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Editorial overview

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Peter Simmonds has been a Professor of Virology at Edinburgh University since 1995. His varied research programme embraces a common theme of the evolution and epidemiology of virus infections, and interactions with their hosts. Current research investigations range from evolutionary studies of virus variability and recombination, molecular epidemiology and investigations of viral pathogenesis and interactions of virus with host cell defences. Much of this work has been associated with the development of a variety of molecular biology and bioinformatic analysis techniques.

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Esteban Domingo has been Professor of Research at Centro de Biología Molecular Severo Ochoa (CSIC-UAM) since 1989. With his group in Madrid he documented high mutability and quasispecies dynamics of the important animal pathogen foot-and-mouth disease virus (FMDV). Work with FMDV and other RNA viruses, in particular vesicular stomatitis virus (VSV) in collaboration with John Holland, established multiple biological implications of quasispecies dynamics. In recent years his research is centered in lethal mutagenesis as a new antiviral strategy.

This volume addresses concisely many of the major issues of virus evolution. Topics reviewed include: intra-host variation with its implications for viral pathogenesis and disease control, the process of viral mutation and selection, reconstruction of viral evolutionary histories and patterns of long-term evolution and their connection with host–virus coevolution. Intra-host evolution is the result of positive and negative selection and random drift acting on mutationally active and ever changing mutant swarms. This is particularly extreme in the case of RNA viruses because (with the exception of the large coronaviruses) they lack a proofreading-repair activity in their RNA-dependent RNA polymerases. Thus, misincorporation errors remain uncorrected, giving rise to genetically complex populations. For some viruses recombination also contributes significantly to the generation of variant genomes.

Population heterogeneity was traditionally characterized through molecular cloning and Sanger sequencing. More recently ultra deep sequencing methodologies have been applied in studies of virus population heterogeneity, providing nucleotide sequences of thousands or tens of thousands of genomes present within a sample. The analytical capacity of this technique has expanded enormously and its ability to detect minor populations and potentially the presence of mutated viral genomes before selection is profoundly changing our view of virus genetics. The future implementation of third-generation whole genome massive sequencing will increase this analytical capacity further and potentially enable whole population studies. However, ultra deep sequencing is not free from potential pitfalls that arise from both the sequencing data and bioinformatic alignment errors and that can distort the quantifications of complexity of viral populations. In this regard, Beerenwinkel and Zagordi provide a valuable discussion of the use of deep sequencing for investigating quasispecies evolution and the computational and statistical advances for read error correction and for the estimation of true population heterogeneity. When applied correctly, these ultra deep sequencing analyses have unveiled extremely large amounts of variants participating in many adaptive pathways, most abortive and a few successful, that viruses explore to overcome selective pressures. The new data reinforce the view that evolution, rather than being timed by some steady accumulation of mutations, emerges from disequilibria in mutant distributions.

It has long been known that elevated mutation rates and the presence of highly dynamic mutant swarms represent serious difficulties for viral disease control. The almost systematic selection of viral mutants resistant either to single or multiple antiviral drugs is a firm observation in clinical practice. Some new antiviral designs are currently being explored in an attempt to

abate the potential of viruses to find fitness-enhancing pathways in the presence of antiviral agents. One such approach is termed lethal mutagenesis, and it consists of provoking an excessive mutational load in viral genomes through mutagenic agents, so as to jeopardize basic viral functions. Unexpected to many only a few years ago, the use of virus-specific mutagenic agents has had its first clinical trial with AIDS patients, a remarkable step forward. Lethal mutagenesis is not without problems, including the requirement that mutagenesis be virus-specific, and the already documented selection of mutagen-resistant viral mutants. However, the studies undertaken to understand the molecular basis of viral extinction by enhanced mutagenesis are helping to unveil complex interactions that can be established among components of a mutant spectrum. Of note, such interactions that can push viruses towards fitness gain or fitness loss can involve not only viruses within an entire organism but also within an organ or even a cell. An understanding of the interplay between mutagenesis and inhibition is also suggesting new means of antiviral intervention, in particular the possibility of sequential rather than combinatory inhibitor-mutagen administration. The current status of lethal mutagenesis is summarized by Perales, Martín and Domingo.

Faria and colleagues have contributed a timely summary of the new and emerging field of viral phylogeography, a method for evolutionary reconstruction that accounts for both temporal and geographical distributions of viral variants. This approach acknowledges that sequence evolution and the accumulation of substitutions over time also differentiate viruses' populations that have become geographically dispersed through founder effects. Evolutionary reconstructions that incorporate spatial differentiation by Bayesian inference methods provide historical perspectives on viral spread that have a number of important applications. These include the potential identification of key reservoir species in emerging virus infections with zoonotic origins and in the evaluation of the effect of animal or human mobility on viral disease spread.

The authors provide a clear, non-mathematical and accessible description of the underlying methodologies for evolutionary reconstruction by these methods, and include examples, such as the spread of influenza virus, where this technique has been recently applied. One issue that will have to be addressed in the longer term is how to improve virus population sampling both temporally and geographically to fully exploit the power of phylodynamics to reconstruct virus population histories. Although there are exceptions, such as internationally coordinated very active investigation of the recent global pandemic of swine-origin influenza virus, most current viral sequence datasets are limited in one or other of these variables. Clearly, the use of much larger scale virus sequencing possible by new technologies and a greater application of these methods application in clinical and environmental surveillance is needed to provide the data the method needs.

Virus evolution, reconstruction of their population histories and our understanding of viral population diversity are liked themes that rely fundamentally on the process of mutation. The misincorporation of nucleotides during replication and the subsequent effects of selection or drift that fix substitutions in populations, represent the fundamental driving force in evolution. Despite its importance, the process itself is not well understood mechanistically and subject to a number of misconceptions by many who work directly in the evolutionary field. The review by Belshaw and colleagues tackles these issues head on. It explains the different ways in which mutation rates have been measured, when and where in the virus replication cycle it occurs and its highly variable relationship with substitution rates that are additionally influenced by fitness selection. It additionally reveals the considerable variability in the use of these terms to describe viral evolutionary processes at different biological scales, be it at the level of the individual infected cell, the host organism or the host population. As the authors point out, these require quite different concepts of the reproductive unit, and nature of the selection processes.

A critical issue that has been controversial for decades has been how the vast complexity of genomes present in any infected individual translates into the patterns of long-term evolution. At an epidemiological level, for acute, non-persistent viruses the spread of an infection can be viewed as a succession of transmission events from infected individuals to susceptible hosts. Transmissions often constitute very effective bottleneck events in which one or a few representatives of a mutant swarm are randomly transferred to a new host to generate new swarms, and so on. Persistent or latent viruses, particularly those that integrate their DNA in cellular DNA, or that are transmitted vertically, often restrict the amplitude of the mutant types that go into successive host generations. True long-term evolution, the one derived from a large number of transmission episodes, poses the additional issue of how to reconcile the unpredictable generation of intra-host mutant and recombinant genomes with the evidence of a certain constancy of evolutionary rates, reflecting the operation of a molecular clock. Virologists are divided into believers and non-believers in the molecular clock, with some quasi-believers when relaxed clock models are used to fit the quantifications. Sceptics of the clock will find justifications in the article by Sharp and Simmonds on timescales of virus evolution. There are astonishing discrepancies in rates of evolution, several orders of magnitude in many cases that create entirely different models for the origins and host relationships of viral lineages. Most pertinent is the possibility of

long-term host–virus coevolution. The match between host and virus phylogenies indeed provides evidence for this process in some viruses such as pegiviruses and retroviruses, but which require a long-term viral substitution rate far slower than conceptualised in current models of RNA virus evolution.

The biological basis of such discrepancies remains a mystery, one that will obviously occupy thoughts, measurements, and experiments over the next few years. Perhaps quasispecies swarms and evolution through population disequilibria (as opposed to evolution through linear accumulation of mutations) have been unduly disregarded. We know now that some mutant distributions can include remarkably divergent sequences, particularly those that approach the rank of hypermutated genomes as a consequence of cellular editing activities, increasingly recognised in the generation of variability. Recombination could act on such sequences to rescue viable, highly divergent genomes that could go unnoticed in some epidemiological surveys. However, there are other possibilities. We do not know how representative the sequences on which we base our studies are. Negative selection may preserve only a relatively constant subset of genomes to survive in future generations. The larger the time interval between viral samplings, the slower will the clock appear. Or perhaps the problem lies in computational procedures applied to successions of consensus sequences that entail uncertainties that we have not been able to grasp. Whatever the reasons, it is perhaps the time that virologists who focus on intra-host, quasispecies mechanisms and those involved in the application of phylogenetic and statistical methods to long-term evolutionary analyses, come closer to each other. This issue of Current Opinion in Virology certainly represents a step forward in this direction.