VIROLOGY

How does variation count?

Alice S. Huang and John M. Coffin

WHAT do virologists, evolutionary biologists, population geneticists, mathematical modellers, epidemiologists and physicians have in common? At a recent EMBO workshop* an area of overlap emerged that could grow into a powerful scientific interface. New approaches were reported for analysing viral disease processes and evolutionary relationships, in which computer simulations were combined with the wealth of molecular data being generated by the sequencing of viral genomes. Complex but manipulatable experimental viral systems were used to test theories of population dynamics. And, almost as an aside, it was firmly established that all RNA viruses, not only human immunodeficiency virus, mutate — but do not necessarily evolve — at a high rate, and that recombination is relatively common among RNA viruses.

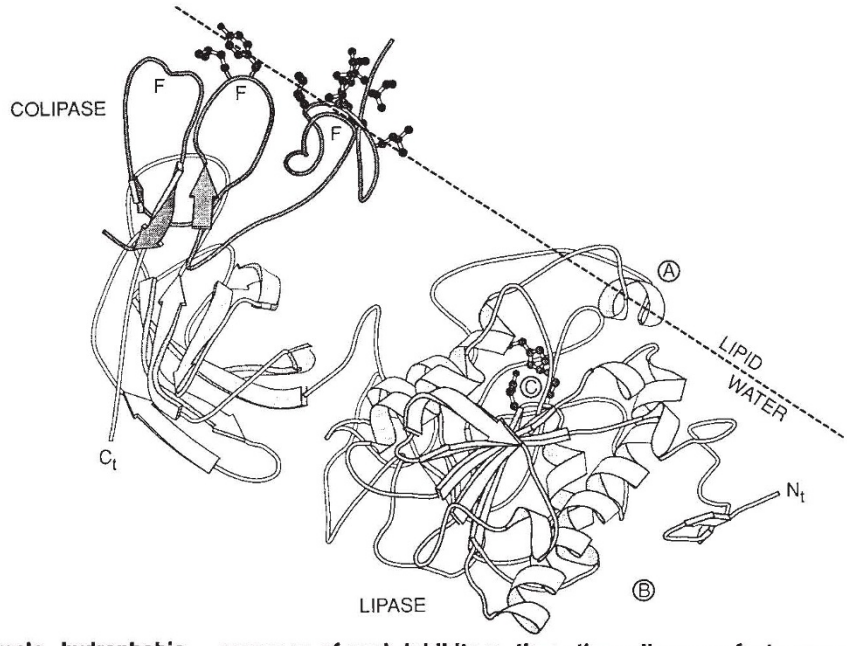
Ever since von Magnus described changes in influenza virus populations almost 40 years ago, investigators have sought the cause of these changes. They have wondered whether such variations, so easily detected in cell culture systems, could account for altered virulence in the infected host. Sparked by what seems to be a remarkable genetic plasticity among human immunodeficiency virus and the annual emergence of threatening new influenza virus strains, a closer inspection was made of what is currently known about mutation rates for viruses. Rates of mutation for RNA genomes are now estimated to be 1,000-fold higher than for DNA genomes (J. Drake, National Institute of Environmental Health Sciences). Furthermore, the rates are highly variable from site to site in the same genome (T. Kunkel, National Institute of Environmental Health Sciences; B. Preston, Rutgers; M. Ricchetti, Pasteur Institute). This microheterogeneity leads to a population of viral genomes known as quasispecies, which are closely related but different from one another. The proportions of these quasispecies change, depending on both intrinsic error rates in polymerases and selective forces that may vary according to the host environment. From the composition of different quasispecies, viral replication rates, fitness selection and mutation rates can all be estimated in that virus population (M. Nowak, University of Oxford).

Using improved molecular technology, particularly the ready amplification of nucleic acid sequences by the polymerase chain reaction, mutational heterogeneity is easily detected among different specimens taken at the same time and place from different patients, and in sequential specimens taken from the same patient. Although genetic variation may itself be important for pathogenesis, the immune system provides an important selective force (R. Zinkernagel, University of Zurich; R. Phillips, John Radcliffe Hospital, Oxford; S. Carpenter, Iowa State University; A. Leigh-Brown, University of Edinburgh). Disappointingly, there were no clear-cut correlations between the degree of heterogeneity and the course of disease. It may well be that genetic change among viruses is intrinsic and the number of mutations simply related to the number of replication cycles (S. Wain-Hobson, Pasteur Institute), resulting in a genetically diverse population capable of responding to very small selective forces.

Besides the heterogeneity resulting from single base mutations, a range of strange and wonderful variations among viruses was presented. These included satellite viruses of plant and animal viruses, where there is no commonality of sequence between the parasite and its

*EMBO Workshop on Variation and Molecular Evolution of Viruses, Grignon, France, 7–11 July 1992.

Lifting the lid on enzymatic breakdown of lipids



LIPASES are enzymes that hydrolyse triacylglycerol lipids into free fatty acids and glycerol — a crucial step in breaking down ingested fats in the gut. The pancreatic lipase enzyme's activity is greatly increased at the lipid–water interface, when a short amphipathic helix, or lid (A), in the amino-terminal domain of the lipase (B), pops open and exposes the active site (C) of the enzyme to its lipid substrate (a phenomenon known as interfacial activation). The interaction of the lipase enzyme with the lipid interface is very sensitive to the chemical nature of the lipid surface; in the presence of amphiphiles such as bile salts and phospholipids, the binding of the lipase to the lipid surface requires a small protein cofactor, colipase. The complex of these two molecules reveals that colipase binds to the carboxy-terminal domain of the pancreatic lipase molecule (see the paper by H. van Tilbeurgh *et al.* on page 159 of this issue). The colipase's three hydrophobic fingers (F), here ball-and-stick groups, are sample hydrophobic amino-acid side chains that point away from the lipase molecule but are on the same face of the complex as the lid that covers the active site of the lipase molecule. The binding of lipase, but not colipase, to the lipid–water interface is inhibited by amphiphiles (like bile salts). In the presence of such inhibitors, then, the colipase cofactor may bring the larger lipase enzyme into close contact with the interface and allow opening of the lid to expose the active site, so facilitating the enzymatic breakdown of lipids. (Figure adapted from P. J. Kraulis *J. appl. Crystallogr.* 24, 946–950; 1991.) G. R.

helper (G. Bruening, University of California at Davis; W. Carman, Institute of Virology, Glasgow); viroids, which are small RNA replicons, containing duplicated sequences (P. Keese, CSIRO, Canberra); illegitimate recombinants among viruses containing either RNA or DNA genomes (H. Temin, University of Wisconsin; M. Lai, University of Southern California; G. Smith, University of Oxford); hypermutation of G to A in retrovirus genomes (J.-P. Vartanian, Pasteur Institute); nonreplicative modification of nucleotides in RNA of paramyxoviruses (M. A. Billeter, University of Zurich); and the insertion of host sequences into the RNA of a flavivirus, bovine viral diarrhoea virus. The insertion introduces a change in a cleavage site, producing a new protein that changes the course of disease in persistently infected cows to death from mucosal peeling within a few hours (N. Tautz, Federal Research Centre for Virus Disease of Animals, Tübingen). Results like these promise that differences in disease potential may indeed be due to heterogeneity in viral populations in which chunks of genetic material are being inserted, deleted or otherwise altered. The challenge will be to dissect out population changes in hosts during the evolving pathogenic events and to relate attenuation or virulence to each of the particular variations found in these viruses.

Hundreds of sequences from viral isolates and more distantly related viruses are grist to the mill for an evolutionary biologist or mathematical modeller. Different algorithms for analysing sequence relationships among viruses yield evolutionary trees relating one isolate to another within families of viruses. These trees relate isolates from individuals, obtained during an epidemic or during pandemics evolved over a century, with isolates from related host species that span from thousands to millions of years (A. Leigh-Brown; W. M. Fitch, University of California at Irvine; M. Eigen, Karl-Friedrich-Bonhoeffer Institute). Gaining recognition is the application of pattern recognition to sequences in search of similar catalytic functions among diverse proteins (M. McClure, University of California at Irvine). The problem of applying a timescale to such trees is severe. Molecular clocks based on neutral theory are inappropriate for viral quasispecies: RNA viruses can be genetically very stable despite high mutation rates (E. Domingo, Universidad Autonoma de Madrid) and the rate of change of influenza virus is quite different in different hosts (W. M. Fitch; O. Gorman, St Jude's Hospital). Observations like these call into question recent estimates of the age of human immunodeficiency virus and its relatives. One group is even attempting, so far without success, to approach this issue by analysis of monkey bones from Egyptian tombs for simian immunodeficiency virus-like sequences (A. van der Kuyl, University of Amsterdam).

To population theorists, quasispecies, recombinations and deletion mutants, coupled with the relatively short generation times of viruses, offer interesting systems to test hypotheses *vis à vis* real data. For instance, there is Muller's ratchet, a hypothesis that states that mutation will move a small population inexorably more distant from the most fit genotype; to ensure survival, such driven populations gain stability through increasing their population size or through recombination. Continuous passaging of an RNA phage under nonselective conditions and measuring mutants and recombinants led to complete support for this hypothesis (L. Chao, University of Maryland).

Another successful use of mathematical modelling was based on the predator–prey relationship described for a defective interfering particle and its helper standard virus. Rather than regular cycling between defective interfering particles and standard virus, there was chaotic unpredictability even in a deterministic model (T. B. L. Kirkwood, National Institute for Medical Research, Mill Hill). When mutations were added to the model, computer-simulated viral passages showed that new defective interfering particles periodically emerged and drove the level of replication-competent virus so low that there was continual pressure for viruses to evolve and escape interference.

Unlike most evolutionary studies, there is immediate and important practical value to understanding the evolution of viruses. Close examination of examples of recently emergent viruses implies that the key is altered conditions permitting efficient spread of virus in its new host rather than the initial infection events themselves (S. Morse, Rockefeller University). Armed with knowledge of evolutionary processes, perhaps we can head off the next pandemic. Mathematical modelling based firmly on experimental results has considerable potential for understanding viral diseases, and evolving populations and virus systems will provide tools for mathematical biologists to test their theories. Certainly more workshops at this interface and other multidisciplinary interfaces are needed. □

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DAEDALUS

Know thyself!

THE victorian art of phrenology divided the brain into compartments. One part dealt with hope, another with constructiveness, another with language and so on. The strength of each faculty could be judged by the enlargement of the skull at that point. Feeling their clients' skulls with sensitive fingers, popular phrenologists provided vocational guidance, character assessment and psychiatric diagnosis. They even tried to detect an overactive faculty from the warmth of the skull over the relevant region. Nowadays, these skills have practically died out.

Daedalus plans to revive them. He points out that modern techniques such as computed X-ray tomography give us direct access to the shape and size of the brain. Nuclear magnetic scanning, and especially positron-emission imaging, can even map its metabolic activity. So DREADCO technicians are now setting up a laboratory of Positron-Emission Phrenology to test the old ideas with modern rigour and explore new ones.

Volunteers for the new study will simply lie in the PEP machine and respond to requests to think about a particular topic or exercise some specific emotion. The site of sudden metabolic activity, or raised concentration of the implicated neurotransmitter, will then be displayed on the machine's brain map. For example, classical phrenology located the sexual instinct at the back of the head, rather below the level of the ears. Since (it is claimed) the average male thinks about sex every ten minutes, the metabolism of this region in males should show a ten-minute periodicity.

Even more revealing, volunteers will be allowed to watch their own brain display. Simply by letting his thoughts range, a subject will quickly discover what thoughts or mental efforts give rise to what activity where. By rapid biofeedback exploration, he will soon map out his own personal phrenology. He should then be able to learn how to stimulate particular regions of his brain, and to think himself into little-used areas. Those who normally rely on the left side of their brains will discover how to exercise their creative right side. Those out of touch with their feelings, or psychologically burdened with repressed memories, may be able to explore their brain map until they have tracked them down and inspected them. Those tortured by obsessive thoughts will find out at last where they lurk in the brain. They may then learn how to avoid stimulating that region, or how to swamp it with a mass of numbing platitudes. A few minutes on the machine could save you years on the couch. David Jones