GRADUATE FELLOWSHIP FOR STUDY AND RESEARCH IN BIOGENETIC ENGINEERING

FINAL REPORT

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### Graduate Fellowship for Study and Research in Biogenetic Engineering

**Title:** Infectious bovine rhinotracheitis virus, recombinant DNA, cosmid cloning, thymidine kinase, M13 sequencing

**Abstract:**

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 *Escherichia coli* 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...
IBRV genome with the exception of both termini. The individual recombinant cosmid clones were then to transform E. coli tdk- mutant strains, Ky895 or C600 tdk- 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- harboring pIBRTK partially restores the tk activity by exhibiting a three and a half fold increase in the level of the incorporation of [3H]thymidine into bacterial DNA over that of C600 tdk- 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 32p-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.
2. PROBLEM STUDIED:

a) PURPOSE OF THE STUDY

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 mutat 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 150C in a Beckman SW27 rotor at 24K rpm. The polyA⁺ RNA was enriched with a oligo(dT) chromatographic column. The total RNA or polyA⁺-enriched RNA samples were then applied to a nitrocellulose paper presetted in a S&S (Schleicher ans Schuell) slot blotter Minifold II apparatus. The blotted filter was then hybridized to
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 6 hr 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 expression in our system. The RNA samples were also analyzed by northern blot hybridization followed the procedures recommended by Amersham. A messenger with approximately 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 manipulate viral DNA genetically 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:


4. LISTS OF PERSONNEL:

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5. BIBLIOGRAPHY:


