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REVIEWS

Spotlight

Ebola Virus Replication Stands Out

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A recent study by Deflubé *et al.* (*Proc. Natl. Acad. Sci. U. S. A.* 2019;116:8535–8543) reported that Ebola virus genomes have variable 3′ terminal nucleotides. This finding is a departure from other nonsegmented, negative-strand RNA viruses (NNSVs) that have been studied to date and has broad implications for filoviral replication and innate immune evasion.

Rare filovirus disease (FVD) outbreaks are often associated with high case fatality rates, sometimes up to 90% [1]. Although factors determining patient survival are poorly understood, clinical observations suggest that a high probability of death is associated with high viral load due to uncontrolled viral replication [2]. While progress has been made towards understanding filoviral innate immune evasion mechanisms, much work remains to be done to better define filoviral RNA synthesis [1]. These gaps limit the development of therapeutics, such as direct-acting antivirals (DAAs) targeting the viral RNA synthesis complex.

Much of what is assumed for Ebola virus, a member of the family *Filoviridae*, is largely borrowed from studies of other NNSVs, including respiratory syncytial virus (RSV) [3] and vesicular stomatitis virus (VSV) [4]. The Ebola virus RNA genome is encapsidated by nucleoprotein (NP), which controls access to the genomic RNA by the RNA synthesis complex. The viral genome is transcribed to generate subgenomic capped and polyadenylated mRNAs for viral protein translation. Subsequently, the viral genome is replicated to generate antigenomic and genomic RNAs, which are then bound by NP [1].

These two major processes, transcription and replication, are carried out by the RNA synthesis complex, which includes the viral RNA-dependent RNA polymerase (RdRp or L protein) and viral protein 35 (VP35), an essential cofactor that bridges the interaction with NP [1]. However, the exact roles that *cis*-regulatory elements in the genome and viral proteins play in viral replication are not well understood, in part due to the complexity and multifunctional nature of these components.

A new study by Deflubé and colleagues [5] sheds light on a key part of the puzzle by discovering that the 3′ ends of genomic and antigenomic RNAs are variable. This is intriguing, as the polymerase needs to processively replicate the whole template, approximately 19 kb long, and recognize the 3′ ends of both genome and antigenome for initiation. Variable 3′ terminal sequences could possibly implicate different initiation mechanisms if all of these 3′ ends serve as initiation templates. To gain mechanistic insights of initiation, the authors used a modified minigenome assay that abolishes transcription and that is restricted to the antigenome synthesis step of replication. The results are striking. The polymerase always begins synthesis opposite the first C of the 3′ CCUGUG template, regardless of substitutions, deletions, or additional nucleotides added to the 3′ end. In addition, RNA synthesis is abolished if the first C is mutated, suggesting that the polymerase precisely locates its binding site. This observation is in contrast to what is currently known about RSV and VSV replication initiation, which involve *de novo* initiation from the exact 3′ end of templates [3,4]. In the case of RSV, both replication and transcription begin at the *leader* (*le*) promoter region at the 3′ end of the viral genome. However, initiation occurs independently at different sites within the *le* (genome replication from position 1U and mRNA transcription from position 3C), with the same L protein capable of selecting both sites. Although the promoter sequences of RSV do not align with other viruses within the same family, their sequences all begin with 3′ UG, suggesting that the replication product is initiated at 5′ AC. While the overall genomic structures and polymerase active sites in Ebola virus are similar to those of other NNSVs, the Ebola virus genomic ends are unconventional [6].

How do Ebolaviruses generate these variable 3′ ends with additional nucleotides? Two possible mechanisms are proposed by Deflubé *et al*. First, sequencing results of some extended 3′ ends support a back-priming model where delayed encapsidation of RNA by NP at the termini results in formation of RNA hairpins that allow for templated addition of 3′ ends. The complex kinetics involved in RNA encapsidation, RNA secondary structure formation, and enzymatic reactions offer ample space to explain the variable 3′ ends generated. Second, terminal transferase activity has been demonstrated in some RdRps in positive-sense RNA viruses [7]. Ebola virus may utilize similar mechanisms [8], but additional studies are needed.

Why do Ebola viruses have these variable 3′ ends with additional nucleotides? It appears that additional nucleotides may offer a replication advantage. Given that a major hurdle in replication is stabilization of the initiation complex, these additional nucleotides may stabilize incoming nuleotide triphosphate (NTPs) for the first few reactions to happen. In addition, a variable 3′ end may facilitate innate immune evasion by impairing recognition by cytosolic RIG-I like receptors (RLRs) that are sensitive to 3′ and 5′ overhangs [9]. Previous studies revealed that dsRNA binding by VP35 is important for Ebola virus interferon (IFN) antagonism. Structural and functional analyses show that the VP35 C terminal interferon inhibitory

domain (IID) can recognize RNA, including those with overhangs. Mimicry of cellular RIG-I like receptors is part of the arsenal of Ebola virus immune evasion tactics [10].

Unique replication products defined by Deflubé *et al.* are the latest findings that shed light on the unusual replication initiation mechanism utilized by Ebola viruses, which, not too long ago, was considered to be similar to that of other NNSVs.

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Spotlight

Bacteriophage's Dualism in Therapy

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Careful selection of bacteriophages for phage therapy is needed to avoid undesirable consequences. Different approaches to phage therapy are compared: from the use of multispecies industrially produced phage mixtures with wide range of antibacterial activity to the 'magistral phage' approach in which bacteriophages are selected for treating individual patients.

Prolonged use of new antibiotics, consistently administered for therapy, inevitably leads to the emergence of resistant variants of pathogenic bacteria; this necessitates a search for alternative approaches to antibacterial therapy. One such approach is the use of phage therapy, that is, the use of viruses that infect bacteria; these viruses are called bacteriophages (phages). The initiator of phage therapy was the French scientist F. D'Herelle, one of the discoverers of phages. His collaboration with Georgian researchers in the 1930s promoted the introduction of phage therapy into clinical practice and led to the beginning of the industrial production of phage as a medical preparation. Since then, phage therapy has been successfully applied in Russia and in some countries of Eastern Europe, being used mostly against surface infections – for example, in the treatment of infected wounds. Now several industrial enterprises in Russia (e.g., 'Microgen') produce preparatons of therapeutic phages which display high-level activity against several of the bacterial species most frequently encountered in clinical practice. As an example, the phage mixture 'Pyophage' includes viruses of different species that are active against strains of bacteria such as *Staphylococcus, Streptococcus, Proteus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* – which are the most common pathogens. Maintaining the lytic activity of commercial phage preparations is achieved by continually updating their composition by the introduction of new phages that are active against the newly emerged phage-resistant bacterial variants. As a result, phage preparations released under the same name at different times, or by different enterprises, may differ significantly in activity with respect to a given set of strains isolated from a particular patient.

At the same time, phages of some species should be considered as obviously unacceptable for therapy. This would include those that are a powerful factor in imparting pathogenicity and virulence to bacteria or that may cause undesirable evolutionary changes to pathogenic bacteria.

An example of a phage that is unacceptable for therapy is the filamentous phage Pf, which is active on *P. aeruginosa.* In a series of studies, the results of which were summarized in a recent study [1], a critical role of this bacteriophage was shown to be suppression of immunity against bacterial infection; the presence of Pf was frequently associated with chronic nonhealing wound infections involving *P. aeruginosa*. When phage Pf is internalized by human and murine immune cells it stimulates maladaptive viral pattern-recognition receptors and suppresses bacterial clearance from infected wounds. Accordingly, the authors hypothesized that vaccination against Pf phage virions could be a potential strategy to prevent at least some *P. aeruginosa* infections. It would be interesting to assess the possible effect of Pf phage on the course of bacterial infection of the lungs in an animal model.