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Collective properties of viral infectivity

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Individual virions typically fail to infect cells. Such decoupling between virions and infectious units is most evident in multicomponent and other segmented viruses, but is also frequent in non-segmented viruses. Despite being a well-known observation, the causes and implications of low single-virion infectivity often remain unclear. In principle, this can originate from intrinsic genetic and/or structural virion defects, but also from host infection barriers that limit early viral proliferation. Hence, viruses may have evolved strategies to increase the per-virion likelihood of establishing successful infections. This can be achieved by adopting spread modes that elevate the multiplicity of infection at the cellular level, including direct cell-to-cell viral transfer, encapsulation of multiple virions in microvesicles or other intercellular vehicles, virion aggregation, and virion binding to microbiota. In turn, increasing the multiplicity of infection could favor the evolution of defective viruses, hence modifying the fitness value of these spread modes.

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One is not enough: poor infectivity of individual virions

The virion or viral particle has been traditionally viewed as the minimal viral infectious unit. However, typically the majority of virions are non-infectious. Viral titers obtained by standard methods such as the plaque assay or the median infectious dose can be tens or even hundreds of times lower than the actual number of viral particles in a given suspension. This deviation can be measured using the particle to plaque forming unit (PFU) ratio [1]. High particle-to-PFU ratios are often attributed to lack of some genetic material inside the virion, structural defects in the capsid and/or envelope, or lethal mutations.

Segmented viruses are particularly prone to non-infectiousness. In principle, one may expect genome packaging processes to ensure the incorporation of all segments in each virion. Tight control of segment encapsidation has been indeed reported in some viruses such as bacteriophage φ6 [2], yet other viruses surprisingly appear to package segments quasi-randomly. For instance, in Rift Valley fever virus, FISH analysis of virions and infected cells revealed that up to 90% of viral particles lack at least one of the three segments, despite each segment being essential [3]. Another example of such apparent lack of regulation is provided by birnaviruses [4]. This issue is aggravated in multipartite viruses, because each segment is packaged in a different particle and, presumably, a high multiplicity of infection (MOI) is necessary for productive infection [5,6].

In recent years, the causes of influenza virus high particle-to-PFU ratios have been investigated in some detail. For instance, single-cell analysis and stochastic modelling suggested that up to 90% of cells infected by a single viral particle produce little or no progeny [7••]. Similarly, it was found that approximately 90% of the particles fail to express at least one segment [8•]. These appear to differ from classical defective interfering particles (DIPs) [9] in that they initiate cellular infection but fail to complete it, and have been termed semi-infectious particles (SIPs) [10]. Influenza virus infectivity increases strongly when the MOI is high enough to ensure coinfection of cells with multiple SIPs. However, surprisingly, the presence of SIPs does not seem to require a high MOI. This suggests that SIPs frequently appear *de novo*, or that they propagate across cells in association with other particles. The relevance of DIPs and/or SIPs is supported by *in vivo* work showing that influenza virus particles that lack segments can undergo multiple infection cycles in the upper respiratory tract of guinea pigs [11]. Sequencing of nasopharyngeal specimens from infected humans indicated frequent DIP production and suggested that DIPs can undergo inter-host transmission [12].

Per-virion cell invasion efficiencies have also been suggested to be unexpectedly low in non-segmented viruses, such as tomato mosaic virus (ToMV) undergoing cell-to-cell spread through plasmodesmata. ToMV was labeled with sequence tags or fluorescent markers to quantify the population bottleneck experienced during transfer between cells [13•]. Whereas plasmodesmata should allow for the passage of hundreds or thousands of viral genomes, the authors suggested that the vast majority of these genomes fail to give progeny, and that each cellular infection is effectively initiated by only 2–7 viral

genomes, on average. This sieving is a poorly understood phenomenon and could obey to lack of infectivity, but also to competition or even to altruistic interactions among viral genomes at the intracellular level [14•]. Another study with vaccinia virus used microfluidics to place a specific number of viral particles in individual cells [15••]. Most cells receiving a single particle were uninfected, whereas infection probability increased disproportionately (logistically) with the number of particles placed per cell, suggesting a cooperative initiation of the infection cycle.

Spatial clutering and MOIs

A monodisperse viral population (i.e. showing no spatial structure) will fail to reach sufficiently high MOIs during the early stages of population growth. This is because typically only a minuscule fraction of particles present in a given individual host colonize new hosts [16,17], and bottlenecks also operate at the intra-host level, as revealed by sequence analysis of well-studied pathogens such as HIV-1, influenza A virus, and hepatitis C virus [18–20]. Importantly, though, high MOIs are reached much earlier if the population exhibits spatial clustering. As a result of the diffusion process of free viral particles, most virus grow in the form of infection foci. Even stronger clustering can be achieved if the virus uses cell-to-cell spread, which allows for direct transfer of multiple viral genomes between cells and has been described in many viruses including most plant viruses, HIV-1, human T-cell leukemia virus, measles virus, vaccinia virus, and herpes virus [21,22•]. However, in most cases, this spread mode is local, and systemic dissemination probably relies on free virions. Therefore, the high-MOI regime would be interrupted during systemic dissemination and inter-host transmission, purging out semi/non-infectious particles. In some cases, though, the cell-to-cell mode may also operate during systemic dissemination, notably in the case of blood-borne viruses such as HIV-1 [21,23•]. Inter-host transmission in a cell-associated manner is an understudied process, and may be more common than often assumed. Again, a well-studied case is HIV-1, for which the cell-associated route is known to contribute significantly to inter-host transmission [24].

Despite a likely role of limited diffusion and cell-to-cell spread in the maintenance of semi/non-infectious particles, it was inferred in cell cultures and in humanized mice that cells co-infected with GFP-encoding and mCherry-encoding HIV-1 had only a 6–14% chance of transferring both variants by the cell-to-cell route using virological synapses [25]. Although this was far more efficient than free virion-dependent coinfection, these data suggest that cell-to-cell spread may not allow for the sustained co-transmission of different virus variants throughout multiple cycles. However, further work is required in this area.

Collective infectious units as coinfection vehicles

If high cellular MOIs help overcome the low infectivity of individual particles, viruses might benefit from maintaining relatively high MOIs even in the presence of the strong population bottlenecks associated with dispersal. As outlined above, the case of multi-partite viruses is particularly extreme. Since very high viral population densities would be required for ensuring that a full set of independently diffusing segments is delivered to at least a fraction of cells, there should be mechanisms leading to the linked spread of segments, at least for viruses with more than three segments [5,6]. One possible such mechanism is inter-segment RNA–RNA interactions [26]. Interestingly, packaging does not appear to be necessary for systemic dissemination of brome mosaic virus, since uncoated viral RNAs can move long distances, probably in the form of ribonucleoprotein complexes involving cellular factors and the viral movement protein [27]. Systemic dissemination in the form of ribonucleoproteins has also been shown for potato mop top virus [28]. In some plant species, not all viral RNA segments are required for spread at the intra-host level, and it is therefore likely that the RNA–RNA–protein interactions mediating physical segment linkage involve only a subset of segments, mainly those encoding the replication machinery and other essential factors. However, this leaves unanswered the problem of how multipartite viruses undergo inter-host transmission, as this stage necessitates virions and the concurrence of all segments.

Polyploidy might be yet another strategy for increasing the chances of successful cellular infection. In segmented viruses, aneuploidy can be seen as a trivial consequence of non-selective segment encapsidation. However, and more interestingly, polyploidy might serve as a strategy to increase infectivity. This was first studied using infectious bursal disease virus [4]. The size of the icosahedral capsid of this virus is larger than required for packaging just one copy of each of the two segments, and can easily accommodate two copies of each. This might compensate for non-selective packaging. With room for only two RNA molecules and random packaging, 50% of the capsids would miss one of the two essential segments and hence would not be infectious. In contrast, with room for four RNA molecules, this chance drops to 12.5%. Similar findings were later reported in infectious pancreatic necrosis virus, another birnavirus [29]. Interestingly, polyploidy has also been shown in non-segmented viruses, where the problem of ensuring a full set of segments obviously does not exist. This includes filamentous viruses such as bacteriophage f1 [30] and Ebola virus [31], in which capsids are capable of accommodating extra genetic material, but also measles virus [32], which forms particles containing a flexible helicoidal nucleocapsid surrounded by an external spherical envelope.