Review

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Selective packaging of the influenza A genome and consequences for genetic reassortment

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Influenza A viruses package their segmented RNA genome in a selective manner. Electron tomography, biochemical assays, and replication assays of viruses produced by reverse genetics recently unveiled molecular details of this mechanism, whereby different influenza viral strains form different and unique networks of direct intermolecular RNA–RNA interactions. Together with detailed views of the three-dimensional structure of the viral ribonucleoparticles, these recent advances help us understand the rules that govern genome packaging. They also have deep implications for the genetic reassortment processes, which are responsible for devastating pandemics.

General features of influenza A viruses

Influenza A viruses (IAVs) are the causative agents of recurrent influenza epidemics and occasional severe pandemics in humans and animals. According to the World Health Organization (WHO), IAV infections remain a major public health issue as seasonal epidemics claim 250 000 to 500 000 lives annually (http://www.who.int/mediacentre/factsheets/fs211/en/). The first well-documented human influenza pandemic, the 1918 Spanish flu, caused the death of ~50 million people, and was followed by the 1957 Asian and the 1968 Hong Kong flu pandemics. The first pandemic of the 21st century emerged in 2009, creating considerable impact on global health and economics [1]. Occasional deadly infections of humans by avian influenza viruses, including the recent human H7N9 cases [2], remind us of the constant threat of the next flu pandemic. The wide dissemination of highly pathogenic avian influenza (HPAI) H5N1 viruses [3] has resulted in intraspecies transmission in mammals (zoonotic infections), even if at a limited scale, and in a number of highly lethal human infections (http://www.who.int). This situation, which provides opportunities for the virus to adapt to the new host, might not evolve, but recent studies have nevertheless demonstrated how HPAI H5N1 viruses could reach aerosol transmissibility and thus be a direct source for pandemics by increasing the ability of sustained human-to-human transmissibility [4].

IAVs are members of the *Orthomyxoviridae* family, comprising three types of influenza viruses (A, B, and C) as well as Thogoto-like viruses, Isavirus, and one tentative genus, *Quarjavirus* [5]. IAVs are characterized by a segmented genome that consists of eight single-stranded negative-sense viral RNA molecules (vRNA1 to vRNA8) varying in length between 890 and 2341 nucleotides (nts). The gene segments are numbered from one to eight or named after the main protein they encode (Table 1). The IAV genome expresses ten essential proteins as well as several more recently characterized accessory proteins [6–12] (Table 1). IAVs are classified into antigenic subtypes based on their surface glycoproteins, with hemagglutinin (HA) proteins falling into classes H1 to H18 and neuraminidase (NA) proteins falling into classes N1 to N11. Although only a limited number of these HAs and NAs have been isolated from viruses infecting humans, the H1–H16 and N1–N9 subtypes are all maintained in aquatic bird populations [13], which constitute the natural reservoir of IAVs, and the H17N10 and H18N11 viruses were recently identified in bats [14].

vRNAs all share the same organization (Figure 1A): a central open reading frame that encodes one or more proteins, in antisense orientation, that is flanked by two short (19–58 nts) untranslated regions (UTRs). The 3′ and 5′ termini of the vRNAs consist of 12 (Uni12, U12) and 13 (Uni13, U13) nts, respectively, that are highly conserved between vRNAs and between species (reviewed in [13,15–19]). These conserved sequences are partially complementary and anneal to form a hairpin structure essential for transcription and replication (reviewed in [13,15–19]). Each vRNA is associated to the PB2–PB1–PA polymerase complex, which binds to the vRNA termini, and the RNA-binding protein NP [15,20–22] to form a viral ribonucleoprotein (vRNP).

Genome segmentation confers significant evolutionary advantages to the virus by allowing genetic reassortment, that is, the exchange of gene segments when two, or more,

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Table 1. IAVs gene segments, proteins and their functions

vRNA	Protein name[a]	Main function of the protein
1	PB2	Cellular mRNA cap recognition and binding
2	PB1 PB1-F2* N40*	RNA-dependent RNA polymerase Proapoptotic activity Unknown
3	PA PA-X* PA-N155* PA-N182*	Endonuclease (implicated in cap snatching) Repression of cellular RNA polymerase II-mediated gene expression Probably involved in viral replication Probably involved in viral replication
4	HA	Cellular receptor binding and viral membrane/endosome fusion
5	NP	vRNA binding, vRNP nuclear export, and vRNA replication
6	NA	Neuraminidase activity (cleavage between HA and sialic acid)
7	M1 M2 M43*	Matrix protein, vRNP nuclear export, and virus budding Ionic channel (important for virion acidification and vRNP release) Can functionally replace the M2 protein
8	NS1 NS2-NEP NS3*	Antiviral response inhibition vRNP nuclear export Provides replicative gain-of-function

[a]Auxiliary proteins are marked by asterisks (*).

viruses coinfect the same cell. However, it undeniably complicates virion assembly, as IAV particles must incorporate at least one copy of each vRNA to be replication-competent and thus fully infectious. Evidence has accumulated in the past 10 years to support a selective packaging of the influenza A genome, implying the existence of specific features on each vRNA, allowing for discrimination between the eight vRNAs. Despite the identification of *cis*-acting packaging signals in all eight vRNAs, for two human H1N1 strains, the molecular mechanisms that govern packaging have only recently started to be unveiled. This review will highlight recent advances towards the understanding of the selective packaging process of IAVs as well as the implications for genetic reassortment.

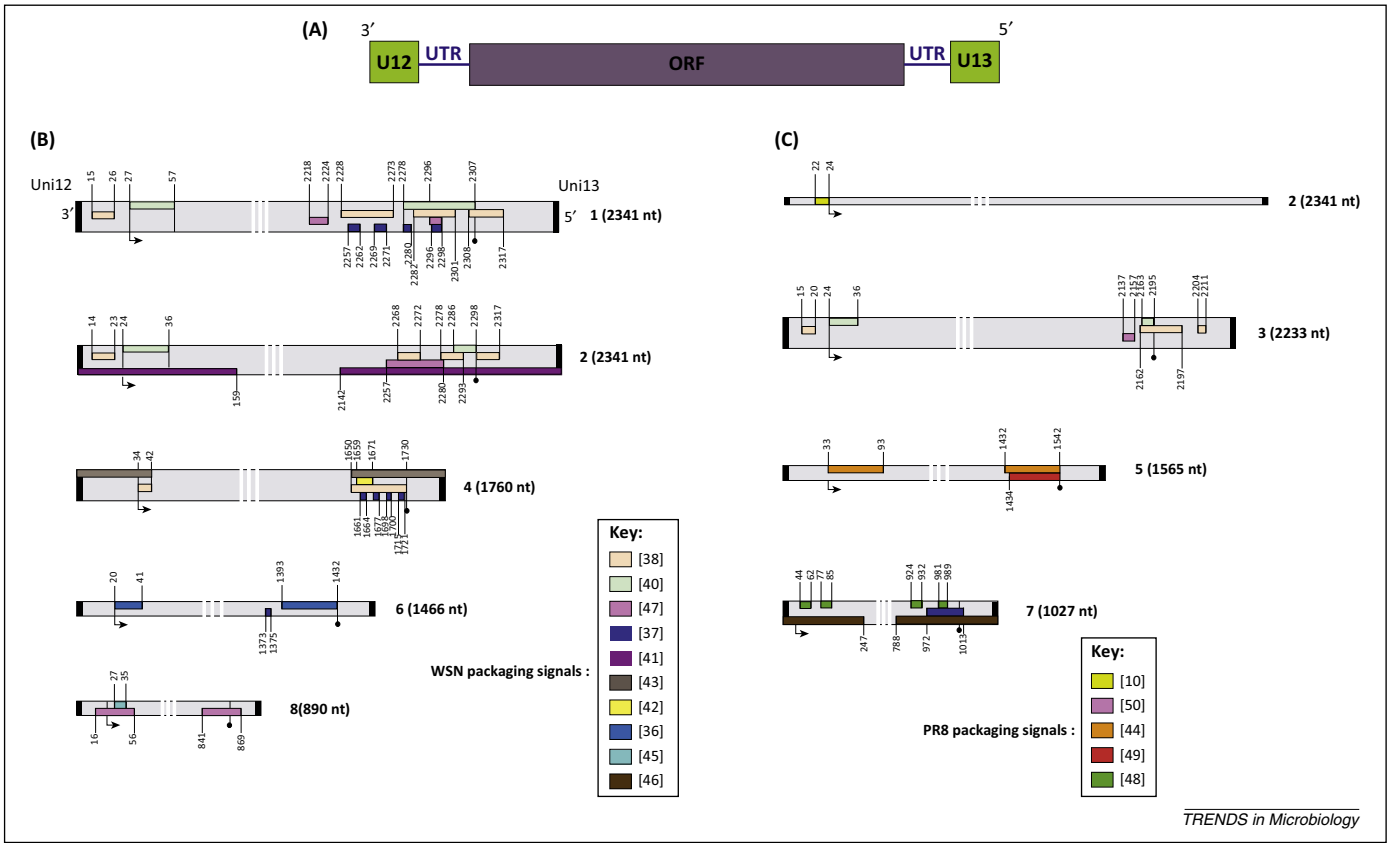

Figure 1. Mapping of the packaging regions for human A/WSN/33 (H1N1) (WSN) and A/PuertoRico/8/34 (H1N1) (PR8) influenza strains. **(A)** Genetic organization of the influenza A viral gene segments. Abbreviations: U12, unique 12 nucleotide (nt) conserved sequence; U13, unique 13 nt conserved sequence; UTR, untranslated region; ORF, open reading frame. **(B,C)** Coding and noncoding regions important for packaging of influenza WSN (B) and PR8 (C) strains. vRNAs are drawn and numbered according to the conventional representation and nomenclature for negative-strand RNA viruses, from 3′ to 5′. The U12 and U13 sequences are represented as black boxes at each end of the vRNAs. The arrows correspond to the translation initiation sites, the black dots to the stop codons. The sequences referenced as important for packaging are color-coded according to the publications that described them [10,36–38,40–46,48–50]. Note that the vRNAs are not drawn to scale. Abbreviation: vRNA, viral RNA.