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MINIREVIEW

Population Diversity and Collective Interactions during Influenza Virus Infection

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ABSTRACT Influenza A virus (IAV) continues to pose an enormous and unpredictable global public health threat, largely due to the continual evolution of escape from preexisting immunity and the potential for zoonotic emergence. Understanding how the unique genetic makeup and structure of IAV populations influences their transmission and evolution is essential for developing more-effective vaccines, therapeutics, and surveillance capabilities. Owing to their mutation-prone replicase and unique genome organization, IAV populations exhibit enormous amounts of diversity both in terms of sequence and functional gene content. Here, I review what is currently known about the genetic and genomic diversity present within IAV populations and how this diversity may shape the replicative and evolutionary dynamics of these viruses.

KEYWORDS RNA virus, diversity, evolution, genome organization, influenza, population biology

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Seasonal influenza A viruses (IAVs) are estimated to cause thousands of deaths and tens of billions of dollars in economic costs every year in the United States alone, despite widespread preexposure and vaccination (1). IAV persists within the human population by continually evolving resistance to herd immunity. This is not a general feature of all viruses: many viruses with mutation rates similar to those of IAVs (e.g., measles virus) do not effectively evolve immune resistance in humans and are effectively controlled by vaccination (2, 3). Identifying the specific factors that influence the evolution of influenza viruses remains a critical open question in virology, one that must be addressed in order to design next-generation escape-resistant vaccines and therapeutics.

New technologies and approaches have begun to reveal in higher definition the extent to which IAV populations exist as highly diverse swarms of genetically and phenotypically heterogeneous particles. Beyond physical pleomorphy (reviewed elsewhere [4]), IAV populations exhibit enormous amounts of both genetic and genomic diversity (Fig. 1). Genetic diversity refers to the abundance of nucleotide sequence polymorphisms that arise from the relatively high mutation rate of the viral polymerase, a characteristic that IAV shares with most other RNA viruses. Genomic diversity refers to variation in the gene coding capacity of individual viral particles, i.e., which viral genes are or are not successfully expressed upon infection. This minireview will cover what we know about the genetic and genomic diversity within IAV populations and will discuss the implications for understanding the replication and evolution of these critical pathogens.

GENETIC DIVERSITY

IAV populations exist as swarms of closely related yet genetically distinct minor sequence variants often (but not always accurately) referred to as a "quasispecies" (5).

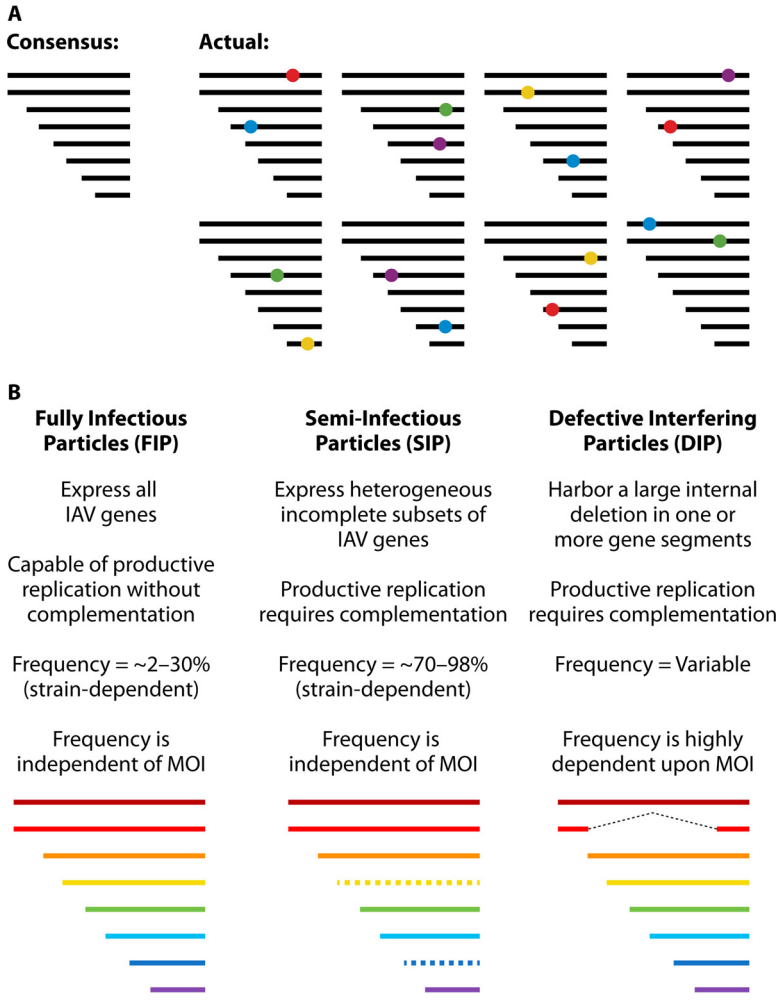

FIG 1 Genetic and genomic diversity. (A) Simplified representation of genetic diversity within an IAV population. Black lines represent the consensus sequence, colored dots represent single nucleotide variants (SNVs) present within individual virions. (B) The distinct particle types present within IAV populations that contribute to genomic diversity.

The effects of this diversity on viral fitness and pathogenicity have been extensively explored in other RNA virus systems (6–8). Preexisting genetic variation provides the fuel for adaptation in the form of mutations that may confer enhanced fitness upon encountering new selective pressures (9–11). This process of evolutionary innovation facilitates both the persistence of seasonal influenza virus strains within the human population and the emergence of zoonotic strains with pandemic potential into human circulation, as both processes depend upon the accumulation of beneficial substitutions (12–14). The enormous potential benefits of this diversity are not without costs, as the majority of random mutations are actually deleterious (15). The evolutionary success of a virus likely depends in part on balancing the beneficial and deleterious effects of maintaining a large degree of standing diversity.

The mutational landscape present within IAV populations is the product of the mutation-prone replication process and is shaped by selection and other evolutionary forces such as genetic drift. Here I will outline what is known about the virologic features and the evolutionary mechanisms that govern the genetic makeup of IAV populations.

ORIGINS OF GENETIC DIVERSITY

Similar to most other RNA viruses, IAV encodes an RNA-dependent RNA polymerase (RdRp) that lacks proofreading capability, resulting in significantly lower genome

replication fidelity than what has been observed for organisms with DNA genomes (16, 17). Numerous studies have estimated a mutation frequency for IAV populations in the range of 1.5×10^{-5} to 2.0×10^{-6} mutations per nucleotide per infectious cycle (18–22). Mutation frequencies may underestimate the actual mutation rate of a virus, as mutations that decrease fitness will likely be underestimated (23). To account for this, Pauly et al. recently developed a Luria-Delbrück fluctuation test for IAV that measured the rate at which a defined set of fitness-neutral substitutions are generated (24). This approach estimated mutation rates of 1.8×10^{-4} and 2.5×10^{-4} substitutions per nucleotide per RNA strand copied for H1N1 (A/Puerto Rico/8/34) and H3N2 (A/Wisconsin/03/07), respectively. This is greater than the inverse of the IAV genome size of ~13 kb, suggesting that newly synthesized viral genomes could be expected to harbor up to two mutations on average.

Further, the rates at which specific mutation types generated are not equivalent, suggesting that the IAV RdRp may be biased toward some classes of mutations over others (24). This is in line with previous studies of poliovirus and hepatitis C virus which found that the frequencies (not rates, and thus subject to effects of selection) of specific types of mutations could vary by more than 2 orders of magnitude (25, 26). In addition to the inherent tendencies of the viral replicase complex, it is possible that mutation rates may be further influenced by template sequence context or host cell factors (27–29). The existence of inherent mutational tendencies may constrain the ability of IAV populations to traverse fitness landscapes, as some potentially beneficial substitutions may exhibit especially low frequencies of *de novo* generation.

There is some evidence that the mutation rate may vary among viral strains; Suárez et al. isolated mutator mutants within wild-type IAV populations that exhibited apparent mutation rates approximately four times higher than the population mean (20). A more recent study isolated a ribavirin resistance mutation (V43I) in PB1 that resulted in a roughly 1.5-fold decrease in the measured mutation frequency (30). Increasing the fidelity of the IAV RdRp appears to be attenuating *in vivo*, as has been observed for poliovirus and Chikungunya virus (6, 7, 30, 31). It remains to be seen whether IAV mutants with decreased fidelity will also be attenuated, though it may be expected based on studies with picornaviruses and alphaviruses (32–35).

MUTATIONAL TOLERANCE

Beyond the mutation rate, the amount of standing diversity within viral populations is influenced by the distribution and magnitude of mutational fitness effects, a property known as mutational robustness (36–38). A higher degree of mutational robustness would allow a gene or population to maintain more genetic diversity, as mutations will be on average less susceptible to purifying selection (39). It has been hypothesized that the sustained antigenic evolution of hemagglutinin (HA) is partially enabled by a high degree of mutational robustness within neutralizing antibody epitopes on the HA globular head, minimizing the fitness tradeoffs associated with immune escape substitutions (40, 41). Multiple studies that examined the relative abilities of different regions of the HA gene to tolerate either single amino acid substitutions or five-amino-acid insertions have suggested that the HA globular head is in fact more robust than the rest of the gene (40, 42–44).

A recent study by Visher et al. examined the genetic robustness of IAV by precisely measuring the fitness effects of a panel of point mutants that spanned the genome of the H1N1 strain A/WSN/33 (15). They found that, similar to other RNA viruses, the majority of IAV point mutations have deleterious effects on fitness and that roughly 30% of mutations are lethal (15, 45–48). Further, the HA and neuraminidase (NA) genes appeared to be more robust than the rest of the genome (a strong but not statistically significant trend), and the HA globular head appeared to be more mutationally tolerant than the stem region, thus corroborating the findings of the previous studies.

REASSORTMENT

Genetic diversity within IAV populations is further shaped by the process of reassortment, in which mixed-genotype progeny are generated through the intermixing of genome segments delivered by multiple coinfecting viral particles (49–51). For compatible strains, reassortment occurs with high frequency both *in vitro* and in infected guinea pigs, with little to no preferential segregation of segments (52–54). Similar patterns of rampant reassortment between strains have been observed in wild bird populations (55, 56). In some cases, incompatibilities between the packaging signals or the individual proteins of different strains can restrict reassortment or heavily bias it to produce specific gene segment combinations (57–61).

There are limited data on reassortment during human infection, though one study has suggested that the effective reassortment rate may be relatively low during acute infection (62). At the host population level however, reassortment appears common among circulating seasonal IAVs of the same subtype and may have played a role in increasing the severity of some seasonal epidemics (63–68).

Reassortment is generally considered in the context of coinfection between different strains or subtypes, as this process has been central in the emergence of nearly every influenza pandemic strain of the past century (69). Reassortment within single viral populations also plays an enormous role during IAV evolution however, as it serves as the primary means of recombination for IAV, since copy choice recombination appears to be exceedingly rare for these viruses (70). By breaking apart and rearranging different gene segment constellations, reassortment can decrease linkage between the individual genome segments, allowing the separation of beneficial alleles from deleterious alleles on other segments. As such, reassortment can facilitate the rapid shuffling of viral gene segment variants within a population into optimally fit combinations and may potentially accelerate the process of adaptation (71).

INTRAHOST DIVERSITY AND SELECTION

Beyond the generation and reassortment of variation, the IAV mutant swarm is further shaped by evolutionary forces acting at multiple scales of infection: intracellular, intrahost, and between hosts. These forces can include positive and purifying selection, as well as the stochastic influence of genetic drift. Advances in next-generation population sequencing have greatly expanded our ability to examine intrahost diversity in experimental animal systems as well as human samples.

Initial studies of intrahost diversity in dogs, pigs, horses, and birds suggested rapid emergence of single nucleotide variants (SNVs) within individual hosts and evidence for transmission of those SNVs between hosts in some cases (72–74). Efforts to define the effects of prior vaccination on intrahost diversity in experimental settings failed to identify any clear signatures. HA substitutions within antigenic sites were observed at greater frequency in vaccinated versus naive dogs and horses; however, no such differences were observed in pigs (73, 75, 76). Surprisingly, measures of selection (such as the ratio of nonsynonymous to synonymous substitutions [*dN*/*dS* ratio]) were similar for vaccinated and naive animals in all of these studies.

A handful of recent studies have examined intrahost genetic diversity during human infection. Poon et al. assessed intrahost variation within a cohort of patients naturally infected during the 2009 H1N1 pandemic (77). Comparison of SNVs between samples suggested that SNVs (including antigenically significant substitutions located within HA) were commonly transmitted between individuals sharing a household. Sobel et al. examined a set of human patients experimentally challenged with seasonal H3N2 and found strong evidence for purifying selection limiting the emergence of coding SNVs during human infection (78). Similarly, Dinis et al. reported a dominant role for purifying selection in limiting HA diversity during natural infection (79). Finally, Debbink et al. used a large set of samples collected during a placebo-controlled vaccine efficacy trial to assess the effects of vaccination on intrahost variation during natural infection (80). This study also observed a dominant role for purifying selection, similar to the previous studies. Surprisingly, host humoral immune status (as measured by HA inhibition [HAI]

and NA inhibition [NAI] activities in serum) had no significant effect on the SNV profile. This finding suggests that the immune mechanisms often thought of as the primary drivers of IAV evolution in humans (namely, HA- and NA-inhibiting antibodies) may not exert as much of an effect at the intrahost scale as expected.

One potential explanation for the discordance between evolutionary dynamics observed at the intrahost and between-host scales is that the studies outlined above have focused on a relatively narrow swath of the human host population. Individuals at extremes of age, with conditions that limit viral clearance, or variation in immune repertoire may all give rise to intrahost evolutionary dynamics distinct from those captured in the above studies. For instance, there is strong evidence that the antibody repertoires of different age groups vary in their recognition of different antigenic sites on HA (81). It is possible that antigenic drift substitutions may emerge globally from a specific subset of hosts in which intrahost and between-host selection are more closely aligned.

This issue was examined recently by Xue et al. in a study that examined HA evolution in a cohort of immunocompromised patients that suffered from chronic H3N2 infections (82). This study observed that a cluster of antigenically significant HA substitutions emerged at high frequencies in multiple patients, albeit generally over a span of weeks. Intriguingly, intrahost emergence of these substitutions presaged their emergence on a global scale in subsequent years. The contrast between the results of this study and other studies of intrahost variation may be explained by the significantly longer duration of infection in immunocompromised hosts. A longer time frame may have permitted weakly selected antigenic substitutions to emerge above the threshold of detection. Alternatively, antigenic substitutions may have been allowed time to accumulate additional compensatory mutations that increased their overall fitness.

Adding to the complexity of understanding intrahost selection, selective pressures are not uniform throughout the host, or even within a single organ. The mammalian respiratory tract includes a diverse spectrum of cell types and tissue microenvironments, each imposing distinct constraints on the virus. This tissue/cellular heterogeneity could result in compartmentalization of variants as the population explores multiple niches. The structuring of IAV populations into genetically distinct subpopulations could significantly affect the adaptive potential of the population as a whole (83). Lakdawala and colleagues recently revealed that differences in the distribution of specific sialic acid receptor structures across the respiratory tract can lead to compartmentalization of IAV populations *in vivo* (84). These results suggest that an accurate accounting of the genetic diversity of a population and the mechanisms of adaptation will require sampling of multiple tissue subcompartments.

GENOMIC DIVERSITY

Beyond genetic diversity, IAV populations exhibit a high degree of genomic diversity, or diversity in the gene coding capabilities of individual virions. This is a direct function of the division of the IAV genome into eight distinct, negative-sense RNA genome segments. The individual genome segments delivered to a cell by a virion are each physically associated with a viral polymerase complex, allowing them to function as distinct transcriptional units, largely independent of one another (85). This means that the absence or inactivation of one viral genome segment does not necessarily preclude replication and expression of the others.

A productive replication cycle requires expression from all eight segments, and for many years, it was generally believed that close to 100% of virions carried a copy of each of the eight genome segments, thus ensuring their replicative potential (86–88). While it is clear that IAV encodes a highly selective mechanism for genome packaging, a number of studies have suggested that the situation may be more complicated than dogma suggested (89, 90). Some of the earliest evidence comes from a pair of studies that suggested that traditional measures of infectivity, such as the plaque assay, substantially underestimated the true infectious potential of IAV populations (91, 92). Going further, the work of Marcus and colleagues revealed that the number of IAV

particles capable of triggering apoptosis or inducing or suppressing type I interferon (IFN) production greatly outnumbered the number of particles capable of forming plaques (93–95). These findings suggested that non-plaque-forming IAV particles can still exert significant and highly heterogeneous effects on host cells.

SEMI-INFECTIOUS PARTICLES

A potential explanation for this heterogeneity was provided in a study by Martin and Helenius that suggested that a fraction of infectious IAV particles failed to express the full set of viral genes (96). Subsequent work by Hutchinson et al. observed a similar pattern of infected cells that fail to replicate a given viral RNA (vRNA) segment (97). We extended these findings to demonstrate that the vast majority of infectious IAV particles express variable, incomplete sets of viral gene products (defining infectious as the ability to transduce cells to express any detectable viral protein) (98).

Using a flow cytometry-based method that allows the quantification of single virion gene expression patterns and examining the laboratory-adapted H1N1 strain A/Puerto Rico/8/34 (PR8), we determined that the viral HA, nucleoprotein (NP), NA, and NS1 genes were each individually expressed by only 70 to 80% of infectious particles (98). In accordance with this finding, we also observed that close to 90% of infectious PR8 particles are incapable of multiround replication under low-MOI (multiplicity of infection) conditions. We coined the term semi-infectious particles (SIPs) to differentiate these particles from the small fraction of fully infectious particles (FIPs) (commonly measured as PFU) that do express the full set of viral genes and are capable of independent replication. Importantly, despite constituting ~70 to 98% of biologically active particles within IAV populations, SIPs are generally not accounted for by traditional infectivity assays (98).

The relative production of SIPs versus FIPs is independent of the cell line, animal, or MOI conditions used during virus production and did not significantly differ when we used different cell lines as target cells (98). This suggests that SIP production is an intrinsic feature of the viral replication process that is minimally affected by host biology; however, it is entirely possible that a more comprehensive survey will reveal host conditions that do affect SIP production and/or the gene expression patterns of individual virions. Importantly, there are numerous non-mutually exclusive viral mechanisms that could explain the incomplete expression patterns of SIPs, including gene-lethal mutations and failure of either gene segment packaging or nuclear delivery (15, 99, 100).

We and others have since shown that SIPs are produced by a wide variety of H1N1 and H3N2 strains (98, 101–103). Intriguingly, we observed a significant amount of diversity in the ratio of SIPs and FIPs produced by different IAV strains (101). This suggests the existence of strain-specific genetic determinants of SIP production and raises the possibility that increased or decreased SIP production may be selected for over the evolutionary histories of some IAV genotypes. How these differences in SIP production and gene expression may contribute to differences in replication, transmission, and pathogenicity between IAV strains remains poorly understood.

Some initial insight into this issue comes from efforts by Ince et al. to identify mechanisms that contribute to species adaptation (71). By serially passaging a maladapted H1N1 strain in guinea pigs, they identified a single amino acid substitution in nucleoprotein (F346S) that is sufficient to confer enhanced fitness and transmissibility. Subsequent examination revealed that this single substitution in NP results in a selective decrease in NA vRNA abundance, NA protein expression, and the incorporation of NA protein into virions (101). Importantly, the NP F346S substitution also results in a threefold reduction in the relative number of NA genome segments packaged into virions. This selective decrease in genome packaging comes with a proportional increase in the number of SIPs produced relative to FIPs. This study demonstrated both that SIP production can be rapidly modified through a single point mutation outside of the canonical IAV packaging determinants and that decreased genome packaging efficiency can actually be associated with increased *in vivo* fitness and transmissibility.

Association does not necessarily imply causality, and more work is needed to understand how SIP production influences viral fitness and transmission.

EFFECTS OF SIPs ON POPULATION-LEVEL PHENOTYPES

One potential consequence of SIP production for IAV populations is that it may promote reassortment by necessitating that a large fraction of the population replicate through coinfection (89, 98). Fonville et al. directly tested this hypothesis and demonstrated that artificially increasing the proportion of SIPs present in a viral population increases the frequency of reassortment (102). As reassortment can accelerate IAV adaptation, SIP production could influence the rate of adaptation by IAV populations (71).

Variation in gene expression potential at the population level may also facilitate selective regulation of viral gene expression through gene dosing effects, as has been demonstrated for multipartite plant viruses (104, 105). Changes in the percentage of particles that carry a functional copy of a given IAV genome segment will alter the stoichiometry of gene segments delivered to multiply infected cells. We demonstrated that stoichiometric changes in gene segment abundance at the population level are sufficient to significantly alter the relative expression levels of the viral glycoprotein genes within multiply infected cells (101). It remains to be seen whether SIP-mediated regulation of IAV gene expression through gene dosing actually plays a significant role in the real world, however.

DEFECTIVE INTERFERING PARTICLES

Complicating matters further, IAV populations can also include defective interfering particles (DIPs). DIPs were first described more than 60 years ago as a common product of high-multiplicity IAV replication and were defined both by a requirement for coinfection with wild-type virus to replicate and the ability to interfere with the productive replication of wild-type virus, hence the name (106–109).

DIPs are characterized by the presence of large internal deletions within one or more viral genome segments that eliminate much of the protein coding capability of the segment while retaining the genome packaging signals (110–112). As a result, DIP genome segments can outreplicate full-length segments while also competing with them for packaging into virions (113, 114). This competition can suppress replication of full-length infectious genomes; thus, the presence of DIPs is generally considered to be detrimental to IAV populations. Importantly, DIPs are distinct from SIPs, which do not interfere appreciably with replication, do not harbor large internal deletions, and whose production is not MOI dependent.

The presence of IAV DIPs can influence the host environment in ways that we still do not fully understand. DIPs can suppress the cytopathic effect induced by normal virus infection *in vitro* and can limit virus-induced pathology in mice (115–118). There is substantial evidence that DIPs can modulate the host inflammatory response to infection, potentially due to differential detection of DIP RNAs by cytosolic RNA sensors (119). Much more work is needed to better define how the presence of DIPs alters the host response to infection and what the consequences of this are for both the virus and host.

While most studies of influenza virus DIPs have been carried out *in vitro*, a recent study revealed that DIPs are regularly generated during natural infection in humans and suggested that DIP genomes can potentially be transmitted from person to person (120). This would suggest that the presence of DIPs may not hinder viral transmission as much as would be expected. Given how little we still know about the biology of IAV DIPs, it is likely that their effects on viral populations may be more complicated and more interesting than previously appreciated.

IMPORTANCE OF COINFECTION AND COLLECTIVE INTERACTIONS

The high degree of genomic diversity within IAV populations results in the vast majority of virions being dead-end products under low-MOI conditions. This suggests

that coinfection and complementation likely play important roles during natural infection by facilitating the productive replication of SIPs through multiplicity reactivation, a phenomenon that has been long observed with IAV (91, 92).

How widespread is coinfection during *in vivo* infection? The issue of *in vivo* MOI during viral infection remains poorly characterized due to the technical challenges involved in measuring it. Fukuyama et al. used a mixture of IAV strains expressing different fluorescent reporters to reveal that ~30% of IAV-infected cells are coinfected with multiple genotypes in mice (121). This is likely an underestimation however, as it does not capture coinfection by virions expressing the same reporter. To estimate *in vivo* coinfection rates without the need for reporter genes, we took advantage of the predictable relationship between the effective MOI and the coexpression frequency of IAV genes that arises from complementation of SIPs. We found that conditions of widespread coinfection are rapidly established following intranasal inoculation of guinea pigs, comparable to an *in vitro* MOI of ~5 to 15 virions per cell (101). Importantly, this measurement is from a single time point, and the effective MOI of IAV during natural infection likely varies over both time and space within a given host.

Frequent coinfection would allow for the emergence of collective interactions between virions and between gene segments within IAV populations (122). Collective interactions within a population could range from antagonistic, as typified by DIP competition with wild-type virus, to cooperative. Cooperative interactions include complementation and multiplicity reactivation of SIPs, as well as more complex, mutually beneficial interactions between distinct variants of a given gene segment. Instances of cooperativity between variants within complex populations have been previously described for coxsackievirus and measles virus (123, 124). A more recent study by Xue et al. demonstrated that similar cooperative dynamics could emerge within IAV populations as well (125). In this study, the authors observed the frequency-dependent selection for a mixture of two distinct NA variants rather than a pure population during *in vitro* passage of a particular H3N2 strain. The growth advantage of the NA variant mixture over pure populations was only clearly observed under high-MOI conditions, indicating that frequent coinfection was required for the effect. More work is needed to explore the extent and significance of collective behaviors during IAV replication and adaptation.

GENOME ARCHITECTURE AND THE FUNCTIONAL ORGANIZATION OF IAV POPULATIONS

There is still a great deal we do not know about how the diverse genome architectures of different virus families influence their replication and evolution. Among segmented viruses, genome packaging efficiencies vary widely, ranging from apparently near-perfect copackaging for reoviruses and phi6 bacteriophage to completely independent (or multipartite) packaging for some plant viruses (104, 126–128). IAV appears to fall closer to the latter end of this spectrum, as the vast majority of IAV particles are incapable of expressing the full set of essential viral genes and thus have a partially multipartite genome (98). The organization of the IAV genome is unique (as far as we know) among human pathogens; however, a segmented Jengmenvirus isolated from mosquitoes, Guaico Culex virus (GCXV), was recently found to have a similar, partially multipartite genome organization (129). There is also evidence that Rift Valley fever virus, a zoonotic bunyavirus that is capable of lethally infecting humans, produces significant numbers of particles that lack the full set of genome segments (130). The origins of this diversity in genome organization remain mysterious. Are they simply the result of historical chance or are they adaptive? If segmentation and multipartite genome organization are adaptive, what pressures drive their emergence and maintenance?

To date, everything we have learned about the diversity and complexity of IAV populations necessitates a reconceptualization of the viral genome. Rather than the textbook image of eight gene segments contained within a single virion, the effective IAV genome is the variable collection of gene segments delivered to a given cell by a

genetically and genomically diverse population of virions. The distribution of the eight individual segments across host cells is dynamic over time and space and is shaped by coinfection rates and differences in the gene coding patterns of individual virions, resulting in varying states of ploidy. This view has significant implications for understanding the replicative and evolutionary potential of IAV populations and calls for further experimental and theoretical exploration.

FUTURE QUESTIONS

Recent breakthroughs in population sequencing and other experimental approaches have revealed the enormous amount of genetic and genomic diversity present within IAV populations. We are still in the early stages of dissecting how this heterogeneity influences the replication and evolution of IAV populations, and a number of outstanding questions remain.

What forces drive the global emergence of antigenic substitutions? While viral targets of adaptive immunity are clearly under strong positive selection at the global scale, the same does not appear to be true at the intrahost scale (131–135). Multiple studies suggest that purifying selection limits genetic diversification and that antigenic variants rarely emerge at a high frequency within an individual host over the short span of an acute infection, regardless of immune status. The relatively limited emergence of antigenic escape variants observed within vaccinated individuals suggests that selection to escape neutralizing antibody pressure may not always be the dominant evolutionary force acting on intrahost IAV populations. If selection at the between-host and intrahost levels is discordant, how and where do these variants emerge from low frequencies within individual hosts to achieve fixation at the global scale?

What role does genetic drift play during intrahost and between-host IAV evolution? Numerous studies have suggested that IAV populations undergo significant bottlenecks during natural transmission, such that most infections are initiated by at most a couple hundred genomes (77, 136, 137). The repeated bottlenecking of IAV populations may result in a significant role for genetic drift in both limiting the forward transmission of beneficial variants and in increasing the abundance of deleterious variants. Further work should be aimed at defining how transmission bottlenecking and stochasticity influence IAV adaptation across different scales.

Have influenza viruses evolved to generate and structure both genetic and genomic diversity at the population level to maximize replicative and/or adaptive potential? It has been suggested that RNA virus mutation rates have been selected to balance evolutionary innovation with genome integrity and replicative viability (138). It remains to be seen whether and how genomic diversity is modulated by selection. A related question is the extent to which IAV strains and subtypes differ in the specific virologic parameters that determine the genetic and genomic makeup of populations, such as the propensity for reassortment, the production and gene expression patterns of SIPs, virion aggregation rates, spatial diffusion of virions, and the frequency and distribution of coinfection. Between-strain variation in these parameters could result in differences in transmissibility or evolutionary potential and could determine the potential for collective interactions.

How do differences in the functional organization of IAV populations affect emergent population-level phenotypes such as transmissibility and adaptability? While there is experimental evidence that changes in SIP production and gene expression patterns can be associated with changes in reassortment frequency and transmissibility (101, 102), mechanistic connections between the genomic makeup of IAV populations and their emergent phenotypes remain elusive.

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