Virus evolution during persistent infection

from C. R. Pringle

In certain circumstances cells in culture can survive infection by viruses which normally kill them, and the cells continue to multiply still harbouring the infecting viral genome. Nuclear DNA viruses and reverse transcribing RNA viruses persist by integration into the host genome, or by regulated association with the host cell nucleus. The phenotype of the cell may be changed as a consequence and the cell may become oncogenic. However, many cytocidal RNA viruses like the rhabdoviruses and parainfluenza viruses which multiply entirely in the cytoplasm are able to establish a persistent infection in susceptible cells. The molecular mechanisms concerned in the moderation of normally lethal infections in which there is no nuclear involvement are attracting increasing interest. Three factors have been associated with persistent infection. These are temperature-sensitive (*ts*) mutation which impairs virus replication at normal incubation temperature, defective interfering (DI) particles which have portions of the viral genome deleted and suppress the multiplication of homologous complete virus, and production of interferon. The most recent analyses indicate that these factors are interdependent, and that interferon production may hold the balance. Sekellick and Marcus (*Virology* **85**, 175; 1978; **95**, 36; 1979) have shown that many *ts* mutants of the rhabdovirus vesicular stomatitis virus (VSV) are good inducers of interferon in competent cells at restrictive and semi-permissive temperatures, and that the ability of DI particles to protect cells from the lethal action of infectious virus (as distinct from their ability to inhibit viral replication), is mediated by interferon. Interferon levels in persistently infected cultures are usually low, but addition of anti-interferon serum frequently results in an increase in virus production and cell destruction (Youngner *et al. J. Virol.* **28**, 6 1978; Ramseur and Friedman *Virology* **85**, 253; 1978). However, persistent infection can be established in cell lines such as Vero and BHK-21 which are genetically deficient in some component of the interferon system. Another important factor may be the progressive selection of a population of semi-refractory cells, because a change in karyotype has been observed in a culture of BS-C-1 cells persistently infected with respiratory syncytial virus (Pringle *et al. J. Virol.* **28**, 199: 1978).

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Persistent infection has been given new significance by the results of surveillance over five years of a BHK-21 cell culture persistently infected with the Indiana serotype of VSV, with oligonucleotide fingerprinting used to detect changes in the genetic constitution of the virus (Holland *et al. Cell* **16**, 495; 1979). The data reveal that the virus has undergone extensive and progressive non-lethal mutational change during this period. The implication is that the mechanisms which moderate virulence to produce persistent infection may simultaneously promote rapid virus evolution.

VSV seems to be genetically stable during repeated cycles of lytic infection. Clewley *et al.* (*J. Virol.* **23**, 152; 1977) found that strains of VSV derived from different field isolates were readily distinguishable by oligonucleotide fingerprinting, although most of the oligonucleotides were conserved. On the other hand one strain maintained independently in six laboratories for 16 yr was unaltered. Likewise Holland and his colleagues could detect no changes in their strain of VSV after eight sequential cycles of cloning in BHK-21 cells, forty cycles of lytic infection in the same cells, and five lethal infections of adult mice. The virus also remained unaltered after growth for 2 months in *Aedes albopictus* cells, although passage in insect cells was known to generate high freequencies of *ts* mutants. These results are not unexpected, however, because 85–90% of all sequence changes would not be detectable by oligonucleotide mapping, because only the larger oligonucleotides (10–15% of the total) obtained by T1 ribonuclease digestion would produce unique spots after polyacrylamide gel electrophoresis in two dimensions.

A very different pattern was observed in the persistently infected culture. This carrier culture had been established by a maturation-defective *ts* mutant of VSV (*ts* G31) in the presence of a DI particle derived from this same mutant. Thus all the genome alterations observed in this culture were a consequence of mutation in the progeny of a single virus particle. The DI particle associated with *ts* G31, which is the smallest of the VSV DIs with approximately 90 % of the genome deleted, was considered to be an essential factor in establishing persistence, although during propagation of the culture it was subsequently replaced by a succession of other DI particles. These DI particles had different oligonucleotide patterns from the original DI of *ts* G31. Infectious virus recovered from the culture after continuous propagation for 14, 21 and 42 months showed 5, 8 and 11 map changes, respectively. At any particular time one DI particle predominated and different clones of infectious virus isolated from the culture at the same passage level had identical oligonucleotide patterns. Because 90 % of sequence changes would have occurred in the non-unique small oligonucleotides, it is clear that at least 50–100 mutations had occurred by 3.5yr, although only a proportion would result in changes in the amino acid composition of the viral proteins. Nevertheless, it was evident that the VSV genome had experienced extensive and progressive mutational change during persistent infection. The genome changes induced by persistent infection seemed to be stable because virus isolated from the carrier culture after 5yr showed no alteration during ten cycles of lytic infection in susceptible cells.

These observations suggest that persistent infections are a major source of genetic variation for viruses like the parainfluenza viruses and rhabdoviruses, which are unable to acquire new variation by recombination. Holland *et al.* also suggest that persistent infection is an important factor in the evolution of influenza A virus. A given strain may show little variation during periods of acute infection, and extensive sequence alterations (with or without reassortment of subunits) may only accumulate in the interval between pandemics when the virus is sequestered in foci of persistent infection in individual hosts.

One of the most interesting properties of these carrier cells was that they could be propagated in nude (athymic) mice, and it was observed that persistent infection by VSV suppressed the normal tumorigenicity of BHK-21 cells (Reid *et al. J. gen. Virol.* **42**, 609; 1979). Analysis of this anti-tumour response may shed light on the difficulties encountered in propagation of certain types of human and other tumours in nude mice. □

A hundred years ago

Intellect in Brutes

The following instance of sagacity in a cat has just been related to me by a friend who knew both the cat and its owner well. The latter, who lived at Ragusa Vecchia, in Dalmatia, was too poor to be able to provide food for the cat; the animal was therefore obliged to cater for himself, and was well known as a thief in the neighbourhood. One day one of the children was being sent off to school without any breakfast; the cat, hearing him sobbing for hunger, immediately went off, and returned with a piece of bread he had stolen from a baker hard by, and brought it to the child. The same thing happened another day, and he came back, dragging along a piece of meat bigger than himself. On crossing the threshold a bit of bone caught in a hole, so puss miawed till some one came to his help. This same cat, who was constantly catching birds on the roof, slept with some pet birds in a cage without attempting to touch them.

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MARGARET EVANS

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