSHIP FOR STUDY AND RESEARCH IN  
BIOGENETIC ENGINEERING(U) CALIFORNIA UNIV DAVIS  
J S MANNING ET AL 30 JUN 86 ARO-20401 1-LS-F

1/1

UNCLASSIFIED

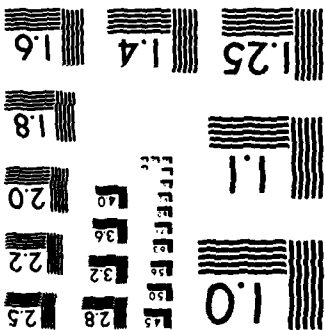
DAGG29-83-G-0004

F/G 6/13

NL



MICROCOPY RESOLUTION TEST CHART  
NATIONAL BUREAU OF STANDARDS-1963-A



GRADUATE FELLOWSHIP FOR STUDY AND RESEARCH IN BIOGENETIC ENGINEERING

FINAL REPORT

JA RUE S. MANNING  
Y.-S. LIU  
JOHN L. INGRAHAM

AD-A170 322

June 30, 1986

U. S. ARMY RESEARCH OFFICE

DAAG29-83-G-0004

DTIC  
ELECTE  
JUL 28 1986  
S  
E  
D

UNIVERSITY OF CALIFORNIA AT DAVIS

APPROVED FOR PUBLIC RELEASE;  
DISTRIBUTION UNLIMITED

DTIC FILE COPY

THE VIEW, OPINIONS, AND/OR FINDINGS CONTAINED IN THIS REPORT ARE THOSE OF THE AUTHOR(S) AND SHOULD NOT BE CONSTRUED AS AN OFFICIAL DEPARTMENT OF THE ARMY POSITION, POLICY, OR DECISION, UNLESS SO DESIGNATED BY OTHER DOCUMENTATION.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER <b>ARO 20401.1-LS-F</b>	2. GOVT ACCESSION NO. <b>AD-A170 322</b>	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) <b>GRADUATE FELLOWSHIP FOR STUDY AND RESEARCH IN BIOGENETIC ENGINEERING</b>		5. TYPE OF REPORT & PERIOD COVERED <b>Final 04/01/83 to 03/31/86</b>
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) <b>JaRue S. Manning, Y.-S. Liu and John L. Ingraham</b>		8. CONTRACT OR GRANT NUMBER(s) <b>DAAG29-83-G-0004</b>
9. PERFORMING ORGANIZATION NAME AND ADDRESS <b>The Regents of the University of California University of California, Davis Campus Office Of Research, 275 Mrak Hall, Davis, CA 95616</b>		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS <b>U. S. Army Research Office Post Office Box 12211 Research Triangle Park, NC 27709</b>		12. REPORT DATE <b>June 30, 1986</b>
		13. NUMBER OF PAGES <b>4</b>
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) <b>Unclassified</b>
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report)  <b>Approved for public release; distribution unlimited.</b>		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)  <b>NA</b>		
18. SUPPLEMENTARY NOTES <b>The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.</b>		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) <b>Infectious bovine rhinotracheitis virus, recombinant DNA, cosmid cloning, thymidine kinase, M13 sequencing</b>		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  <b>In an attempt to understand the gene expression of the infectious bovine rhinotracheitis virus (IBRV), the viral thymidine kinase gene (tk), a well regulated viral gene has been constructed in <u>Escherichia coli</u> HB101 by cloning partially Sau3A-digested DNA fragments into a cosmid vector, pJB8. Recombinant cosmids were further analyzed by restriction digestions and by Southern blot hybridization. Results showed that this plasmid library comprised all of the</b>		

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

IBRV genome with the exception of both termini. The individual recombinant cosmid clones were then to transform E. coli tdk<sup>-</sup> mutant strains, Ky895 or C600 tdk<sup>-</sup> for the selection of the IBRV tk gene. The clones able to grow on the selection plates containing 5-fluorouracil, uridine and thymidine were selected and further characterized. The physical location of the viral DNA inserts of one of the clones, pIBR5, was determined and sequences complementing the tk activity were isolated by subcloning. The plasmid, pIBRTK, was shown to grow on selection plates and therefore, retained the ability to complement the tk gene. The E. coli mutant strain C600 tdk<sup>-</sup> harboring pIBRTK partially restores the tk activity by exhibiting a three and a half fold increase in the level of the incorporation of [<sup>3</sup>H]thymidine into bacterial DNA over that of C600 tdk<sup>-</sup> mutant. The plasmid, pIBRTK was also used as the probe to examine the expression of IBRV-tk gene in infected MDBK cells. The results showed that the IBRV-tk gene was expressed as a beta group gene and the expression of the gene was dependent upon the alpha proteins. A species of mRNA from infected cells with a molecular weight of 2.2 kb was obtained when the total RNA or polyA-enriched RNA were electrophoresed in agarose gels and hybridized with <sup>32</sup>P-pIBRTK DNA.

## 1. FOREWARD:

Infectious bovine rhinotracheitis virus (IBRV) is a member of bovine herpesvirus family. The virus infects cattle and causes severe upper respiratory disease. It shares all the characteristics with other members of herpesvirus family. Especially, the herpesvirus can establish latency in animals. Our interest in this virus is not only limited to its pathological effects but also the molecular biology of the virus itself. The study of molecular biology of the virus will certainly help us to understand the virus-host interaction and pathogenesis. Eventually it will help us to establish better methods for treatment and prevention. The IBRV contains dsDNA of approximately 150 kb in length and potentially can encode for more than 100 genes. The viral genes expressed in a cascade fashion. The immediately early or alpha genes express without translation of any viral proteins. The early(beta) genes only express after the translation of alpha genes and prior to viral DNA replication. The late gene expression requires the expression of both alpha and beta genes. This makes IBRV genes to be one of the ideal model for study of eukaryotic gene expression.

Acceptance	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	



## 2. PROBLEM STUDIED:

### a) PURPOSE OF THE STUDY

*infectious bovine rhinotracheitis*

In an attempt to study regulation of IBRV gene expression, we chose one of the well known genes, thymidine kinase (tk) gene. The viral thymidine kinase is different from cellular TK in substrate specificities, pH and temperature optimum, etc (Weinmaster et al., 1982; Kit & Qavi, 1983). The TK is a beta protein and expressed only after the translation of alpha genes. In addition, in herpesvirus infection, TK activity was thought to be associated with the reactivation of the disease (Tenser et al., 1979). The direct approach to attack the problem is to clone the IBRV-tk gene followed the similar task used in the identification of HSV-tk gene by transfecting the tk-containing DNA fragment into tk defective mouse cells (Wigler et al., 1977). But the isolation of the IBRV-tk gene was considered to be difficult due to the incapability of converting the  $Ltk^-$  cells into  $tk^+$  phenotype. In this report, we took a novel approach to identify the gene and prove the gene isolated expressed as a beta group gene and encoded a message in IBRV-infected MDBK cells.

### b) SUMMARY OF MOST IMPORTANT RESULTS

We have previously generated a genomic library of IBRV DNA with the cosmid pJB8 system. One of the recombinant clones pIBR5 was able to complement the *E. coli*  $tdk^-$  mutant cells, Ky895 (Hiraga et al., 1967) and C600tdk (Strauss et al., 1983), and was able to grow on the selection plates containing 5-fluorouracil.

The recombinant cosmid pIBR5 was then subcloned to a smaller plasmid, pIBRTK which has a molecular weight of 4.2 kb and when transformed into *E. coli*  $tdk$  mutant cells was shown to have ability of forming colonies on drug selection plates. In addition, the Ky895 or C600tdk harboring pIBRTK showed the ability to partially restore the TK activity by incorporating [ $^3H$ ]-thymidine into bacterial cells followed the procedure reported by Strauss et al. (1983).

The expression of tk gene in IBRV-infected MDBK cells was examined. The total RNA from IBRV-infected cells was isolated by Guanidine Thiocyanate method (Maniatis et al., 1982) at different time points post infection (p.i.). The RNA pellet was obtained by centrifugation through a 5.7M CsCl solution overnight at 15°C in a Beckman SW27 rotor at 24K rpm. The polyA<sup>+</sup> RNA was enriched with a oligo(dT) chromatographic column. The total RNA or polyA-enriched RNA samples were then applied to a nitrocellulose paper presettled in a S&S (Schleicher and Schuell) slot blotter Minifold II apparatus. The blotted filter was then hybridized to



alpha-<sup>32</sup>P nick-translated pIBRTK DNA (Rigby et al., 1977). The results have shown that upon the normal infection, the tk-mRNA could be detected at 4 hr p.i. and reached at maximal expression at 6hr p.i.. The treatment with cycloheximide in IBRV-infected MDBK cells at earlier stage of infection blocked the tk gene expression, but addition of drug at 4 hr p.i. resulted in no effect on tk expreswsion in our system. The RNA samples were also analyzed by northern blot hybridization followed the procedures recommended by Amersham. A messenger with approximatedly 2.2 kb in size was detected.

The sequences of IBRV tk gene in pIBRTK have been analyzed by M13 sequencing methods recommended by Amersham. The plasmid pIBRTK was linearized and cloned into polylinker regions of M13mp18 and M13mp19. From those recombinant M13 phages, a series of overlapping deletion mutants were generated with the Rapid Deletion Subcloning system from IBI. The sequences showed to be G+C rich and have open reading frames. The establishment of complete nucleotide sequences is in progress.

The above inforamtions considering the IBRV-tk gene should provide us (1) a model system to study the gene regulation in bovine herpesviruses; (2) an opportunity to examine the cis-acting regulatory region of a bovine herpesvirus gene at molecular level; (3) a feasible approach to identify trans-acting factors involving gene expression upon the viral infection; (4) a possibilities to manipunate viral DNA geneticaaly with relative ease for the purpose of the disease control and prevention; and (6) a biochemical tool to study the latency and reoccurrence of bovine herpesvirus infection.

### 3. PUBLICATION:

Katie Yu-sun C. Liu and JaRue S. Manning. Identification of the thymidine kinase gene of infectious bovine rhinotracheitis virus and its function in Escherichia coli hosts. (GENE, in press).

### 4. LISTS OF PERSONNEL:

Yu-sun (Katie) C. Liu; Degree received. Ph.D. June, 1986.

JaRue S. Manning (Professor); Supervising Investigator.

John Ingraham (Professor); Principal Investigator.

### 5. BIBLIOGRAPHY:

Weinsmaster, G.A., Misra, V., McGuire, R., Babiuk, L.A. and DeClercq, E.: Bovid herpesvirus type-1 (Infectious bovine rhinotracheitis virus)-induced thymidine kinase. Virol. 118 (1982)191-201.

Kit, S. and Qavi, H.: Thymidine kinase (TK) induction after infection of TK-deficient rabbit cell mutants with bovine herpes virus type 1 (BHV-1): Isolation of TK<sup>-</sup> BHV-1 mutants. Virol. 130 (1983)381-389.

Tenser, R.B., Miller, R.L. and Rapp, F.: Trigeminal ganglion infection by thymidine kinase-negative mutants of herpes simplex virus.

Science 205(1979) 915-917.

Wigler, M., Silverstein, S., Lee, L.S. Pellicer, A., Cheng, Y.C. and Axel, R. Cell 11(1977) 223-232.

Hiraga, S., Igarashi, K. & Yura, T.A. : A deoxythymidine kinase-deficient mutant of E. coli. I. Isolation and some properties. Biochemica ET Biophys. Acta 145 (1967) 41-51.

Strauss, M. Kiessling, U. and Kahler, R.: Plasmids expressing the herpes simplex virus thymidine kinase gene in mammalian and bacterial cells. Mol. Gen. Genet. 191 (1983) 154-157.

Maniatis, T., Fritsch, E.F. and Sambrook, J.: Molecular Cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1982.

END

DTIC

9-86