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Initiation and regulation of paramyxovirus transcription and replication

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ABSTRACT

The paramyxovirus family has a genome consisting of a single strand of negative sense RNA. This genome acts as a template for two distinct processes: transcription to generate subgenomic, capped and polyadenylated mRNAs, and genome replication. These viruses only encode one polymerase. Thus, an intriguing question is, how does the viral polymerase initiate and become committed to either transcription or replication? By answering this we can begin to understand how these two processes are regulated. In this review article, we present recent findings from studies on the paramyxovirus, respiratory syncytial virus, which show how its polymerase is able to initiate transcription and replication from a single promoter. We discuss how these findings apply to other paramyxoviruses. Then, we examine how *trans*-acting proteins and promoter secondary structure might serve to regulate transcription and replication during different phases of the paramyxovirus replication cycle.

Introduction

The family *Paramyxoviridae* is large and diverse. It encompasses viruses that infect reptilian, avian and mammalian hosts, and includes a number of human pathogens, such as respiratory syncytial virus (RSV), mumps (MuV), measles (MeV), parainfluenza viruses (PIV 1–5), and the newly emerged Nipah and Hendra viruses. The family is divided into two subfamilies, the *Pneumovirinae and Paramyxovirinae*, which contain two and seven genera, respectively (King et al., 2012). The paramyxoviruses have a single stranded, negative sense RNA genome, and so are members of the non-segmented, negative sense (NNS) RNA virus order. The paramyxoviruses also share a similar (although not identical) cohort of genes as each other. During their replication cycle, the viral genome is transcribed to produce subgenomic, capped and polyadenylated mRNAs and replicated to produce encapsidated antigenome and genome RNAs (Lamb and Parks, 2007). Despite the fact that approximately two-thirds of the paramyxovirus genome encodes proteins involved in performing and regulating gene expression and genome replication, paramyxoviruses only encode one polymerase. This raises questions that have puzzled researchers for more than three decades, namely how does the polymerase become committed to either mRNA transcription or genome replication, and how can these processes be differentially regulated? In this review, we attempt to address these questions. The review is divided into three parts. Part 1 presents an overview of paramyxovirus transcription and replication and discusses previously proposed models. In Part 2, we describe relatively new findings regarding RSV transcription and replication, propose a revised model that fits these data, and discuss if this revised model can be applied across the paramyxovirus family. With models to describe possible mechanisms of transcription and replication initiation, it is possible to consider how these processes might be regulated during infection and in Part 3 we describe information available regarding regulation of transcription and replication, highlighting similarities and differences across the family.

Part 1: paramyxovirus transcription and replication

Overview of paramyxovirus transcription and replication

The general strategy of paramyxovirus transcription and replication is similar to that of other NNS RNA viruses (Lamb and Parks, 2007; Whelan et al., 2004) and much of what we know has been as a result of studies on another virus in the order, vesicular stomatitis virus (VSV), a member of the family *Rhabdoviridae*. However, there is a considerable body of research on paramyxoviruses, as reviewed previously (Lamb and Parks, 2007), and this is described below.

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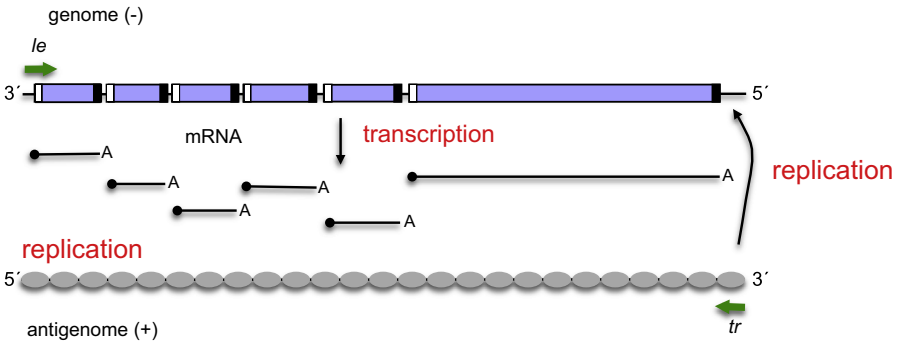

Fig. 1. Schematic diagram illustrating a representative paramyxovirus genome and transcription and RNA replication products. The genes are represented by purple boxes, and *gs* and *ge* signals are illustrated with white and black boxes, respectively. The *le* and *tr* promoters at the 3′ ends of the genome and antigenome, respectively, are indicated with green arrows. The genome acts as a template for mRNA and antigenome synthesis, and the antigenome as a template for genome RNA synthesis. The mRNA caps are indicated with black circles. The antigenome is shown covered with gray ovals, representing N protein, to indicate that it is encapsidated. The genome is also encapsidated, but this is not shown so that the *cis*-acting signals can be clearly seen.

The core polymerase is a complex of two proteins, the large polymerase subunit L, which contains the enzymatic domains involved in RNA synthesis, capping and cap methylation, and the phosphoprotein, P, which is an essential cofactor (Morin et al., 2013; Horikami et al., 1992; Noton et al., 2012; Mazumder et al., 1994). The polymerase executes transcription and replication by recognizing and responding to a number of essential *cis*-acting elements within the virus genome (Fig. 1). At the beginning and end of each gene are short (~10–13 nt) conserved signals termed *gene start* (*gs*) and *gene end* (*ge*) signals, respectively, and between each gene is a short, non-transcribed intergenic region. At the 3′ end of the genome, before the first gene, is a *leader* (*le*) promoter region, which is ~40–55 nt long, and at the 5′ end is a trailer region which is variable in length, depending on the virus (Lamb and Parks, 2007). An important feature of the genome template is that it is associated along its length with an interlinking polymer of nucleoprotein (N) to form a helical nucleocapsid, such that *cis*-acting RNA elements are buried within the N-RNA structure (Cox et al., 2014; Ruigrok et al., 2011; Tawar et al., 2009). Most likely for this reason, when the polymerase transcribes the genome to produce mRNAs, it cannot access individual genes independently. Instead, it first engages the template at or near the 3′ end of the genome, within the *le* promoter. The polymerase then moves along the genome, presumably with the N subunits of the nucleocapsid being displaced and replaced as the polymerase passes by. As the polymerase proceeds, it responds to the *gs* and *ge* signals it encounters to generate the subgenomic mRNAs: at a *gs* signal, the polymerase initiates mRNA synthesis (opposite the first nucleotide of the *gs*) and at the *ge* signal, it releases the RNA (Lamb and Parks, 2007). The polymerase can then scan the intergenic region to locate the next *gs* signal and begin mRNA synthesis of the next gene (Fearns and Collins, 1999). This allows the polymerase to generate subgenomic RNAs. The mRNAs are also modified to contain a 5′ methyl cap and 3′ poly A tail. Work with VSV and the paramyxovirus, Sendai virus (SeV) indicates that the complement of the *gs* signal, which lies at the 5′ end of the mRNA, contains a signal that directs the capping reaction and methylation of the cap (Ogino et al., 2005; Wang et al., 2007; Stillman and Whitt, 1999). The *ge* signal contains a poly U tract, and it is thought that stuttering of the polymerase on this U-stretch leads to polyadenylation of the mRNA (Whelan et al., 2004). Similarly to cellular capping, there is evidence that addition of the cap is important to allow the transcribing polymerase to transition into an elongation mode: in the case of RSV, if capping is inhibited, the polymerase aborts RNA synthesis approximately 45–50 nt after initiating at the *gs* signal (Liuzzi et al., 2005).

To replicate the genome, the polymerase also initiates RNA synthesis at the *le* promoter. In this case, it must initiate precisely opposite the first nucleotide of the template. During replication, the polymerase does not respond to the gene junction signals, but instead elongates the nascent RNA along the complete length of the genome to produce a positive sense antigenome. The 3′ end of the antigenome contains the complement of the trailer, referred to here as *tr* promoter. The *tr* promoter in turn signals the polymerase to initiate and perform genome RNA synthesis. The antigenome and genome RNAs are not capped, but instead are encapsidated with N protein, which is delivered to the elongating RNA in a complex with P (N_0P, where N_0 is a monomer of N) (Horikami et al., 1992). It is thought that concurrent encapsidation causes the polymerase to enter a super-processive mode, allowing it to disregard the *ge* signals and extend to the end of the template (Vidal and Kolakofsky, 1989; Gubbay et al., 2001; McGivern et al., 2005). Encapsidation initiation appears to be dependent on *cis*-acting elements in the *le* and *tr* promoters (McGivern et al., 2005). These elements could function in the context of the promoter within the template strand, to recruit a specific pool of polymerase that is capable of delivering N protein onto the RNA that it is synthesizing. Alternatively, they could function at the 5′ end of the nascent RNA product to signal an initial nucleation event that begins polymerization of N protein onto the growing RNA chain.

What emerges from this description of transcription and replication is that the *le* and *tr* promoters and the *gs* signals are all multifunctional entities, which are not only important for directing initiation of RNA synthesis, but also directing modification of the RNA products. These modifications enable the polymerase to elongate the RNA and also serve to protect the RNA from nucleases. This multifunctional nature of the *cis*-acting signals complicates analysis of the initial events in RNA synthesis, particularly in cell-based assays in which abortive (*i.e.* prematurely released), unmodified RNAs might be unstable, and this is the reason why understanding mechanisms underlying transcription and replication initiation and regulation has proven difficult.

NNS RNA virus transcription and replication initiation models

A complexity in understanding mechanisms by which the polymerase is coordinated between transcription and RNA replication is that it is difficult to conclusively define where transcription begins on the viral genome. The mRNA for the first protein-coding gene is initiated at the first *gs* signal (at ~nt 40–55), but how the polymerase accesses this signal has been the focus of debate (Curran and Kolakofsky, 2008; Whelan, 2008; Banerjee, 2008). Three models have been proposed to explain how this could happen, based largely on studies with paramyxoviruses (mainly SeV, PIV-3 and RSV) and VSV.

Model 1: according to this model, transcription and replication are both initiated in exactly the same way, opposite the first nucleotide of the *le* promoter (Vidal and Kolakofsky, 1989; Kolakofsky et al., 2004). In its simplest version, this model postulates that a single pool of

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