

Analysis of the Human Parainfluenza Virus Replication Promoter

Leif Magnusson

Faculty Sponsor: Michael Hoffman, Department of Microbiology

ABSTRACT

Human Parainfluenza Virus type 3 (HPIV3) has been shown to be a major cause of lower respiratory tract infections in children, elderly, and immunocompromised individuals. HPIV3 is a member of *Paramyxoviridae* family which includes many other virulent species such as the mumps, measles and canine distemper viruses. A region of the HPIV3 genome, bases 13-28, has been identified as important for the replication of the RNA genome of HPIV3. Simian Virus 5 (SV5), another member of the *Paramyxoviridae* family, has been shown to have a region that appears to be similar to HPIV3 13-38 region. This SV5 region has been shown to remain functional when its position within the genome is shifted. To better understand the characteristics of the HPIV3 13-28 element, and its similarity to the other sequences involved in replication of members of the *Paramyxoviridae*, the effects of moving HPIV3 bases 13-28 was examined. The 13-28 region was shifted six and twelve bases and checked for function. The resulting mutants showed a significant decrease in the replication of HPIV3, demonstrating that the 13-28 region of HPIV3 is not mobile within the genome. This indicates that the HPIV3 13-28 region may not be homologous to the SV5 region.

INTRODUCTION

Human parainfluenza virus (HPIV) is an enveloped, single-stranded, RNA virus belonging to the family *Paramyxoviridae*. There are four types of human parainfluenza viruses (HPIV1-4), all of which are spread through the respiratory route. HPIV3 is a major cause of lower respiratory tract diseases such as pneumonia, bronchiolitis, and croup in infants, young children, elderly and immunocompromised individuals, but usually presents as a common cold in healthy individuals (1).

HPIV3 virus particles consist of six major proteins: Nucleocapsid (N), Phosphoprotein (P), Matrix (M), Fusion (F), Hemagglutinin-Neuraminidase (HN), and Polymerase (L). The N protein is responsible for encapsidating the RNA genome, while P and L combine to form the functional RNA polymerase. These three proteins are the only viral proteins needed for replicating the viral genome. For replication of the viral RNA genome, the genomic promoter (GP) (at the 3' end of the genome, Fig. 1) serves as the binding site for the RNA polymerase when the polymerase begins to copy (replicate) the genome into a complementary strand. This complementary strand is termed the antigenome. Then, the 3' end of the antigenome, called the antigenomic promoter (AGP), is bound by the viral RNA polymerase to copy the genome from the antigenome. These new genomes can be packaged into new virus particles (1).

To thoroughly understand the process of viral genome replication, the Hoffman lab has investigated the GP in more detail (Fig. 1) (2,3). Within the GP, discrete, sequence elements have been found to be necessary for replication of the genome. These necessary elements are bases 1-12 and 79-96. Another element, bases 13-28, has been found to be important but the function is unknown. A related virus, Simian virus 5 (SV5), has been shown to have an element, bases 51-66, which appears to be similar to HPIV3 bases 13-28. The element in SV5 has been shown to still function when moved within the promoter. To see if HPIV3 element 13-28 is similar in function to SV5 element 51-66, HPIV3 bases 13-28 were also shifted and examined for the effect on replication.

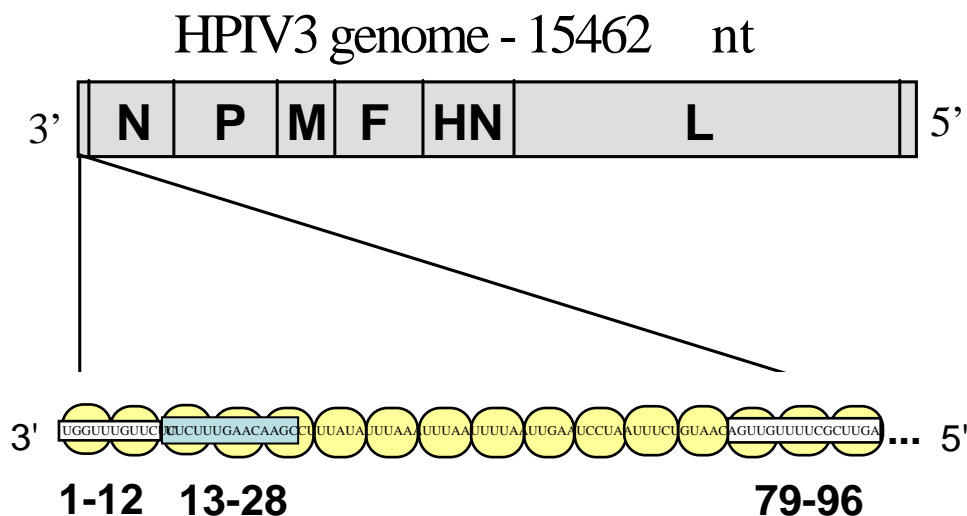


FIG 1. Organization of the HPIV3 genome showing an enlargement of the genomic promoter (GP). Important elements are highlighted (1-12, 13-28, and 79-96). The ovals represent the nucleocapsid protein, which binds the entire length of the genomic RNA.

MATERIALS AND METHODS

Plasmid Construction.

To study HPIV3 transcription and replication, the Hoffman lab uses an HPIV3 minigenome (pHPIV3-MG(-)) system (Fig 2). The pHPIV3-MG(-) plasmid encodes both the AGP and GP with a marker gene (luciferase) in between. Construction of the negative sense minireplicon, pHPIV3MG(-), was described previously (2). The T7 RNA polymerase-produced transcript from pHPIV3MG(-) contains 91 nucleotides from the 5' terminus of the HPIV3 genome, a negative sense copy of the luciferase gene, 97 nucleotides from the 3' terminus of the genome and the hepatitis delta virus antigenomic ribozyme. Mutations in pHPIV3MG(-) were made by megaprimer PCR-directed mutagenesis (6). Mutations made in the minigenome plasmid shifted the 13-28 element 6 and 12 bases within the GP. These mutants were designated +6 and +12, respectively. Megaprimer PCR products containing mutations were digested with *Sac* I and *Bst*X I and inserted into the same sites of pHPIV3MG(-). All mutants were confirmed by sequencing.

Transfection.

The effects of the mutations on genome replication was detected by transfecting HeLa cells with the parental and +6 and +12 mutated pHPIV3-MG(-), along with plasmids encoding for the N, P, and L proteins. After 24 hours the cells were treated with Actinomycin D, which inhibits cellular transcription. After 48 hours the cells were lysed and the RNA was extracted using Trizol (Invitrogen).

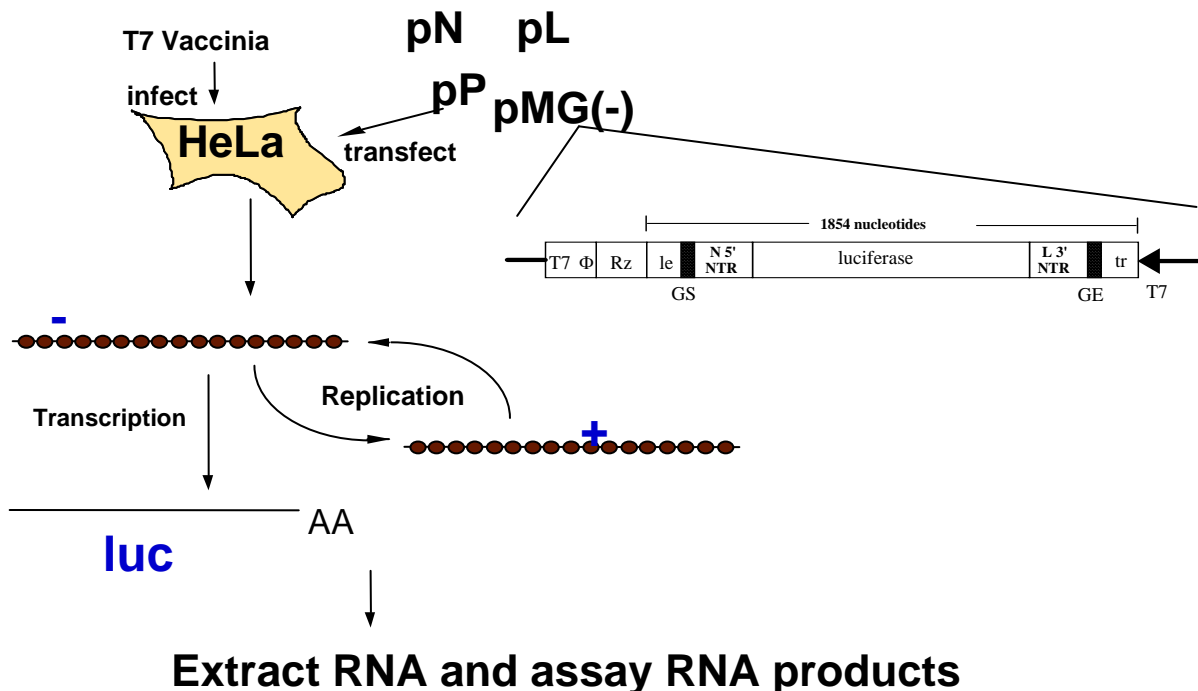


FIG 2. Minireplicon system for assay of HPIV3 replication.

Primer Extension Analysis.

Detection of antigenomic RNA was done using a negative sense, [γ - 32 P]ATP end-labeled oligomer that primes at nucleotide 2 of the luciferase gene. This primer is extended 47 nt when annealed to luciferase mRNA, and 101 nt when annealed to the antigenomic RNA. Detection of the genomic RNA was done using an end-labeled oligomer that primes at nucleotide 1649 of the luciferase gene, which is 101 nucleotides from the 5' end of the genomic RNA. These primers were used with 33% of the S7-treated extract in standard reverse transcription reactions with Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (New England Biolabs) at 44°C for 1 h. The extension products were separated on a 6% acrylamide/7M urea gel and analyzed by autoradiography and/or quantitation on a Molecular Dynamics PhosphorImager. (3).

RESULTS

After transfection of the HeLa cells, replicated minigemomic RNAs were isolated and quantitated by primer extension and phosphorimager analysis. A distinct band showing the replication of the minigemome RNA was present when the transfection included the wt (parental) pHPIV3-MG(-). Replication products were not visible, however, from HeLa cells transfected with the mutants containing the 6 and 12 base shifts of HPIV3 element 13-28 (Fig. 3). The lack of visible replication products indicates that shifting the HPIV3 element 13-28 prevented replication from the GP.

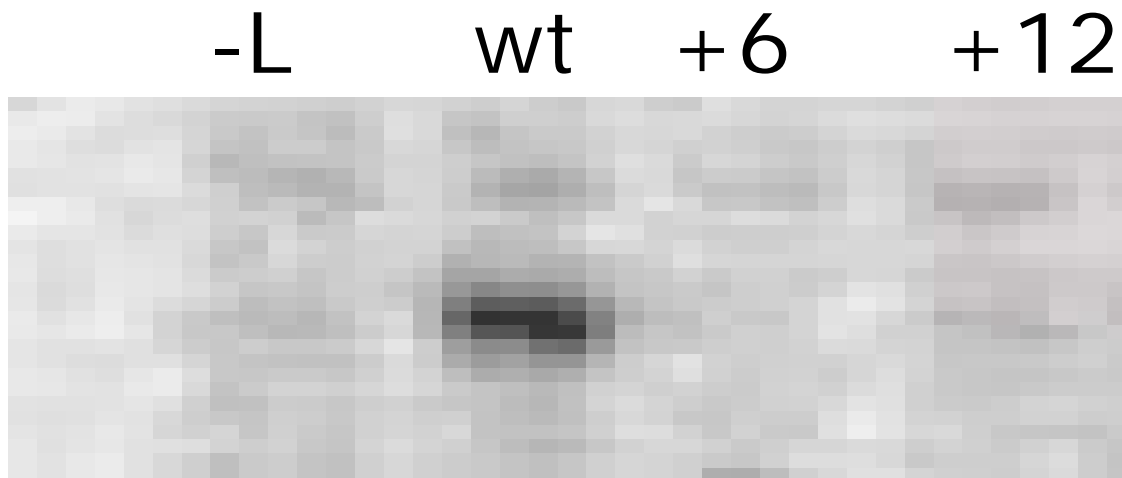


FIG 3. Effects of changing the position of HPIV3 element 13-28. Mutations were made to move the 13-28 element away from the 3' end. Analysis of S7 treated RNA (for replication to the antigenome) was isolated from cells transfected with the indicated minigenome and N-, P-, and L-encoding support plasmids. The RNA was detected by use of primer extension using a positive-sense primer to detect replicated genomic RNA. The gel shows the results of the primer extension. The lane marked -L indicates the L plasmid was omitted as a negative control. Lanes marked +6 and +12 indicate shifts of six and twelve bases from the 3' end. Replication products were seen on the wild type lane only.

CONCLUSION

The lack of replication with the +6 and +12 mutants is consistent with a previous finding. In earlier work, moving portions of the 13-28 element destroyed the ability of the HPIV3 minireplicon to replicate (3). With this current work, it now appears definitive that HPIV3 bases 13-28 cannot be moved and still retain function. This is similar to the other important elements in the HPIV3 GP. Bases 1-12 and 79-96 cannot be moved and retain function either. The strict spacing requirements are perhaps because these sequence elements must be positioned precisely (properly spaced) relative to one another to form a functional template for replication.

Since the HPIV3 13-28 element and the SV5 51-66 element have distinct properties, they may not be related. Still, the ultimate test to determine the relatedness of these two elements will be to determine the precise functions of these two elements in viral genome replication.

REFERENCES

- Collins, P. L., R. M. Chanock, and K. McIntosh. 1996. Parainfluenza viruses. *Fields Virology*. 3:1205-1233.
- Hoffman, M. A., and A. K. Banerjee. 2000. Precise mapping of the replication and transcription promoters of Human Parainfluenza virus type 3. *Virology*. 296: 201-211.
- Hoffman, M. A., L. M. Thorson, J. E. Vickman, J. S. Anderson, N. A. May, and M. N. Schweitzer. 2006. Roles of human parainfluenza virus type 3 bases 13-78 in replication and transcription: Identification of an additional replication promoter element and evidence for internal transcription initiation. *Journal of Virology*. 80: 5388-5396
- Lamb, R. A., and D. Kolakofsky. 2001. *Paramyxoviridae: The viruses and their replication*, p. 1305-40. In D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roisman, and S. E. Straus (ed.), *Fields Virology*, Fourth ed, vol. 1. Philadelphia, Philadelphia.
- Murphy, S. K., Y. Ito, and G. D. Parks. 1998. A functional antigenomic promoter for the paramyxovirus simian virus 5 requires proper spacing between an essential internal segment and the 3' terminus. *J Virol* 72:10-9.
- Sarkar, G., and S. S. Sommer. 1990. The "megaprimer" method of site-directed mutagenesis. *Biotechniques* 8:404-7.