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Cooperation: another mechanism of viral evolution

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RNA viruses evolve rapidly under selection pressure as a result of the high error rates of viral RNA polymerase. 'Cooperation' between wild type and variant measles virus (MV) genomes through the heterooligomer formation of a viral protein has recently been shown to act as a mechanism of viral evolution. This type of cooperation between genomes producing a new phenotype may have implications for various aspects of evolution, including the expansion of viral tropism and host range, the emergence of segmented viral genomes, and the evolution of heteromultimeric molecules. It also lends support to the concept of the quasispecies acting as a unit of selection.

RNA virus evolution

The high error rates of the viral RNA-dependent RNA polymerase lead to the error-prone nature of genome replication in RNA viruses, which causes virus populations to form mutant spectra (mutant clouds or quasispecies) [1–5]. This feature is thought to enable the rapid evolution of many RNA viruses under selection pressure, and endow them with the ability to change their cell tropism and host range as well as developing resistance to host immune responses and antiviral therapeutic agents [5]. As a consequence, it remains problematic to control infections of some RNA viruses, including HIV and hepatitis C virus [2,4,5].

It is generally thought that growth competition occurs between different virus variants and the fittest clones predominate under given conditions. However, recent studies have provided evidence that cooperative and interfering interactions also take place within mutant spectra. For example, a narrow mutant spectrum was reported to restrict the neuropathogenicity of poliovirus due to the lack of cooperative interactions within viral populations [6]. Increases in the replicative ability of the West Nile virus in mosquito cells were found to correlate with increases in the size of the mutant spectrum, rather than changes in the consensus sequence [7]. Furthermore, it was demonstrated that co-infection with West Nile virus can lead to complementation, which acts to maintain phenotypic and genotypic diversity, and that cooperative interactions may lead to mutant spectrum fitness levels that exceed the fitness levels of any individual genotype [8]. A model based on group-level selection acting on genetically complementary mutant spectra, rather than individual-level selection, was shown to nicely simulate the observed evolution of HIV [9].

In addition, specific replication-competent mutants of the foot-and-mouth disease virus were found to exert an interfering activity on the replication of wild type viral RNA, and the extent of interference did not correlate with the types and number of interactions involving affected mutations [10,11]. These cooperative and interfering interactions within mutant spectra support the notion that the mutant spectrum acts as a unit of selection. However, the molecular mechanisms that underlie internal interactions within mutant spectra have not been well defined.

A recent study has revealed a new mechanism of viral evolution, 'cooperation' through the heterooligomer formation of a viral protein [12]. In this opinion article, we first describe how this phenomenon was uncovered, and then discuss several topics related to the concept of 'cooperation', including viral tropism and host range, the quasispecies theory, and genome segmentation. The possible role of cooperation in the evolution of heteromultimeric molecules will also be considered.

Cooperation through the heterooligomer formation of a viral protein

MV, an enveloped non-segmented single-stranded RNA virus in the *Paramyxoviridae* family, enters host cells by fusing its envelope with the host cell membrane. Two envelope glycoproteins, a hemagglutinin (H) and a fusion (F) protein, mediate virus-to-cell fusion and, when expressed on the surface of infected cells, cell-to-cell fusion (syncytium formation). Binding of the H protein to a cellular receptor is thought to induce conformational changes in the adjacently located F protein, thereby leading to membrane fusion [13].

Glossary

Complementation: production of the normal phenotype in a defective virus mediated by gene products supplied by another functional or defective (in other gene products) virus.
Fusion assay: a plasmid-mediated assay to evaluate the fusogenicity of viral envelope glycoproteins (H and F proteins in the case of MV) by examining syncytium formation in receptor-positive cells transfected with those proteins.
Gene duplication: an evolutional event causing duplication of genes resulting from homologous recombination, retrotransposition, or duplication of the whole genome.
Polyploid: having multiple sets of complete genetic information (genome) within a single viral particle.
Quasispecies: dynamic distribution of nonidentical but related viral genomes as a result of the high error rates of viral RNA polymerase.
Segmented genome: a genome in which separated segments, not a single molecule, are necessary to carry the whole genetic information of a virus, as is the case with influenza A virus containing eight RNA segments.

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In general, only cells expressing appropriate receptors allow membrane fusion, thus entry and syncytium formation.

A recombinant MV containing a tagged H protein was found to be non-fusogenic in previously-susceptible cells, presumably because the added tag interfered with the function of the H protein. After passaging this non-fusogenic recombinant virus in the same cells, several mutant viruses emerged that had regained the ability to induce syncytium formation. Most of them had single amino acid substitutions in the F protein. However, one mutant virus was found to contain two different types of genomes within a virion (virus particle), one encoding the wild type F protein and the other encoding the mutant F protein with a glycine to arginine substitution at position 264 (G264R) [12].

Paramyxoviruses are known to have the capacity to enclose several genomes within a single envelope, termed polyploidy [14–16], which must be responsible for the observed mixed genomes. Because the F protein forms functional trimers [17,18], the MV with the mixed genomes must have heterotrimers comprised of two different F proteins. A fusion assay (see Glossary) indicated that heterotrimers of wild type and G264R F proteins induce syncytia, whereas homotrimers of the wild type or G264R F protein do not [12]. More importantly, the heterotrimeric F protein, when expressed together with the wild type (nontagged) H protein, exhibited enhanced fusion activity as compared with the wild type F protein. Furthermore, the heterotrimeric F protein, in conjunction with the wild type H protein, induced syncytia even in receptor-negative cells (Figure 1A), and the recombinant MV with the mixed genomes encoding respective F proteins and the wild type H protein spread in hamster brains that lacked efficient receptors (Figure 1B). Thus, cooperation between different genomes produced a new phenotype (cell-to-cell fusion and *in vivo* spread in receptor-negative cells), which the wild type virus does not exhibit.

In this article, we define the phenomenon 'cooperation' as a distinct concept from 'complementation'. In cooperation, neither of the two genomes complements the defects of the other, but two genomes produce a new phenotype through a cooperative interaction between variant proteins (heterooligomer formation). Furthermore, cooperation, which provides an advantageous phenotype over the wild type, may be subject to positive selection even at a low multiplicity of infection. Under such a condition, complementation is more likely disfavored although it can still occur through mutations arising during genome replication within a single infected cell.

It should be noted that polyploidy contributed to cooperation between different MV genomes [12]. Polyploidy is characteristic of enveloped paramyxoviruses [16] and non-enveloped birnaviruses [19], and most other viruses contain only a single copy of each gene (haploid). Although retroviruses contain two copies of the genome [20–22], only one copy is subjected to reverse transcription, thus referred to as pseudodiploid [23]. Nevertheless, cooperation could occur in any viruses other than those with polyploidy through co-infection of the same cell with different virions. For example, long-term transmission of defective RNA genomes has been documented in patients infected with dengue virus, a member of the *Flaviviridae* family [24]. A recent study has reported that a mathematical model predicts efficient dengue virus transmission due to the presence of defective genomes [25]. This phenomenon is thought to be caused by complementation [24,25], but may actually be more complicated, with the potential for cooperation between the defective and wild type genomes producing an advantageous property. Thus, the concept of cooperation may be applied to a broad range of virus families, and we should keep the possibility of cooperation in mind, when we study virus populations.

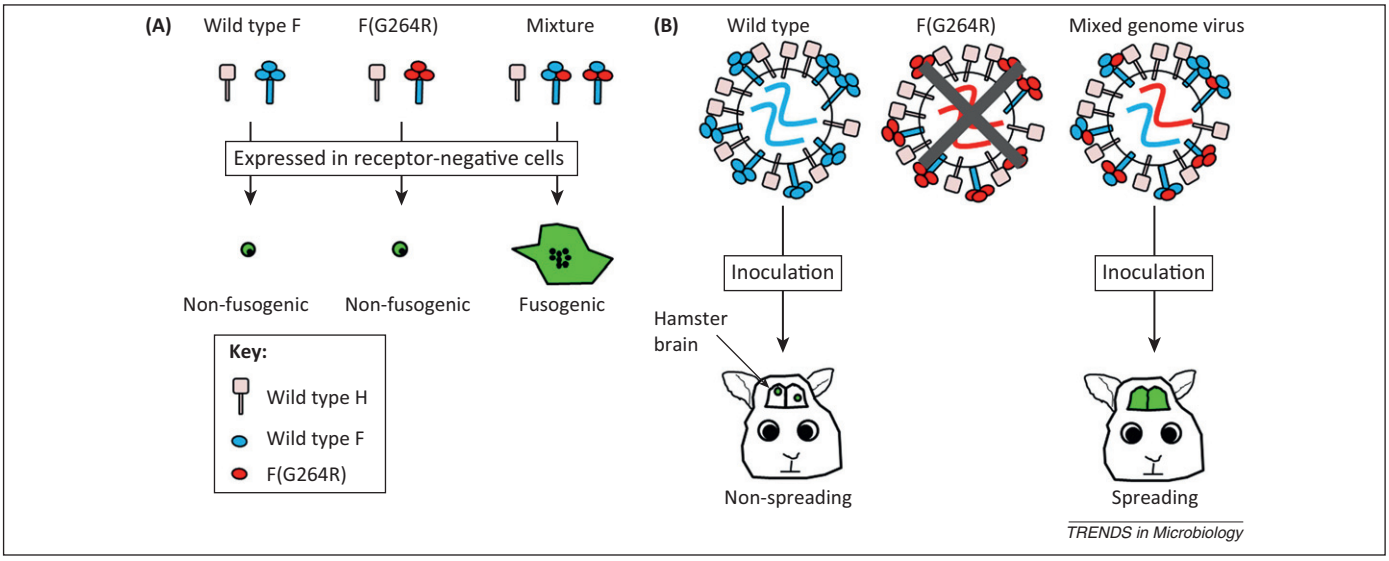

Figure 1. Cooperation between different genomes confers a new phenotype on a virus. **(A)** When both wild type and G264R F proteins were expressed together with the wild type H protein, syncytium formation was observed even in receptor-negative cells [12]. By contrast, no syncytium was observed when either F protein alone was expressed with the wild type H protein. **(B)** Three types of recombinant measles virus (MV) containing wild type F, G264R F, or both F proteins were recovered [12]. The mixed genome virus spread in suckling hamster brains lacking efficient receptors, whereas the wild type virus did not. The recombinant virus bearing only the G264R F protein exhibited no infectivity even in receptor-positive cells.