Replication and Transcriptional Regulation Review of Respiratory Syncytial Virus (RSV)

Md Ashrafuzzaman1*, Zannatul Naim1, Md Mustahsan Billah2, S M Masud Rana1*

1Department of Biomedical Engineering, Military Institute of Science and Technology (MIST), Dhaka, Bangladesh
2Department of Pharmacy, Dhaka International University, Dhaka, Bangladesh

*Corresponding Author: Md Ashrafuzzaman and S M Masud Rana, Department of Biomedical Engineering, Military Institute of Science and Technology (MIST), Dhaka, Bangladesh.

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Abstract

Respiratory Syncytial Virus shortly RSV is virus that reason for respiratory disease in people. About 90% of infants and young children by the age of 2 years are affected by RSV. This is a seasonal virus. The Respiratory Syncytial Virus genome consists of a single strand of negative sense RNA. RNA-dependent RNA polymerase in the cytoplasm of the host cell is responsible for replication and transcription. Respiratory Syncytial Virus transcription is regulated by the N, P, L and M2-1 and M2-2 Proteins. The main aim of the review is focus on replication process of RSV and how transcription process is regulated.

Keywords: Respiratory Syncytial Virus; Replication; Transcriptional Regulation

Introduction

Respiratory syncytial virus (RSV) is a single –strand negative sense RNA virus [1] and is classified in the Order Mononegavirales Family Paramyxoviridae. Previously it known was as chimpanzee coryza agent (CCA).

RSV strains are classified into genotypes within 2 major RSV subgroups, RSV-A and RSV-B.

RSV is the leading viral cause of acute lower respiratory tract infections, including bronchiolitis and pneumonia, among infants and young children global [2].

It is a common virus that infects the linings of the airways - the nose, throat, windpipe, bronchi and bronchioles (the air passages of the lungs).

Approximately two thirds of infants are infected with RSV within their first year and 90% have been infected by the age of 2 [3].

Discovery

Respiratory syncytial virus (RSV) was isolated as a novel virus in 1956 from laboratory chimpanzees with common cold infection [4]. Then Channock., et al. [5] isolated it from children with pulmonary disease (pneumonia and bronchiolitis) in Baltimore, USA after few years later.

Size and Structure

The RNA of RSV is consisting of 15,191 base pairs (https://microbewiki.kenyon.edu/index.php/Human_respiratory_syncytial_virus). Average size 120 - 200 nm which enveloped with lipoprotein (nucleocapsid N protein within a lipid) coat and a linear. The RSV genome is composed of 15000 nucleotides and encodes 10 viral proteins including structural and non-structural [6]. The virion of the RSV is enveloped with a lipid bilayer contains three surface
glycoproteins, G- the attachment protein, SH- the small hydrophobic protein and F- fusion protein which are separated from each other and can be seen as “spikes” that project out of the virion. The glycoproteins can be measured to be about 11 - 20 nm in size, while the virion appears to be about 150 - 300 nm in diameter [6]. The glycoprotein, G, is a type II transmembrane glycoprotein and is the major RSV attachment protein [7]. It contains a single hydrophobic region which serves as a signal peptide and also as a membrane anchor. The small hydrophobic SH protein is a short integral membrane protein whose function is unknown. However, it is suggested that the SH protein enhances the function of the attachment protein and or fusion protein. The major function of the F protein is to direct viral penetration by the fusion between the virion and the host plasma membrane. Another RSV protein is the matrix protein M, located in the inner layer of the lipid bilayer; and is found to play a role in the formation of virus-like particles. These four proteins are used to form the viral envelop. RSV also consists of two non-structural proteins, NS1 and NS2, these proteins enhance viral growth but are not essential [6]. The virion consists of a nucleocapsid which is contained in the lipid bilayer. The nucleocapsid has a symmetrical helix shape and is measured to be about 12 – 15 nm in diameter. There are four nucleocapsid proteins inside the virion which carry out the replication and transcription of the RSV genome: [3]

1. The nucleocapsid protein N,
2. The phosphoprotein P,
3. The antitermination factor M2-1, and
4. The large polymerase subunit L

The RSV nucleocapsid N protein binds to genomic and antigenomic RNA to provides protection of the viral RNA from the toll-like receptors and RNA recognition helicases that initiate immune responses. The P protein acts as a chaperonin for the N protein, without it the N protein would be incapable to binding to minigenome RNA. The L protein is responsible for all enzymatic activity and the M2-1 protein is a transcription antitermination factor which is crucial for viral viability. Proteins M2-1 and M2-1 are both play important roles in balancing transcription and RNA replication [6].

Classification

Group: Group V ((-)ss RNA)
Order: Monoegvirles
Sub Family: Pneomovirus
Family: Pneumoviridae, Paramyxoviridae (Collins et al 2001).
Genus: Orthopneumovirus  
Species: Human respiratory syncytial virus

**Diagnosis [8]**

RSV infection can be either diagnosed by cell culture techniques or by the identification of viral antigen through rapid diagnosis techniques. Rapid diagnosis is significant for the initiation of proper infection control procedures and for possible antiviral chemotherapy. It also diagnosed by serological tests but these require a long time for the result to become available.

**Rapid Diagnosis**

**Immunofluorescence**

*Advantages:* Both direct and indirect IF utilizing either polyclonal or monoclonal antibodies are available which possess a high degree of sensitivity and specificity. The general sensitivity of IF is 80 - 90% and for monoclonal antibody 95 - 100%. IF techniques are fast and easy to perform.

*Disadvantages:* Interpretation of results is subjective and the specimen must contain adequate nasopharyngeal cells.

**ELISA**

*Advantages:* Objective interpretation, speed, and the possibility of screening a large number of specimens.

*Disadvantages:* Generally poorer sensitivity and a “grey zone” of equivocal results, which requires confirmation by a time-consuming blocking ELISA procedure.

**Cell culture**

Human heteroploid cells, such as HEP-2 and HeLa generally provide the best tissue culture for the isolation of RSV. RSV produces a characteristic CPE consisting of syncytia formation and appears in 4 to 5 days.

**Serology**

Detecting antibody rises in acute and convalescent sera, the length of time required. The serological response in young infants may be poor and not detectable by some antibody assays. It may require 4 - 6 weeks.

RSV infection can be confirmed using tests for antigens or antibodies, or viral RNA by reverse transcription PCR. Quantification of viral load can be determined by various assay tests.

![Syncytial formation caused by RSV in cell culture. (Courtesy of Linda Stannard, University of Cape Town, SA.](image)

**Figure 2:** Syncytial formation caused by RSV in cell culture. (Courtesy of Linda Stannard, University of Cape Town, SA.)
Treatment

Patient with severe RSV infections usually need to be admitted to the hospital. They are will be treated with IV fluids (e.g. Normal Saline), oxygen, and humidified air in hospital.

Bronchodilators may be used, but they are not helpful in every case as a medicine. Some patients with extremely severe condition may need to be placed on a mechanical support (e.g. artificial ventilator) to help them breathe.

Normal saline administration may be effective for the patients, one study noted a 26% reduction in length of stay: 2.6 ± 1.9 days without treated normal saline, compared with 3.5 ± 2.9 days in the normal-saline treated group (p = 0.05) [9].

Pathogenesis

RSV enters the body through the mucous membranes in the eye, nose, throat and mouth (rarely) via the respiratory mucosa [10]. RSV is spread by direct exposure to large droplet secretions through coughing and sneezing and by direct contact with contaminated surfaces [11]. The virus attacks the epithelium of the respiratory tract, by cell to cell transfer. The virus may produce bronchiolitis, pneumonia in the lower respiratory tract after spread. During bronchiolitis, a peribronchiolar inflammation with lymphocytes (early stage), characteristic necrosis and sloughing of the bronchiolar epithelium occur. This sloughed necrotic material creates an obstruction to the flow of air by plugging the bronchioles. Air trapped to the sites, causing the characteristic hyperinflation of bronchiolitis. It may take approximately six or seven days. The incubation period of RSV disease has been reported as being 2 - 8 days [6].

Epidemiology

RSV has two heterotypic strains, group A and group B, which both spread simultaneously during an epidemic. [12]. Both RSV A and B have various subgroups; the antigenic properties of the G surface glycoprotein create the major difference between these subgroups [13]. It has been suggested that Group A viruses are associated with more severe disease and this group viruses tend to predominate [14,15].

It is a seasonal virus, e.g. during the rainy season in tropical climates occurring with outbreak [16].

In the UK, epidemics of RSV happen in a distinct seasonal pattern between the months of November and April, peaking in December, January and February, with outbreaks lasting an average of 22 weeks [16].

There is no particular age-group that is not at risk for RSV infection, although infants and children’s are more susceptible to RSV infection: It affects about 90% of infants and young children by the age of 2 years e.g. in Houston, USA, Infection rates were 68-8 per 100 child-years in infancy, and 82-6 per 100 child-years in the second year of life [17]. RSV infection (e.g. lower respiratory-tract infections) in ten developing countries: Argentina, Colombia, Guatemala, Kenya, Nigeria, Pakistan, Papua New Guinea, the Philippines, Thailand, and Uruguay were very common under 5 years of age children [18].

Risk of RSV disease can be increased various factors such as: prematurity [19], multiple births, congenital heart diseases [20] and immunodeficiency [21]. Environmental factors, such as household crowding, school-age siblings, day- care attendance, passive exposure to smoke and malnutrition, also further predispose to RSV infection [22].

Replication

Viral gene expression and replication occurs in the cytoplasm. Once the virus is in the cytoplasm the nucleocapsid and the genome is released. The M2-2 gene governs the transition from transcription to production of genomic RNA. The polymerase then enters the genome at its 3’ end and the genes are transcribed into mRNAs by the start-stop-restart synthesis. This creates a polar transcription gradient in which the promoter starting genes are transcribed more frequently than the genes which are downstream. Replication generates a complete positive-sense RNA complement of the genome called the antigenome, which acts as a template for genome synthesis [23]. The genome and the antigenome are both coated with the N protein at all time which serves as the template for RNA synthesis. The M protein.
regulates the assembly of the RSV by interacting with the envelope proteins F and G and with the nucleocapsid proteins N, P, and M2-1. The new synthesized proteins then self-assembly and budding occurs, acquiring an envelope from the membrane.

The RSV genome consists of a single strand of negative sense RNA, which is transcribed and replicated by the virus RNA-dependent RNA polymerase in the cytoplasm of the host cell (Figure 3).

The first step in viral replication is attachment of the viral particle to the host cell in the nasal epithelium. The viral RNA enters the host cell along with the viral enzymes that direct production of a new viral RNA and proteins. Multiple new viruses are assembled within the cell, which is ultimately destroyed. The destruction of ciliated epithelial cells lining the airways ultimately causes the symptoms characteristic of the infection. For most individuals, if epithelial cell destruction is limited, RSV is restricted to the upper respiratory tract producing influenza-like illness or appearing as a persistent cold that is self-limiting. However, in some individuals where large amounts of epithelial cells are destroyed, they release a number of pro-inflammatory mediator substances, including cytokines, which cause increased capillary permeability and elevated secretion production. Chemokines are released, attracting additional pro-inflammatory cells, such as macrophages, neutrophils, eosinophils and natural killer cells, to the site of infection (Van Schaik, et al. 2000). Increased capillary permeability results in leakage of plasma proteins into interstitial areas, small airways and alveoli. This causes generalized interstitial swelling and appears to inhibit pulmonary surfactant function. The combination of increased secretion production, decreased secretion clearance due to compromised mucociliary elevator function, and ineffective surfactant function results in small airways filling with secretions and debris from destroyed cells. The release of bronchoconstrictor substances may cause small airways to narrow even further, resulting in increased airway resistance, air trapping and wheezing, which are characteristic of severe lower respiratory-tract infections (Van Schaik, et al. 2000).

Figure 3: The RSV replication cycle. The virus enters by direct fusion at the plasma membrane, releasing the encapsidated genome RNA (blue) and RNA dependent RNA polymerase (green) into the cytoplasm. The polymerase uses the genome as a template to produce capped and polyadenylated mRNAs, which are translated into viral proteins, and encapsidated antigenome and genome RNAs. The resulting encapsidated genomes are assembled with other viral proteins and bud from the plasma membrane to produce progeny virus particles. (Courtesy of Rachel Fearns, Ph.D. Associate Professor of Microbiology, School of Medicine, Boston University, USA). ref: http://www.bumc.bu.edu/microbiology/people/faculty-old/rachel-fearns-phd/
Replication produces antigenome and genome RNAs, which are full-length and encapsidated with virus nucleoprotein. Replication is initiated from a single promoter region at the 3’ end of the genome and it is currently unclear how the polymerase is controlled between these two activities.

MicroRNAs (miRNAs) are small single-stranded RNA molecules involved in the regulation of gene expression at the post-transcriptional level.

**Transcriptional Regulation**

Transcription and replication are regulated by a unique promoter in the 31 leader (Le) region of the genome. Transcription by the RNA-dependent-RNA-polymerase composed of L and P proceeds directly from the negative sense (31-51) genome through the production of capped/polyA monocistronic mRNAs [24].

RNA transcription by Respiratory Syncytial Virus (RSV) is directed by the N, P, and L Proteins; some additional factor supplied by RSV superinfection appeared to be involved in transcription [25].

![Figure 4: Transcriptional Regulation by different protein of RSV/ Regulation through transcription factor 281 such protein.](image)

Five RSV proteins (N, P, L, M2-1, and M2-2) are involved in transcription or RNA synthesis (21).

The nucleoprotein N is essential factor that provides protection of the viral RNA from the toll-like receptors and RNA recognition helicases that initiate immune responses by binding to genomic and antigenomic RNA.

The P protein acts as a chaperonin for the N protein, without it the N protein would be incapable to binding to minigenome RNA.

The Polymerase or Large protein L is the major polymerase subunit and contains the catalytic domains and responsible for all enzymatic activity.

The Phosphoprotein P is an essential cofactor in Transcription (31). The M2-1 and M2-2 proteins are factors involved, in transcription (42) that is balancing between transcription and RNA replication Collins 2001 (9). RSV, processive transcription depends on the M2-1 protein, which is essential for viral viability (42). The M2-1 protein is also crucial for viral viability.

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Four other RSV proteins (G, F, SH and M) associate with the lipid bilayer to form the viral envelope (21).

The matrix M protein lines the inner envelope surface and is important in virion morphogenesis (147). The heavily glycosylated G, fusion F, and small hydrophobic SH proteins are transmembrane surface glycoproteins (Figure 3).

G and F are the only virus neutralization antigens and are the two major protective antigens (21). The G glycoprotein plays a major role in viral attachment (148). It contains several N-linked carbohydrate side chains and an estimated 24 to 25 O-linked side chains. G is anchored in the membrane by a signal/anchor sequence near the N terminus and also is expressed as a secreted form. This secreted form arises from translational initiation at the second methionine (codon 48) in the open reading frame followed by proteolytic trimming to yield a final form that lacks the N-terminal 65 amino acids, including the entire signal/anchor (129). The G ectodomain contains a highly-conserved domain of 13 amino acids whose significance is unknown (146). This conserved sequence overlaps a disulfide bonded tight turn that is called a cystine noose and contains a CX3C motif that is discussed later.

The F protein mediates fusion of infected cells with their neighbors to form syncytia and directs viral penetration by membrane fusion.

The remaining two RSV proteins, NS1 and NS2, are small species that do not appear to be packaged significantly in the virion. As described below, they are nonessential accessory proteins involved in modulating the host response to infection.

Gene expression and RNA replication by RSV broadly follow the mononegavirus model, although admittedly there are substantial gaps in our understanding of these processes even for prototypical mononegaviruses (25). The polymerase enters the genome at or near its 3' end, and the genes are transcribed into individual mRNAs by sequential start-stop-restart synthesis that is guided by short transcription signals flanking the genes. There is a polar gradient of mRNA abundance due to polymerase fall-off. RNA replication involves synthesis of the full-length positive-sense antigenome that in turn is copied into progeny genomes. RSV adds some complexity of its own with the M2-1 and M2-2 proteins, which are found only in close relatives.

On the other hand, MicroRNA (miRNAs) are small single-stranded RNA molecules involved in the regulation of gene at the post-transcriptional level of RSV during airway cells infections.

**Conflict of Interest**

No conflict of interest.

**Bibliography**


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