Infectious laryngotracheitis virus growth, DNA replication, and protein synthesis

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Summary. The polypeptides associated with infection of primary chicken kidney (CK) cells with infectious laryngotracheitis virus (ILTV) were examined by metabolic labelling with [³⁵S]methionine and SDS-PAGE. Polypeptide synthesis was followed over the first 24 h post-infection (p.i.) as this was shown to be the period of viable virus production. A total of 16 ILTV encoded or induced polypeptides were identified using metabolic labelling. The use of inhibitors of protein and DNA synthesis in conjunction with metabolic labelling and viral DNA replication studies enabled a cascade pattern of gene expression, similar to that of herpes simplex virus type 1 (HSV-1), to be established for ILTV. Representatives of alpha, beta, gamma 1 and gamma 2 classes of genes were identified. In contrast to infection with HSV types 1 and 2, which leads to a rapid inhibition of total host cell polypeptide synthesis, ILTV infection of CK cells did not result in a complete inhibition of cellular protein synthesis, with only a small number of host cell polypeptides absent from infected cells.

Introduction

Infectious laryngotracheitis virus (ILTV) is a herpesvirus which causes an acute upper respiratory-tract infection of economic significance in commercial poultry. In Australia infectious laryngotracheitis (ILT) is presently controlled by a live attenuated vaccine, which still retains some pathogenicity particularly in younger chickens [1]. A safer, more easily administered molecular based vaccine would provide significant advantages to the intensive poultry industry.

The genome of ILTV, an alphaherpesvirus, is linear double stranded DNA of approximately 155 kilobase pairs (kb) and has a similar structure to that of pseudorabies virus [2, 14, 16, 18, 28]. From random DNA sequencing of ILTV DNA Griffin [10] was able to identify 21 ILTV genes by sequence homology to other herpesviruses. Of the 21 genes identified 20 had significant sequence
homology to genes of varicella-zoster and 19 to genes of herpes simplex virus type 1 (HSV-1) both alphaherpesviruses; 12 genes were found to have significant sequence homology to genes of Epstein-Barr virus, a gammaherpesvirus. Sequence data of the thymidine kinase gene and the upstream overlapping genes of ILTV also provide evidence that homology exists at the DNA level between ILTV and other alphaherpesviruses [11].

Infection of cells with HSV-1 and HSV-2 results in a shut-off of host cell protein synthesis. Inhibition of host protein synthesis is a multiphase process (reviewed in [9]) initially involving a component of the virion. The second phase requires the expression of the viral genome. The inhibition of host protein synthesis is accompanied by a sequential expression of viral genes. Three groups of HSV-1 proteins have been defined based on their temporal regulation, and functional requirements for gene expression [12]. The synthesis of HSV-1 proteins is regulated in a cascade fashion. As the later viral genes are expressed, the synthesis of preceding genes is in turn down regulated (reviewed in [26]). The first class of genes expressed are the alpha genes, which do not require prior viral protein synthesis for expression, but are enhanced by a component of the infectious particle [5, 7, 19, 20]. Five HSV-1 alpha genes have been identified to date, all appear to have regulatory functions with the possible exception of alpha 47 (reviewed in [27]).

Synthesis of the beta genes follows, and is dependent on the presence of functional alpha gene products. Beta gene expression starts early in infection and declines after DNA replication. The third class of genes to be expressed are the gamma genes, which are divided into two groups based on their requirement for prior viral DNA replication. The gamma 1 genes are expressed at low levels in the absence of viral DNA replication, whilst expression of the gamma 2 genes is completely or almost completely absent when virus DNA synthesis is blocked [6]. Cascade patterns of temporal gene regulation have also been demonstrated for several other herpesviruses including cytomegalovirus [3, 29], pseudorabies virus [13], herpes saimiri virus [23] and equine herpes virus type 1 [4].

This paper reports the results of studies on gene expression of ILTV in primary chicken kidney (CK) cells. The effect of various metabolic inhibitors on the synthesis of viral proteins was used to demonstrate a cascade pattern of gene expression similar to that of HSV-1.

**Materials and methods**

**Cells and viruses**

Primary CK cells were prepared by trypsinization of kidneys isolated from two to four week old specific pathogen free (CSIRO, SPF Poultry Unit, Maribyrnong, Victoria) chickens as described previously [8]. ILTV SA2 vaccine strain was obtained from Arthur Websters Pty. Ltd. (Castle Hill, N.S.W., Australia). ILTV was grown on CK cells in Eagle’s basal medium (Gibco Laboratories) supplemented with 5% bovine calf serum (BCS) and 10 mM HEPES.