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Opinion

Chameleon-Like Mimicry among RNA Viruses Possessing Ambisense Stacking Genes in their Genomes: Possible or Not?

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Abstract

In the RNA genome of influenza viruses and coronaviruses, a novel genes were identified, that, unlike the known canonical genes in these viruses, were encoded in the opposite polarity: positive in influenza virus and negative in coronaviruses (so-called ambipolar genes). The novel ambipolar genes appear to be localized in the genome in the area of canonical genes, the so-called stacking arrangement. The discovery of new viral genes implies the existence of several alternative pathways (strategies) in the realization of the viral genome in a single virus. This variety of strategies gives the virus the ability, like a chameleon, to change the structure of the virion genome and its envelope, consisting of proteins - products of the ambisense genes and/or mosaic with canonical proteins. Structural chameleon-like variants of the virus can arise depending on the biochemical composition of the virus reproduction environment in various organs of the host organism, increasing the adaptive potential and ability of the virus to evade the host immune defense, causing various forms of disease pathogenesis, including the development of latent and persistent forms of the pathological process. The existence of stealth ambipolar viruses is still hidden from researchers, like the "dark side of the moon". Their identification will make it possible to discover novel forms of viral diseases and develop new types of drugs and vaccines.

Keywords: *Viruses; Genome Strategy; Genome Polarity; Ambisense Genes; Influenza; Covid-2019*

A novel class of genes in the RNA genome of viruses and influenza and coronaviruses was described earlier [1-9]. These genes represented extended open reading frames, which are equipped with all the necessary elements for their translation by ribosomes in the mammalian organism.

1. These genes contain the starting AUG (or alternative CUG) codon, which is necessary for ribosomes to recognize and start the synthesis of the corresponding protein molecule [10].
2. In the zone preceding the starting codon, a fragment of the genome, the so-called internal ribosome entry site (IRES), was found to have characteristics of a tertiary petal-like structure with complementary double-helix loops stabilizing its conformation [11].
3. Typical nucleotide sequences, the so-called Kozak motifs, which are recognized by ribosomes for the correct identification of the AUG start codon, were found in the start codon zone ofthe ambisense genes [12].

4. The ambisense stacking genes detected in the genome structure of influenza A viruses and coronaviruses had evolutionary stability. Despite the pronounced and high genome variability of these viruses, the identified ambisense genes were conserved in the natural viral population for a longtime [2]. For influenza viruses, multimetric analysis of virus genomes from different virus isolation periods revealed that the NSP8 ambipolar gene originated in viruses about 125 years ago and was conserved in human influenza virus populations since then, showing high immunological variability [3].
5. The peculiarity of the identified ambisense genes was that they were localized in the genome in the regions that encoded the canonical viral proteins NS, M, NP, PA, PB1, PB2 in the influenza virus and in the gene region of the main structural proteins (S1/S2, N, E, M) and 16 additional regulatory proteins (nsp 1-16) in coronavirus, respectively [8,9]. Notably, this is the so- called stacking (one above the other) localization of the ambisense and canonical genes, wherein the direction of protein translation triplets has the opposite directions in ambisense genes and in canonical genes, respectively [9,13].

The discovery of novel ambipolar genes in the RNA genome of viruses implies two important ideas. The first is that a single virus can have several alternative genome expression strategies, i.e. several viral replication patterns. The second one is that a single virus can form several types of viral particles when, along with canonical virions, there may be other types of viruses, which contain an RNA genome of different polarity and structure and have a protein envelope formed in whole or in part by the proteins of the ambisense genes, the so-called ambipolar virions. The virus can change these replication strategies and form, like a chameleon, viral particles of different compositions both in terms of the polarity of the genomic RNA and in the structure and composition of the protein envelope. These ideas are illustrated in figure 1.

The presence of several alternative replication pathways in a single viral genome and the formation of several structural types of viral particles can be regarded as a kind of process called bet- hedging. Bet-hedging expands the range of viral reproduction pathways, increases the genetic capacity of the viral genome, increases the ability of the virus to adapt to different hosts and evadethe host immune response, and allows the virus to use different mechanisms of pathogenesis (persistent or acute forms of infection) in certain organs in different animals, including humans [13].

So far, these ideas are largely hypothetical, but there are already experimental data confirming the formation of protein products of ambipolar genes in animals infected with the virus. In particular, when mice were infected with the influenza A virus, the formation of cytotoxic lymphocyte clones specifically targeting the viral ambipolar protein NSP8 [14,15], which is encoded by the ambipolar viral gene sequence NSP8 - a product of the RNA segment №8 (segment NS) of the human influenzavirus genome [16,17], was observed. In an earlier work by Zhong., *et al*. (2003), the authors also managed to detect lymphocyte clones recognizing the octapeptide GGLPFSLL (called a hypothetical peptide by the authors) identified in the sequence of the NSP8 ambipolar viral protein (amino acid positions 43 - 50) [18]. Hickman., *et al*. (2019) demonstrated the possibility of NSP8 gene ambipolar expression in mice after infection with recombinant influenza A (H1N1) virus, in which the NSP8 gene contained a protein signal with a highly active fluorescent tag [19,20]. The difficulty in detecting mature protein products of ambisense genes in natural viral infection may be due to their very low level of synthesis and/or short lifespan, as well as the specific regulation of their expression realized only in the cells of certain tissues and organs in the infected host organism [8,13].

The detection of ambisense genes in RNA viruses allows proposing the hypothesis of the existence of stealth virions. Such virions can be formed in certain organs of an infected organism only under specific living and environmental conditions and a certain environment in the microorganism. In their structure, they can have different forms of genome, including +, -, or double-stranded +/- forms of RNA; and the protein envelope may be decorated with proteins that are polypeptides of ambipolar genes. Viruses can disguise themselves (like a chameleon mimicry) and evade the host's immune defenses due to specific properties of ambisense genes and structural and functional

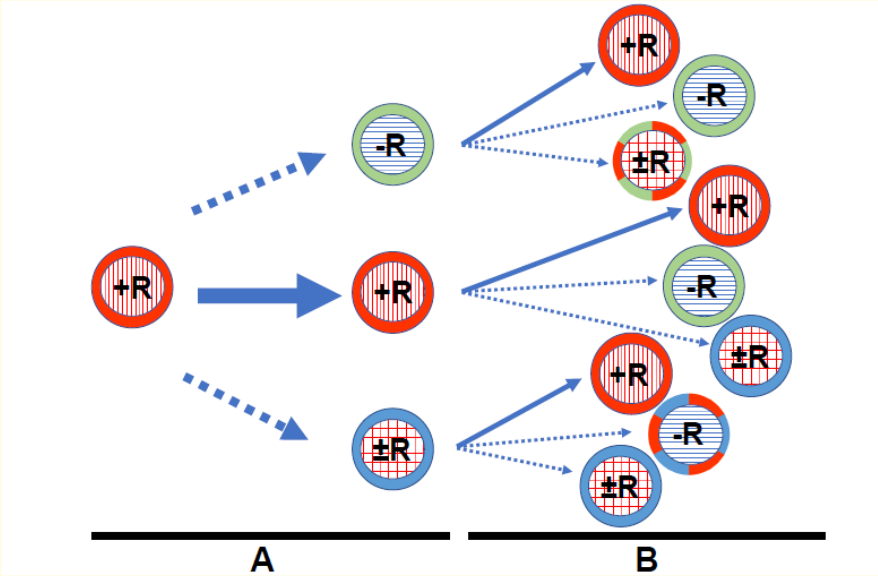

Figure 1: *Chameleon-like mimicry of RNA viruses possessing ambisense stacking genes in their genomes. The scheme shows alternative genome strategies and the formation of ambipolar virions. This scheme has been designed on the model of coronavirus containing canonical positive-sense RNA genome (+R) and is applicable to a wide range of viruses, including orthomyxo-, paramyxo-, pneumo-, rhabdo-, areno-, filoviruses, and other viruses. Major arrow shows the main canonical strategy pathway of the positive-polar RNA of the coronavirus genome (solid arrow). This pathway is initiated by the viral polymerase and forms canonical virions containing the main structural proteins: S1/S2, N, M, E. At the first stage (stage A), under certain environmental conditions and/or in certain hosts, alternative strategies II-III can be expressed with the formation of full-length genomic RNA strands of different polarity (-) RNA (-R) and (+/-) RNA (+/-R), which are packaged into viral particles (dashed arrows). All three classes of full-genome RNA can be decorated with proteins - products of ambipolar genes or have a mosaic composition of canonical and ambipolar proteins (envelope fragments are shown in different colors). The formed ambipolar virions can also use alternative genome expression strategies already in a new round of replication (stage B). The resulting chameleon-like ambipolar viral particles can possess new biochemical and pathogenetic properties and cause novel forms of disease, as well as infect new host species expanding virus life habitat.*

features of their polypeptide products [1,8,21]. So far, this type of viral particle has not been identified, as this would require highly sensitive and highly specific approaches to virus identification in host tissues and organs. The existence of such stealth viruses, so far hidden from researchers like "the dark side of the moon", would allow the virus to expand its ability to adapt to a host or new hosts, using the so-called "bet-hedging" mechanism, and change the forms of its spread by both horizontal and vertical pathways [13]. Further research will clarify the possibility of this yet unknown virus life form and its role in the pathogenesis of latent and opportunistic forms of viral diseases.

Declarations

The work has been done on a personal initiative without any financial support. The authors declarethat they have no competing interests.

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