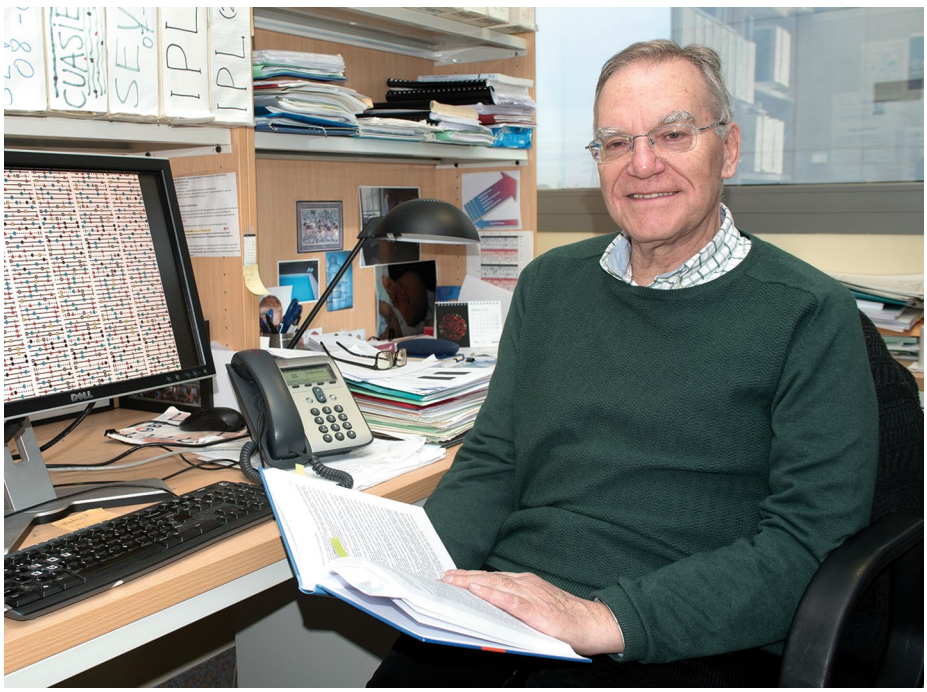
The quest for quasispecies

This month marks 40 years since the publication of 'Nucleotide sequence heterogeneity of an RNA phage population' in *Cell*. We spoke with Esteban Domingo, leading author of this landmark study carried out during his postdoctoral work in Charles Weissman's lab, which proposed RNA viral populations to be quasispecies.



Esteban Domingo

Can you briefly summarize the main findings of this seminal study?

In the 1978 study (E. Domingo et al. *Cell* **13**, 735–744; 1978), we found that in populations of bacteriophage Qβ, each individual genome differed on average from the consensus sequence in one-to-two mutations. Passage experiments showed that genetic heterogeneity was rapidly generated from a single founder genome. We related this finding to a mutation rate of 10^{-4} mutations introduced per nucleotide copied, calculated by myself and Charles with the help of Eduard Batschelet, Professor at the Institute of Mathematics at the University of Zürich. The result of the clonal analyses was unambiguous in that the Qβ genome was not a single-nucleotide sequence. Thus, contrary to the views accepted at the time, we were dealing with clouds of mutants, with an average sequence that perhaps did not even represent any real genome from the population. The connection of our experimental findings with the quasispecies theory of the origin of life — developed by Manfred Eigen and Peter Schuster in Göttingen, also during the 1970s — was established when Charles attended a meeting organized by Manfred. When Charles told me, I was immediately caught by the theory as a base to think of future experiments, and suspected that the findings with Qβ might be valid for other viruses. But this had to be proven.

What was the initial idea behind the experiments?

We were working on site-directed mutagenesis, a pioneer project using an elegant procedure to elongate Qβ RNA in vitro, and to introduce a mutagenic nucleotide at a single, pre-selected site to produce site-specific mutants. I joined Charles' lab when the procedure had been developed by Dick Flavell, Eric Bandle and Donna Sabo, with the great help of Martin Billeter with the mapping of oligonucleotides in the viral genome. We were in pre-sequencing times, and only a few terminal nucleotides of Qβ RNA had been determined using time-consuming RNA-labelling and RNase-cleavage procedures. At the time, mutations affecting a conserved genomic region were thought to be lethal, and the expectation was supported by a first extracistronic mutant constructed by Flavell and colleagues. I was asked by Charles to produce a second mutant, essentially (I suspect) to confirm that it would be lethal. But it was not. I will never forget when I developed the fingerprints that demonstrated that the mutant replicated in *Escherichia coli* (at night sometime in 1975 while we were having dinner in the institute). It was in the course of testing the stability of the mutant that we produced the evidence of quasispecies.

What are the implications of the inherent variability that you discovered in viruses?

Variability at the level we found it provided a molecular basis for the adaptability of RNA viruses to changing environments, a fact that people suspected but for which there was no explanation. It was the first evidence of an increasingly recognized diversity of the biological world, which is now supported by pan-genomic comparisons of cellular and viral populations. Some of the medical implications were brought to my attention by an article published in *Science* by John J. Holland and colleagues in 1982 (J. Holland et al. *Science* **215**, 1577–1585; 1982). The possibility that variant forms of viruses could produce diseases that had unknown aetiology, or atypical forms of known diseases, caught my attention. I must admit that in my lab, we continue to discover new implications of quasispecies to this very day. For example, similarly to how cancer cell heterogeneity influences tumour progression, viral pathogenesis is being increasingly associated with quasispecies dynamics, that is, with the different behaviour of viral subpopulations present in mutant clouds. Quasispecies can be viewed as the first stage of virus diversification in an infected individual, and the starting point of evolutionary processes influenced by selection and random drift. Despite having greater influence on viruses due to their limited genome size and large population numbers, the core concepts of quasispecies apply to many (perhaps most) biological systems.

How was the experimental demonstration of viral quasispecies received by other virologists?

It was received with near silence in the beginning. I suspect that as the first rapid

sequencing methods became available, virologists were interested in sequencing their favourite viruses, without considering the variation within viral isolates. None of the early virology sequencing studies involved comparison of biological or molecular clones. It took time to realize that the clonal variations were important to understand virus behaviour. The impact of quasispecies increased gradually, beginning with the *Science* paper of Holland and colleagues, and with extended use of molecular cloning and nucleotide sequencing, mainly by Holland working with vesicular stomatitis virus, and us working with influenza virus and foot-and-mouth disease virus (FMDV). Also, sadly, with the studies on HIV-1 by the group of Simon Wain-Hobson at the beginning of the AIDS pandemic. I met Richard Jackson at a European picornavirus meeting, and he told me that the Qβ results had arrived 20 years too early — I really did not mind. Indeed, it took more than two decades for quasispecies to become established in general and clinical virology. Fortunately, the concept is now recognized as relevant by experts in widely different fields.

In what ways have technological advances over the last 40 years helped our understanding of the extent and implications of heterogeneity in viral populations?

They have helped in many ways. I find it remarkable that the conclusions made using Qβ were established without rapid sequencing, in vitro DNA recombination, or PCR. When we applied Maxam–Gilbert and Sanger sequencing to molecular and biological clones, we confirmed the genetic heterogeneity that we had seen using oligonucleotide fingerprinting. That was an important moment. A new transition occurred three decades later with the advent of deep sequencing, which provides a detailed picture of mutant spectra. We now have tools to characterize the composition of viral populations in an unprecedented fashion, and at many levels, from environmental samples to individual cells or entire organisms. Furthermore, we can study population dynamics with sequential samples from viruses evolving in controlled lab environments or their natural hosts. Deep sequencing will influence the way we view infection processes and how we design antiviral treatments. Even the concept of lethality should be reconsidered, as what were considered lethal mutations (such as the extracistronic Qβ mutations) may just decrease the frequency of genomes harbouring them, but the mutants may still be replicating at low levels and thus be actors of subsequent evolutionary events. Finally, important progress has also been made in theoretical aspects of quasispecies. For example, the extensions of the theory and its derived error threshold to finite populations in variable fitness landscapes. Peter Schuster has been instrumental in relating experimental and theoretical findings.

At the time the study was published, you were relocating to Madrid to start your own lab. How did you decide what the research focus would be?

When I left Zürich, I asked Charles if he would continue working on Qβ variability and if he could collaborate with my lab in Madrid. He told me that I could take the problem with me because he had other interests. One of the things I had learned from Charles is that when you find something interesting in the lab, you should ask whether the finding extends to other systems. Following the advice of Eladio Viñuela, my mentor in Madrid, we set to find out if the quasispecies concept applied to FMDV and, with the help of Juan Ortín, to human influenza virus. Since these early times, I never doubted that our focus should be on quasispecies and its implications.

What does your lab focus on now?

We have recently determined that high fitness of hepatitis C virus (HCV) populations per se is a determinant of drug resistance. At present, in extremely difficult times for scientific activity in Spain, our team — Celia Perales, Ana Isabel de Avila and Isabel Gallego (in collaboration with other groups) — is working on connecting HCV fitness with virus–host-cell interactions and trying to relate concepts of population dynamics of HCV in cell culture with deep-sequencing analyses of clinical samples, in collaboration with the team of Josep Quer and Josep Gregori at Hospital Vall d'Hebron in Barcelona.

During our collaborations with Holland in the early days, we obtained the first experimental evidence that increases in mutation rate are detrimental to RNA viruses, which established a link with the error threshold concept of the quasispecies theory, and represented the beginning of lethal mutagenesis. We then extended this concept to FMDV and collaborated with several teams on HIV-1 and arenaviruses, and now work on additional viral pathogens. It is quite remarkable that some antiviral agents such as ribavirin or favipiravir may be exerting their antiviral activity through lethal mutagenesis rather than by inhibition of viral replication. We continue to work on the connection between the error threshold and lethal mutagenesis, with an increasing focus on its use as an antiviral strategy, which is a good example of how basic research can find practical applications in the most unexpected manners.

What research directions do you think those interested in viral evolution should be considering in the coming years?

I would suggest relating short-term variations of mutant spectrum composition within infected individuals with viral pathogenesis, choosing good animal models. As I said before, new tools in cellular biology and deep sequencing should provide a new view of infectious processes. I would also recommend to plan interdisciplinary research, and collaboration with theoretical biologists. This theory–experiment connection has been rewarding for me.

You have been a mentor to numerous researchers over the years, what advice would you give researchers setting up their lab today?

To work on what they think is new and important without attending to what is fashionable. Join colleagues with complementary expertise to implement an interdisciplinary approach. Conviction in what you do is essential. Do not force data to publish in high-impact journals. We now have a problem of result reproducibility (for example, in cancer research) that is partly due to pressures to publish brilliantly and prematurely. If these arguments are not convincing, I would convey the advice of Szent-Györgyi: do again an experiment that has already been done and keep your eyes open.

If you could go back to any point in your career thus far and change something, what would it be and why?

I rarely think of possible changes of direction in my scientific career because I am convinced that it is not possible to predict how research plans would have evolved had I changed directions. For example, when I joined Charles' lab in Zürich, Viñuela told me to ask Charles if I could work on Rous sarcoma virus rather than Qβ, as by training in animal virology I could make a valuable contribution to Spanish virology. Charles did not attend to my demand, and I did not insist. Considering the results on quasispecies with Qβ, I doubt that I could have done better! When I was about to leave Zürich, Raul Pérez-Bercoff generously taught me the essentials of picornavirology in the lab, and with the teachings of my colleague Ortín, I compensated a bit for my deficiencies in animal virology. Life and viruses are unpredictable.

Interview by Nonia Pariente

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