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Reprint of "Modeling the intracellular replication of influenza A virus in the presence of defective interfering RNAs☆

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ABSTRACT

Like many other viral pathogens, influenza A viruses can form defective interfering particles (DIPs). These particles carry a large internal deletion in at least one of their genome segments. Thus, their replication depends on the co-infection of cells by standard viruses (STVs), which supply the viral protein(s) encoded by the defective segment. However, DIPs also interfere with STV replication at the molecular level and, despite considerable research efforts, the mechanism of this interference remains largely elusive.

Here, we present a mechanistic mathematical model for the intracellular replication of DIPs. In this model, we account for the common hypothesis that defective interfering RNAs (DI RNAs) possess a replication advantage over full-length (FL) RNAs due to their reduced length. By this means, the model captures experimental data from yield reduction assays and from studies testing different co-infection timings. In addition, our model predicts that one important aspect of interference is the competition for viral proteins, namely the heterotrimeric viral RNA-dependent RNA polymerase (RdRp) and the viral nucleoprotein (NP), which are needed for encapsidation of naked viral RNA. Moreover, we find that there may be an optimum for both the DI RNA synthesis rate and the time point of successive co-infection of a cell by DIPs and STVs. Comparing simulations for the growth of DIPs with a deletion in different genome segments suggests that DI RNAs derived from segments which encode for the polymerase subunits are more competitive than others. Overall, our model, thus, helps to elucidate the interference mechanism of DI RNAs and provides a novel hypothesis why DI RNAs derived from the polymerase-encoding segments are more abundant in DIP preparations.

1. Introduction

Influenza A viruses are highly contagious respiratory pathogens which infect up to 15% of the global population in seasonal epidemics, causing the death of up to 500,000 people annually. Besides annual epidemics, influenza A viruses occasionally cause severe pandemic outbreaks, like the 1918 "Spanish flu", and therefore constitute a permanent threat to global public health. Commonly, virological research is concerned with the infectious influenza virus particles, i.e., virus particles that cause production of progeny virions upon infection, as they represent the primary source of viral pathogenicity. However, the majority of influenza virus particles is non-infectious. As demonstrated by the work of von Magnus in 1954 (Von Magnus, 1954), these non-infectious particles can impair the replication of infectious influenza viruses. In the following years, extensive research revealed that so called defective interfering particles (DIPs) are the main cause of this phenomenon, not only reducing infectious virus titers but also affecting other

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Abbreviations: cRNP, complementary ribonucleoprotein complex; DI RNA, defective interfering RNA; DIP, defective interfering particle; FL, full-length; HAU, haemagglutinating units; hpi, hours post infection; MOI, multiplicity of infection; NEP, nuclear export protein; NP, nucleoprotein; nt, nucleotides; PFU, plaque forming units; RdRp, RNA-dependent RNA polymerase; RNP, ribonucleoprotein complex; STV, standard virus; vRNA, viral RNA; vRNP, viral ribonucleoprotein complex.

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properties of the virus, including its pathogenicity and its evolution (reviewed in Frensing, 2015; Nayak et al., 1985). Due to the efficient reduction of infectious particle production caused by DIPs, they have also been recently proposed as a novel antiviral agent that can overcome the limitations of conventional vaccination and treatment with antivirals (reviewed in Dimmock and Easton, 2014; Marriott and Dimmock, 2010). Besides, there has been a revival of DIP research related to DIP-mediated induction of the immune response (Boergeling et al., 2015; Killip et al., 2015; Tapia et al., 2013), virus-induced apoptosis (Frensing et al., 2014), and the role of DIPs for biotechnology processes (reviewed in Frensing, 2015). However, the molecular mechanism for the interference of DIPs with their standard viruses remains largely elusive.

Influenza A viruses possess a segmented genome comprising eight single-stranded viral RNAs (vRNAs) of negative polarity. These genomic RNA segments are not present as naked RNA but form individual viral ribonucleoprotein complexes (vRNPs) together with the heterotrimeric viral polymerase and several copies of the nucleoprotein (NP). Influenza virus DIPs carry an internal deletion in at least one of their eight RNA genome segments caused by an erroneously translocation of the viral polymerase on the RNA template within the three dimensional RNP structure (reviewed in Frensing, 2015). Influenza virus defective interfering (DI) RNAs are on average 300–500 nucleotides (nt) long and retain approximately 200 nt from each end of the cognate full-length (FL) segment. Consequently, the DI RNA segment contains parts of the coding sequence and the untranslated regions where the viral polymerase binds. Hence, it can serve as a template for viral transcription and replication, and can be packaged into progeny virions (Hutchinson et al., 2010). The deletions in vRNAs seem to occur randomly, are variable in size, and can affect different genome segments (Frensing et al., 2013; Jennings et al., 1983; Nayak et al., 1985). The most abundant DI RNAs, however, originate from segments 1–3, which encode for the three polymerase subunits (Davis and Nayak, 1979; Jennings et al., 1983), and the mechanism behind this observation remains elusive.

Due to their lack in genetic material, DIPs cannot accomplish progeny virion production independently but require cells that are co-infected by their homologous standard virus (STV), which supplies the missing viral protein(s) *in trans*. However, during co-infection, the DI RNA interferes with STV propagation leading to a decrease in the number of infectious progeny virions in favor of DIP production (Marriott and Dimmock, 2010). The molecular basis of this interference is still not fully understood. Yet, several lines of evidence indicate a preferential amplification of DI RNAs over their FL counterparts (Akkina et al., 1984a; Duhaut and McCauley, 1996). In particular, it has been suggested that, due to their reduced length, DI RNAs are synthesized faster, i.e., the viral polymerase can produce more copies of a short RNA per unit time (Marriott and Dimmock, 2010; Nayak et al., 1985). This hypothesis is supported by experimental results obtained by a dual luciferase reporter assay that was performed with influenza virus-like RNAs (Widjaja et al., 2012). In this study, the shorter of two RNA constructs showed a stronger inhibitory effect on luciferase expression compared to the longer reporter segment. This observation was also dependent on other segment properties, like the part of the coding region and the polymerase-binding site that was retained in the DI RNA sequence. Thus, RNA length does not seem to be the only determinant of interference. This is in agreement with other studies where not all DI RNAs accumulated to high levels in co-infected cells (Duhaut and McCauley, 1996). In line with this, some DI RNAs have been shown to be packaged more efficiently into progeny virions (Duhaut and Dimmock, 2002; Duhaut and McCauley, 1996; Odagiri and Tashiro, 1997), and it was furthermore proposed that they compete with FL RNAs for a limiting viral or cellular factor (Marriott and Dimmock, 2010). The latter has also been observed experimentally for both vesicular stomatitis virus (VSV) and influenza virus, where DI and FL genomes compete for viral polymerases (Giachetti and Holland, 1989; Widjaja et al., 2012). Finally, it was proposed that DI RNAs may have lost yet unidentified regulatory elements present in the FL genome, which allows them to accumulate to unusually high levels (Nayak et al., 1985).

To elucidate the interference of DI RNAs with STV propagation in more detail, we developed a mathematical model of DIP replication. In contrast to previous mathematical descriptions which focused on the spreading of DIPs in a cell population (Frensing et al., 2013; Kirkwood and Bangham, 1994), we modeled the intracellular events of virus replication in a single infected cell. In particular, we extended a previously published model of the intracellular influenza viral life cycle (Heldt et al., 2012) by considering the replication of a DI RNA, which has an advantage in RNA synthesis due to its reduced length. Based on this assumption, our model not only captures data from yield reduction assays and experiments testing different timings of successive co-infection. It also counter-intuitively predicts that the intracellular pool of viral NP is depleted first even though we consider a defect in a polymerase-encoding segment. In addition, our simulations indicate that the differential accumulation of regulatory viral proteins, such as the matrix protein 1 (M1) and the nuclear export protein (NEP), can protect an STV-infected cell from DIP-mediated interference in certain infection scenarios. Finally, the model provides a possible explanation for the experimental finding that most DI RNAs of influenza A virus originate from polymerase-encoding segments.

2. Models and methods

Our model of DI RNA replication is based on a previously published description of the intracellular life cycle of influenza A viruses (Heldt et al., 2012). To elucidate the mechanism of interference, we augmented this model by including a DI RNA that possesses a replication advantage in complementary RNA (cRNA) synthesis, as suggested by Odagiri et al. (1994). Below, we outline the changes made to the original model to account for the effect of a DI RNA derived from segment 3, encoding the polymerase acidic protein (PA). We first modeled the replication of a DI RNA derived from one of the polymerase-encoding genes as those are the most abundant DI RNAs in influenza A virus preparations according to literature (Davis and Nayak, 1979; Jennings et al., 1983). A complete list of the equations and a model for a defect in segment 4, encoding hemagglutinin (HA), can be found in the supplementary information. For a detailed discussion of STV replication the reader is referred to the original virus replication model (Heldt et al., 2012).

2.1. Virus entry

The entry of extracellular DIPs (D^{Ex}) into the host cell was modeled as described before for the uptake of STVs (Heldt et al., 2012).

$$\frac{dD^{Ex}}{dt} = k_{Hi}^{Dis} D_{Hi}^{Att} + k_{Lo}^{Dis} D_{Lo}^{Att} - \left(k_{Hi}^{Att} B_{Hi} + k_{Lo}^{Att} B_{Lo}\right) D^{Ex}, \quad (1)$$

$$\text{with} B_n = B_n^{tot} - V_n^{Att} - D_n^{Att} \text{ and} k_n^{Dis} = \frac{k_n^{Att}}{k_n^{Eq}},\ n \in \{\text{Hi,Lo}\}, \quad (2)$$

where we distinguished between binding sites of high affinity (B_{Hi}) and low affinity (B_{Lo}) of which there are B_n^{tot} in total. The rates of attachment to and dissociation from these sites are k_n^{Att} and k_n^{Dis}, respectively. The latter rates follow from the equilibrium constants k_n^{Eq}. Since we assumed a fast recycling of receptors, the number of free binding sites follows from the conservation Eq. (2), where V_n^{Att} and D_n^{Att} denote the number of attached STVs and DIPs, respectively. Here, each virus occupies a single binding site.