
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ELSEVIER

Clinical and Diagnostic Virology 10 (1998) 97–101

Clinical and Diagnostic Virology

# Quasispecies and the implications for virus persistence and escape

Esteban Domingo *

*Centro de Biologia Molecular 'Severo Ochoa', Consejo Superior de Investigaciones Cientificas, Universidad Autonoma de Madrid, Cantoblanco, 28049 Madrid, Spain*

Accepted 28 April 1998

**Abstract**

**Background:** In the 1970s Manfred Eigen and colleagues proposed a new model of molecular evolution to explain adaptability and rapid evolution of simple replicons, as those that probably populated the earth at the onset of life. This model of evolution placed emphasis on mutant generation, to the point of invalidating the concept of wild-type genomes as a defined sequence of nucleotides. In striking similarity with the proposals for such early replicons, present-day RNA viruses consist of complex distributions of nonidentical but closely related genomes termed quasispecies.

**Objectives:** To discuss indeterminations inherent to a quasispecies structure and to the analytical procedures to define it, biological implications of quasispecies, and the need to take into account this type of population structure, in order to design effective strategies to prevent and control diseases caused by highly variable viruses.

**Results:** Quasispecies have many biological implications, extending from viral pathogenesis to the emergence of new pathogens, rapid antigenic variation, and alterations in cell tropism, virulence, host range and viral gene expression.

**Conclusions:** Diseases caused by highly variable RNA viruses prove very difficult to control and vaccine development against such viruses are largely unsuccessful. It is important to understand quasispecies composition and dynamics, as quasispecies are an important step in the natural history of RNA viruses. 

*Keywords:* Quasispecies; Viral swarm; Viral escape; Viral persistence; Hepatitis C virus

*Abbreviations:* FMDV, foot and mouth diseases virus; RT, reverse transcription; PCR, polymerase chain reaction; HIV, human immunodeficiency virus; CTL, cytotoxic T-lymphocyte; AIDS, acquired immune deficiency syndrome; HCV, hepatitis C virus.

* Tel.: +34 1 3978485; fax: +34 1 3974799; e-mail: edomingo@cbm.uam.es

## 1. Introduction

Quasispecies defines a genetic organisation of simple replicons at the population level, first proposed by Eigen, Schuster and their colleagues

0928-0197/98/$19.00 
*PII* S0928-0197(98)00032-4

(Eigen, 1971; Eigen and Schuster, 1979). It means that mutant genomes are generated at such high rates that each individual genomic molecule differs on average in one or a few nucleotides from the other molecules, and each genomic sequence may deviate from the average or consensus sequence. For example, in the proposal of the quasispecies model for phage Q$\beta$ it was found that the most abundant genome amounted to 10–20% of the population. This experimental finding agreed with theoretical predictions of Eigen and colleagues, given the complexity of the Q$\beta$ genome and the mutation rate during its replication (Domingo et al., 1978).

Since this first proposal that bacteriophage Q$\beta$, replicating in *Escherichia coli*, displayed features of a quasispecies, a steadily increasing number of RNA viruses have been shown to share such a population structure (Holland et al., 1982; Domingo et al., 1997). However, there has been a slow realisation of the biological implications of quasispecies. Since adaptability of RNA viruses is key to viral pathogenesis and strategies for disease control, it would seem obvious that quasispecies should have been regarded as highly relevant to these questions (Domingo, 1989, 1996; Domingo and Holland, 1992; Duarte et al., 1994; Novella et al., 1995). This paper updates some observations on quasispecies, and highlights a few critical issues.

## 2. Trying to cope with indetermination

The main departure of quasispecies from previous models of molecular evolution is the consideration of the wild-type not as a genome with a single nucleotide sequence, but as a distribution of genomes which act as a unit of selection. The absence of a defined wild-type has been confirmed experimentally (Domingo et al., 1978, 1995, 1997). A viral genome is statistically defined but individually indeterminate. It is not possible to attribute a biological behaviour to a genomic nucleotide sequence with complete certainty. We must, therefore, rely on the statistical accumulation of data. In a study of antigenic sites of foot-and-mouth disease virus (FMDV) (the example applies to any other virus) the assignment of a neutralising monoclonal antibody to a given epitope necessitates that different clonal preparations of FMDV repeatedly produce escape mutants with amino acid substitutions at the same epitope region (Martínez et al., 1997).

Since it is not possible at present to sequence an individual genomic molecule, a large number of genomes ($10^8$–$10^{11}$) must be gathered, following either biological or molecular cloning, before applying the chemistry for sequence determination. Biological cloning may imply some selection bias on viruses. This led to the timely statement concerning human immunodeficiency virus (HIV)-1 quasispecies analysis "to culture is to disturb" (Meyerhans et al., 1989). Other variations may be found in the mutant spectra derived from reverse transcription (RT) polymerase chain reaction (PCR) amplification procedures, or sequence determination from molecular clones obtained in *E. coli* or other cell-vector systems. Variations and biases may arise from the selective cloning of subsets of genomes, or other mechanisms (Szybalski, 1993; Forns et al., 1997) and cloning in alternative vector-cell systems may be required. RT-PCR errors in amplified genomes must be controlled by using the same protocols for experimental and problem samples (Nájera et al., 1995). It is not surprising, therefore, that the level of genetic heterogeneity of viruses cultured in cells is not significantly different from those estimated using biological molecular cloning or from RT-PCR. Molecular error-prone replication is based on the absence of proofreading-repair, 3′–5′ exonuclease activity (Steinhauer et al., 1992; Sousa, 1996). This applies to the enzymes that replicate or retrotranscribe viral genomes as well as to those that amplify their genomes in vitro often with comparable numbers of replication rounds. Heterogeneities are observed when visualising a consensus sequence, i.e. double bands or similar. In these cases, populations almost systematically segregate into the expected distinct genomes when biological or molecular cloning is undertaken. This finding may be statistically predicted as only a minority of mutations or heterogeneous positions encountered in handling viral genomes will

Table 1
Recent examples of biological implications of quasispecies

| Virus | Observation | References |
|---|---|---|
| Foot-and-mouth disease virus | Altered cell tropism of the minority of the mutant spectrum | Jackson et al. (1996), Sa-Carvalho et al. (1997) |
| | Dispensability of essential motif | Martínez et al. (1997) |
| Vesicular stomatitis virus | Different ability of viral clones to induce interferon | Marcus et al. (1998) |
| Feline calicivirus | Evidence of antigenic evolution in vivo | Radford et al. (1998) |
| Hepatitis C virus | Response to IFN related to quasispecies complexity | Le Guen et al. (1997); Pawlotsky et al. (1998, unpublished data) |

Additional examples can be found in Domingo (1996) and Domingo et al. (1997).

actually have an effect on the result of the biological or cloning assays.

The primary conclusion from published data is that RNA viruses (including viruses which use RNA as replicative intermediate such as hepatitis B virus) behave largely as predicted by the quasispecies model of molecular evolution. Quasispecies swarms, and not any sort of defined genetic entity, are those that replicate in infected hosts as they produce disease (Holland et al., 1992; Domingo et al., 1995; Marcus et al., 1998).

## 3. Biological implications of the quasispecies

Virologists use an extended definition of quasispecies so that recombination can also contribute to the mutant spectrum. Rowe et al. (1997) showed that the complexity of the coronavirus murine hepatitis virus quasispecies, defined by the presence of recombinant genomes and point mutations, influences the pathogenic potential of this virus in mice. In the extended definition, useful to virology, quasispecies are dynamic distributions of non-identical but closely-related mutant and recombinant viral genomes. They are subjected to a continuous process of genetic variation, competition and selection, and act as a unit of selection. The links between the original theoretical quasispecies concept and viral quasispecies have been discussed in several recent papers (Domingo et al., 1995; Eigen, 1996; Domingo and Holland, 1997).

If Eigen's model had not been formulated it would be difficult to reconcile many observations on genotypic and phenotypic variations of RNA viruses. Biological implications of quasispecies extend from viral pathogenesis to the emergence of new viral pathogens (Morse, 1993). They include rapid antigenic variation (antibody-escape and cytotoxic T-lymphocyte (CTL)-escape), alterations in cell tropism, virulence, host range and viral gene expression (Domingo, 1989, 1996; Jackson et al., 1996; Domingo et al., 1997; Sa-Carvalho et al., 1997) (Table 1).

## 4. Types, subtypes and beyond

One of the most obvious consequences of quasispecies structure is the genetic and antigenic diversification of RNA viruses in nature. This has triggered efforts to classify any viral pathogen into different types or subtypes. While classification has clear advantages for diagnostic, clinical and epidemiological work, it tends to assign, to particular viral groups, some biological properties unlikely to be valid as additional isolates are included in the collections. An example is the subtyping of FMDV. This was stopped two decades ago when, after having isolated a representative of subtype 65, it was realised that virtually any isolate could be classified as a new subtype. It was found that additional antibodies allowed increased discrimination among isolates. Viral types and subtypes are just the 'tip of the iceberg' of a more profound and fundamental phenomenon: the continuous dynamics of mutant generation, competition, selection and random

events which push viral quasispecies towards diversification.

In some cases serological classifications (and possibly phylogenetic classifications) may bear a useful relatedness with other biological traits. Based on increasing, but as yet incomplete, evidence it can be suggested that residues of antigenic sites will frequently have some influence on cell receptor recognition (Harber et al., 1995). Amino acid residues of antigenic sites tend to be located at exposed areas on viral capsids and surface proteins, a suitable location to exert influence on the interaction of viruses with receptors. This coincidence of locations may establish some coevolution of antigenicity and host range. In many other cases, however, biological features dependent on one or more nucleotide or amino acid substitutions, will not correspond to any phylogenetic classification based on entire genomes or selected genomic regions.

## 5. Conclusions

Control of diseases caused by highly variable RNA viruses is not currently successful. For important diseases (such as acquired immune deficiency syndrome (AIDS) or hepatitis associated with hepatitis C virus (HCV)) no vaccines are available. Selection of inhibitor-resistant viral mutants is the norm rather than the exception (Domingo, 1989; Domingo and Holland, 1992; Morse, 1993). Failures have triggered strong claims that a radical change in strategy to combat viral disease is needed (Lederberg et al., 1992; Kunin, 1993; Morse, 1994; Casadevall, 1996; Domingo et al., 1997). Part of the problem lies in the quasispecies nature of the RNA viral agents. Another problem is the still rudimentary knowledge of the immune system and immune responses. Quasispecies offer alternatives for improving disease prevention and treatments (Novella et al., 1995; Domingo et al., 1997). Quasispecies structure is an important problem for viral disease control and should not be minimised on account of the indeterminations found when trying to define compositions of mutant spectra. The evolution of an RNA virus may be rapid or slow, but mutant swarms are always hidden in their replicating genome populations. Research to better understand quasispecies composition and dynamics is urgently needed, since quasispecies are an important step in the natural history of RNA viruses.

## Acknowledgements

The author is indebted to John Holland and his colleagues for many years of collaboration and helpful discussions, and to may colleagues and students from the laboratory for contributions to RNA virus quasispecies. This work was supported by grant DGICYT PB94-0034-C02-01, FIS 95/0034-1, FIS 98/0054-00, and Fundación Ramón Areces.

## References

Casadevall A. Crisis in infectious disease: time for a new paradigm? Clin Infect Dis 1996;23:790–4.

Domingo E. RNA virus evolution and the control of viral disease. In: Jucker E, editor. Progress in Drug Research, vol. 33. Basel: Birkhauser, 1989:93–133.

Domingo E. Biological significance of viral quasispecies. Viral Hep Rev 1996;2:247–61.

Domingo E, Holland JJ. Complications of RNA heterogeneity for the engineering of virus vaccines and antiviral agents. In: Setlow JK, editor. Genetic Engineering, Principles and Methods, vol. 14. New York: Plenum, 1992:13–32.

Domingo E, Holland JJ. RNA virus mutations and fitness for survival. Ann Rev Microbiol 1997;51:151–78.

Domingo E, Sabo DL, Taniguchi T, Weissmann C. Nucleotide sequence heterogeneity of an RNA phage population. Cell 1978;13:735–44.

Domingo E, Holland JJ, Biebricher C, Eigen M. Quasispecies: the concept and the word. In: Gibbs A, Calisher C, García-Arenal F, editors. Molecular Basis of Virus Evolution. Cambridge: Cambridge University Press, 1995:171–80.

Domingo E, Menéndez-Arias L, Quiñones-Mateu ME, Holguín A, Gutierrez-Rivas M, Martínez MA, Quer J, Novella IS, Holland JJ. Viral quasispecies and the problem of vaccine-escape and drug-resistant mutants. Prog Drug Res 1997;48:99–128.

Duarte EA, Novella IS, Weaver SC, Domingo E, Wain-Hobson S, Clarke DK, Moya A, Elena SF, de la Torre JC, Holland JJ. RNA virus quasispecies: significance for viral disease and epidemiology. Infect Agents Dis 1994;3:201–14.

Eigen M. Self-organisation of matter and the evolution of biological macromolecules. Naturwissenschaften 1971;58: 465–523.

Eigen M. On the nature of viral quasispecies. Trends Microbiol 1996;4:212–4.

Eigen M, Schuster P. The Hypercycle. A Principle of Natural Self-Organisation. Berlin: Springer, 1979.

Forns X, Bukh J, Purcell RH, Emerson SU. How *Escherichia coli* can bias the results of molecular cloning: preferential selection of defective genomes of hepatitis C virus during the cloning procedure. Proc Natl Acad Sci USA 1997;94:13909–14.

Harber J, Bernhardt G, Ly H-H, Sgro J-Y, Wimmer E. Canyon rim residues, including antigenic determinants, modulate serotype-specific binding of poliovirus to mutants of the poliovirus receptor. Virology 1995;214:559–70.

Holland JJ, Spindler K, Horodyski F, Grabau E, Nichol S, VandePol S. Rapid evolution of RNA genomes. Science 1982;215:1577–85.

Holland JJ, de la Torre JC, Steinhauer DA. RNA virus populations as quasispecies. Curr Top Microbiol Immunol 1992;176:1–20.

Jackson T, Ellard FM, Abu Ghazaleh R, Brookes SM, Blakemore WE, Corteyn AH, Stuart DI, Newman JWI, King AMQ. Efficient infection of cells in culture by type O foot-and-mouth disease virus requires binding to cell surface heparan sulfate. J Virol 1996;70:5282–7.

Kunin CM. Resistance to antimicrobial drugs—a worldwide calamity. Ann Intern Med 1993;118:557–61.

Lederberg J, Shope RE, Oaks SC. Emerging Infections. Microbial Threats to Health in the United States. Washington, DC: National Academic Press, 1992.

Le Guen B, Squadrito G, Nalpas B, Berthelot P, Pol S, Brechot C. Hepatitis C virus genome complexity correlates with response to interferon therapy: a study in French patients with chronic hepatitis C. Hepatology 1997;25:1250–4.

Marcus PI, Rodriguez LL, Sekellick MJ. Interferon induction as a quasispecies marker of vesicular stomatitis virus populations. J Virol 1998;72:542–9.

Martínez MA, Verdaguer N, Mateu MG, Domingo E. Evolution subverting essentiality: dispensability of the cell attachment Arg-Gly-Asp motif in multiply passaged foot-and-mouth disease virus. Proc Natl Acad Sci USA 1997;94:6798–802.

Meyerhans A, Cheynier R, Albert J, Seth M, Kwok S, Sninsky J, Morfeldt-Månson L, Asjo B, Wain-Hobson S. Temporal fluctuations in HIV quasispecies in vivo are not reflected by sequential HIV isolations. Cell 1989;58:901–10.

Morse SS. Emerging Viruses. New York: Oxford University Press, 1993.

Morse SS. The Evolutionary Biology of Viruses. New York: Raven Press, 1994.

Nájera I, Holguín A, Quiñones-Mateu ME, Muñoz-Fernández MA, Nájera R, López-Galíndez C, Domingo E. The *pol* gene quasispecies of human immunodeficiency virus. Mutations associated with drug resistance in virus from patients undergoing no drug therapy. J Virol 1995;69:23–31.

Novella IS, Domingo E, Holland JJ. Rapid viral quasispecies evolution: implications for vaccine and drug strategies. Mol Med Today 1995;1:248–53.

Radford AD, Turner PC, Bennett M, McArdle F, Dawon S, Glen MA, Williams RA, Gaskell RM. Quasispecies evolution of a hypervariable region of the feline calicivirus capsid gene in cell culture and in persistently infected cats. J Gen Virol 1998;79:1–10.

Rowe CL, Baker SC, Nathan MJ, Fleming JO. Evolution of mouse hepatitis virus: detection and characterization of spike deletion variants during persistent infection. J Virol 1997;71:2959–69.

Sa-Carvalho D, Rieder E, Baxt B, Rodarte R, Tanuri A, Mason P. Tissue culture adaptation of foot-and-mouth disease virus selects viruses that bind to heparin and are attenuated in cattle. J Virol 1997;71:5115–23.

Sousa R. Structural and mechanistic relationships between nucleic acid polymerases. Trends Biochem Sci 1996;21:186–90.

Steinhauer D, Domingo E, Holland JJ. Lack of evidence for proofreading mechanisms associated with an RNA virus polymerase. Gene 1992;122:281–8.

Szybalski W. From the double-helix to novel approaches to the sequencing of large genomes. Gene 1993;135:279–90.