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.

whitefly transmissibility. The formation of bipartite begamoviruses in which the DNA B component is not essential for systemic infection, e.g. TYLCV-Th, could represent the first stage in this evolutionary pathway. The DNA B component probably evolved from the DNA A component, as suggested by their almost identical common regions, the low, but significant sequence similarities between the AV1 (CP) and BV1 (NSP) proteins, the fact that both these proteins bind strongly to ssDNA, and the transactivation of transcription of both genes by TrAP. Acquisition of cell-to-cell movement genes on DNA B could have led to the loss of cell-to-cell movement function of the AV2 protein in some Old World bipartite begamoviruses, e.g. ACMV, and loss of this gene completely in the New World bipartite begamoviruses. The greater sequence diversity within the *Mastrevirus* genus than within the *Begamovirus* genus is consistent with the evolution of begamoviruses from ancestral mastreviruses. Sequence comparisons suggest that curtoviruses, such as BCTV and HrCTV, may have arisen by recombination acquiring the CP gene, and hence leafhopper transmissibility, from a mastrevirus and the Rep gene from a begamovirus.

Geminiviruses have some similarities to another group of multipartite ssDNA viruses (proposed *Nanovirus* genus), comprising banana bunchy top virus, subterranean clover stunt virus, faba bean necrotic yellows virus and milk vetch virus, which all have Rep proteins with the Rb-binding LXCXE motif, have intergenic regions containing a putative stem–loop structure with a conserved consensus TAG/TTATTAC (similar to the geminivirus TAATATTAC sequence) sequence and which probably replicate by a rolling circle mechanism. Geminiviruses and nanoviruses also have some affinities to viruses in the *Circoviridae* family of animal ssDNA viruses. Recently it has been found that ToLCV-Aus DNA can replicate in *Agrobacterium tumefaciens* suggesting that plant ssDNA viruses may have arisen from ssDNA bacteriophages or plasmids which employ rolling circle replication. *Agrobacterium* species are widely distributed plant pathogens known to be able, via their Ti plasmids, to insert DNA into plant genomes. Consistent with this theory is the finding that remnants of geminivirus DNA have been found in the genome of some (uninfected) plants which are hosts for geminiviruses.

See also: **Pathogenesis: Plant viruses.**

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GENETICS OF ANIMAL VIRUSES

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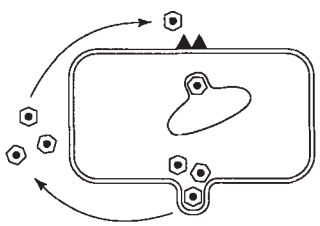

History

Although genetic variation was the basis of some of the earliest work with animal viruses, such as Pasteur's attenuation of rabies virus, the first formal demonstration of the occurrence of mutation in an animal virus did not occur until over 60 years later, when Burnet used limiting dilution methods to clone influenza virus. A few years later, in the early 1950s, Burnet demonstrated 'high frequency recombination' (later called genetic reassortment) between different strains of influenza A virus. Intramolecular recombination was demonstrated with herpes simplex virus in 1955 and with vaccinia virus in 1958 and the first

crude maps (of vaccinia virus) were described soon after. Hirst demonstrated that intramolecular recombination could occur among RNA viruses in experiments with poliovirus in 1962 and Cooper published a genetic map of poliovirus in 1968.

Apart from the demonstration of the remarkable variety to be found in the genomes of animal viruses of different groups, animal virus genetics advanced slowly until the discovery of recombinant DNA by Berg, Boyer and Cohen in 1972. This led to the explosive development of molecular genetics. Since animal viruses multiply only in eucaryotic animal cells, viruses were soon found to be a powerful way of investigating the molecular biology of animal cells, especially after the discovery that the DNA of tumor viruses was integrated into the genome of the host cell.

The Genomes of Animal Viruses

Historically, animal viruses were first classified on the basis of the morphology of the virion, but this criterion failed to distinguish between many small isometric viruses or between several different families of enveloped viruses. Morphology was powerfully supplemented as the basis for classification as the genomes of viruses were analyzed and their extraordinary variety demonstrated. Finally, as work proceeded on the replication mechanisms, a third criterion for classification was developed, the strategy of viral replication. Currently eight families of DNA and 16 families of RNA animal viruses are recognized. The genomes of most DNA viruses are double stranded (ds) and range in size from 3.2 to 280 kbp; others have genomes of ssDNA ranging from 1.8 to 5 kb. Some genomes are linear, others circular; among viruses with ssDNA some are of positive sense, some of negative sense.

Viruses are the only living things that use RNA as the repository of their genetic information. The genomes of the RNA viruses are much smaller than those of most DNA viruses, and different families of RNA viruses show a remarkable diversity in genome structure. All except one family of viruses have haploid genomes; the retroviruses, however, have diploid RNA genomes and they also have an obligatory DNA stage in their replication cycle. Among other families the RNA genome may be a single linear strand varying in size from 3.5 to 24 kb, a circular molecule of 1.7 kb, or it may consist, in different genera, of 2, 3, 7 or 8 pieces of ssRNA, or it may consist of segmented dsRNA in 2, 10, 11 or 12 pieces.

Mutations

The most common and important changes in the nucleic acid sequences of viral genomes are due to mutations. In every viral infection of an animal or a cell culture, one or a small number of virus particles replicate to produce millions of progeny. In such large populations, errors in copying the nucleic acid, i.e. mutations, inevitably occur. Many such mutations are lethal. Whether a particular nonlethal mutation survives in the genotype depends upon whether the resultant change in the gene product affords the mutant virus some selective advantage, or whether it is neutral or disadvantageous.

In the laboratory, reasonable genetic constancy of viral stocks (e.g. those used for making viral vaccines or retained as reference strains) is achieved by: (1) isolating a clone, i.e. a population of viral particles originating from a single particle, usually by growth from a single plaque in cell culture, followed by replaquing; then, (2) growing 'seed' stock from this clone; and (3) as far as practicable avoiding or strictly limiting further passage of the virus.

Mutation Rates

Rates of mutation involving single base substitutions (point mutations) are probably the same in DNA viruses as they are in the DNAs of procaryotic and eucaryotic, since viral replication is subject to the same 'proof-reading' exonuclease error correction as operates in cells. Such errors are estimated to occur at a rate of 10^{-8} to 10^{-11} per incorporated nucleotide (i.e. per base pair per replication). Point mutations in the third nucleotide of a triplet often do not result in an altered amino acid, because of coding redundancy, and some point mutations are lethal, because they produce a stop codon or other aberrant regulatory sequences. Viable mutations that are neutral or deleterious in one host may provide a positive selective advantage in a different host.

The error rate in the replication of viral RNA is much higher than that of viral or cellular DNA, because there is no cellular 'proof-reading' mechanism for RNA. For example, the base substitution rate per incorporated nucleotide in the 11 kb genome of vesicular stomatitis virus is $10^{-3} - 10^{-4}$, which is about a million times higher than the average rate in eucaryotic DNA. Of course, most of these base substitutions would be deleterious and the genomes containing them would be lost. Even so, there is growing evidence that nonlethal mutational changes in the genome of RNA viruses occur very rapidly.

Types of Mutations

Mutations can be classified according to the kind of change in the nucleic acid. The most common are nucleotide substitutions (point mutations), deletions

and insertions. Each point mutation has a characteristic frequency of reversion which can be accurately measured. The physiological effects of mutations depend not only on the kind and location of the mutation but also on the activity of other genes. The phenotypic expression of a mutation in one gene may be reversed not only by a back mutation in the substituted nucleotide but, alternatively, by a suppressor mutation occurring elsewhere in the same, or even in a different gene.

Mutations are usually classified by their phenotype – hence temperature-sensitive, cold adapted, host range, plaque size, etc. mutants have been described. Each of these kinds of mutant has been used for the analysis of viral functions, temperature-sensitive mutants being particularly useful (see later). Cold-adapted and temperature-sensitive mutants have been used extensively in attempts to produce attenuated live virus vaccines. Mutations affecting antigenic determinants of virion surface proteins may be strongly favored when viruses replicate in the presence of antibody, and are of importance both in persistently infected animals (e.g. in equine infectious anemia virus) and epidemiologically, as with influenza virus.

Conditional lethal mutants

These are produced by mutations that so affect a virus that it cannot grow under certain conditions, determined by the experimenter, but can replicate under 'normal' or permissive conditions. Their importance is that a single selective test can be used to obtain and analyze mutants in which mutations may be present in any one of several different genes. The conditional lethal mutants most commonly studied are those whose replication is blocked in certain host cells, or at certain defined temperatures. With temperature-sensitive mutants; the selective condition used is a high temperature of incubation of infected cells. A point mutation in the genome, leading to an amino acid substitution in the translated polypeptide product, results in a structurally abnormal protein which, although functional at the permissive temperature, cannot maintain its structural integrity and functional conformation when the temperature is raised by a few degrees.

Defective-interfering (DI) mutants

Mutants of this class have been demonstrated in all families of RNA viruses and in some DNA viruses. They occur when viruses are passed at high multiplicity of infection, because more cells then receive helper virus to support their replication, and the ratio of defective-interfering to infectious particles increases dramatically on serial passage at high multiplicity. The properties that all DI virus particles have in common are: (1) they are defective, i.e. they cannot replicate alone, but can in the presence of a parental wild-type virus; and (2) they decrease the yield of wild-type virus (interference).

All RNA DI particles studied are deletion mutants. In the case of influenza viruses and reoviruses, which have segmented genomes, the defective virions lack one or more of the larger segments and contain instead smaller segments consisting of an incomplete portion of the encoded gene(s). In the case of viruses with a nonsegmented genome, DI particles contain RNA which is shortened – as little as one-third of the original genome may remain in the DI particles of vesicular stomatitis virus. Morphologically, DI particles resemble the parental virions, having a comparable envelope or capsid, but they are sometimes smaller. Sequencing studies of the RNA of DI particles reveal simple deletions and a great diversity of structural rearrangements.

Mutagenesis

Spontaneous mutations arise because of chance errors during replication, the occurrence of which is probably influenced by natural background ionizing radiation. Mutation frequency can be enhanced by treatment of virions or isolated viral nucleic acid with physical agents such as UV or X irradiation or with chemicals such as nitrous acid, hydroxylamine, ethylmethyl sulfonate or nitrosoguanidine. Base analogues, such as 5-fluorouracil or 5-bromodeoxyuridine, are mutagens only when virus is grown in their presence because they are incorporated into the viral nucleic acid.

Site-directed mutagenesis

This process enables the experimenter to introduce mutations at a selected site in a DNA molecule (a DNA genome or cDNA transcribed from an RNA genome). This technique has opened up new research areas; for example: (1) the function of individual genes and the proteins for which they code, or of particular regions of these genes and proteins, can be dissected; (2) mutations can be introduced into particular genes, e.g. those concerned with viral virulence, to produce mutants suitable for use as attenuated live virus vaccines.

Recombination

When two different viruses simultaneously infect the same cell, several kinds of genetic recombination may occur between the newly synthesized nucleic acid

molecules: intramolecular recombination, reassortment, reactivation (when one of the virions has been inactivated) and marker rescue.

Intramolecular recombination

Intramolecular recombination (**Fig. 1A**) involves the exchange of nucleic acid sequences between different but usually closely related viruses. It occurs with all dsDNA viruses, presumably because of strand switching by the viral DNA polymerase. Intramolecular recombination has been demonstrated among RNA viruses only with foot-and-mouth disease virus, poliovirus and coronavirus.

In rare cases, intramolecular recombination occurs between unrelated viruses; the best example is between SV40 (a papovavirus) and adenoviruses. Both SV40 and adenovirus DNAs become integrated into cellular DNA, so that it is perhaps not surprising to find that when rhesus monkey cells which harbor a persistent SV40 infection are super-infected with an adenovirus, not only does complementation occur (see later), the SV40 acting as a helper in an otherwise abortive adenovirus infection, but recombination occurs between SV40 and adenovirus DNAs to yield hybrid (recombinant) DNA which is packaged into adenovirus capsids.

In addition to recombining with each other, or sometimes, as with SV40, with an unrelated virus (adenovirus), many DNA tumor viruses recombine with the genome of cells and in the process the viral oncogenes that they carry may transform the infected cell to a neoplastic state. Integration of proviral DNA into the cellular DNA by a process of intramolecular recombination is an essential part of the replication cycle of retroviruses. Although the genome of these viruses is positive-sense ssRNA, replication does not occur until this is transcribed into DNA by the virion-associated reverse transcriptase and the resultant copy DNA is integrated into the cell's DNA.

Reassortment

A type of recombination called reassortment (**Fig. 1B**) occurs with viruses that have segmented genomes, whether these are ssRNA or dsRNA and consist of two (*Arenaviridae, Birnaviridae*), three (*Bunyaviridae*), seven or eight (*Orthomyxoviridae*), or 10, 11 or 12 segments (*Reoviridae*). In cells infected with two related viruses, there is an exchange of segments with the production of various stable reassortants. Reassortment occurs in nature, and is epidemiologically important in generating new subtypes of influenza virus for example.

Reactivation

The term multiplicity reactivation is applied to the production of infectious virus by a cell infected with two or more viruses of the same strain, each of which has suffered a lethal mutation in a different gene, e.g. after exposure to UV irradiation. Multiplicity reactivation could theoretically lead to the production of infectious virus if animals were to be inoculated with UV irradiated vaccines; accordingly this method of inactivation is not used for vaccine production. Cross-reactivation or marker rescue are terms used to describe genetic recombination between an infectious virus and an inactivated virus of a related but distinguishable genotype or a fragment of DNA from such a virus.

Interactions between Viral Gene Products

As well as interactions between viral genomes, interactions between viral gene products may occur in mixedly infected cells.

Complementation

The term complementation is used to describe situations where in mixedly infected cells one virus provides a gene product that the other cannot make, thus enabling the latter to replicate. Complementation can occur between different strains of the same species of virus and between unrelated viruses; indeed adeno-associated viruses can replicate only if complemented by genes of an adenovirus.

Phenotypic mixing

This term refers to a situation where, after mixed infections, the envelope or capsid of some of the progeny contains gene products of both parents, such as the spikes of enveloped viruses (**Fig. 1E**), the genomes being those of one or other parent, so that on passage the phenotypically mixed particles produce only virions resembling that parent. Phenotypic mixing is an essential part of the life cycle of defective retroviruses, progeny virions being called pseudotypes, with the genome of the defective parental virus but the envelope glycoproteins of the helper retrovirus.

With nonenveloped viruses, phenotypic mixing can take the form of transcapsidation, in which there is partial or usually complete exchange of capsids (**Fig. 1F, G**). For example, poliovirus nucleic acid may be enclosed within a coxsackievirus capsid, or adenovirus 7 genome may be enclosed within an adenovirus 2 capsid.

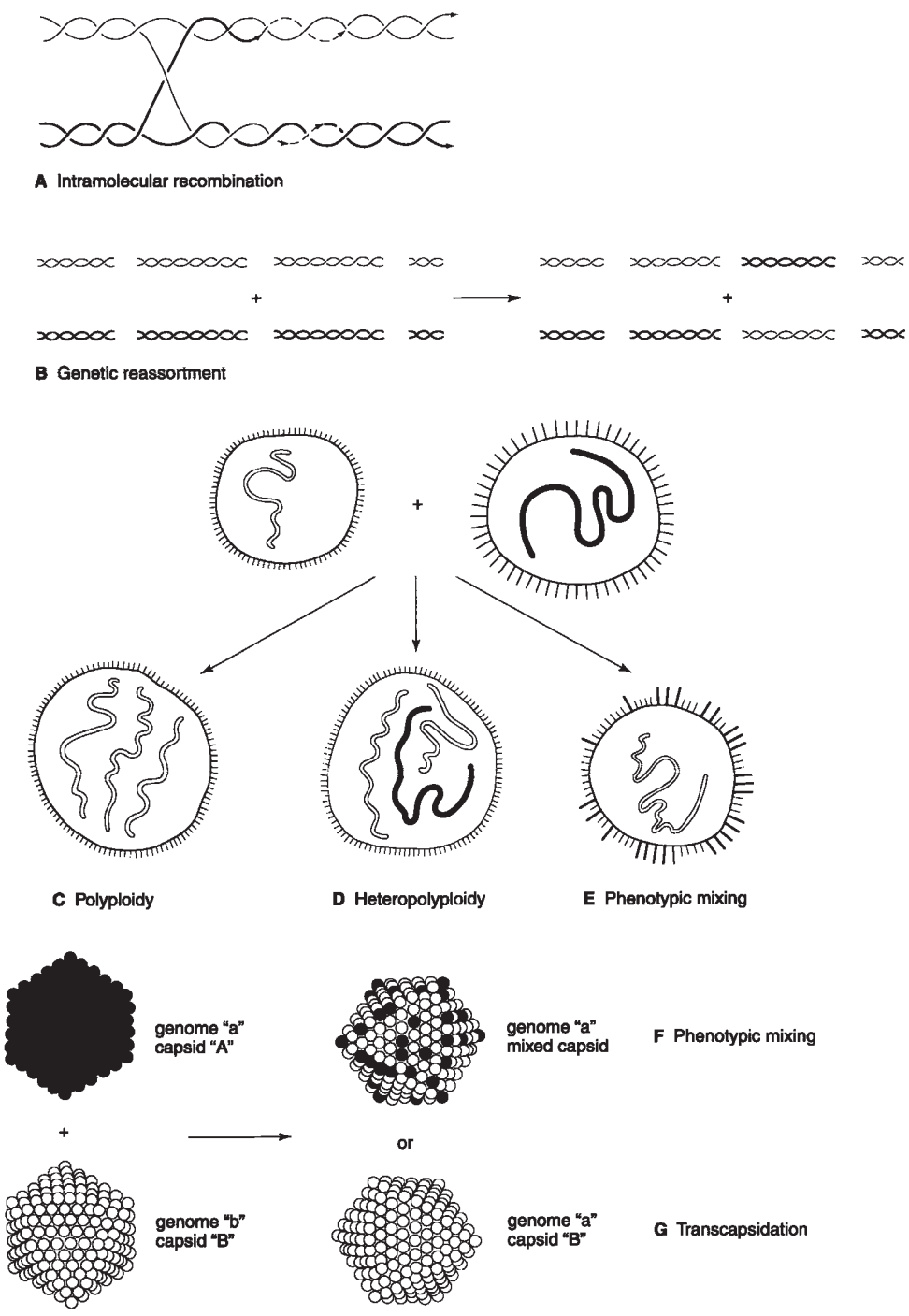

Figure 1 Genetic recombination, polyploidy, phenotypic mixing and transcapsidation. (**A**) Intramolecular recombination. (**B**) Reassortment of genome fragments, as in reoviruses and orthomyxoviruses. (**C**) Polyploidy, as seen in unmixed infections with paramyxoviruses. (**D**) Heteropolyploidy, as may occur in mixed infections with paramyxo-viruses and other enveloped RNA viruses. (**E–G**) Phenotypic mixing: (**E**) with enveloped viruses; (**F**) viruses with icosahedral capsids; (**G**) extreme case of transcapsidation or genomic masking.

Polyploidy

With the exception of the retroviruses, which are diploid, all viruses of vertebrates are haploid, i.e. they contain only a single copy of each gene. However, among viruses that mature by budding from the plasma membrane, e.g. paramyxoviruses, it is commonly found that several nucleocapsids (and thus genomes) are enclosed within a single envelope, i.e.

the virion is polyploid (**Fig. 1C**). If cells are doubly infected with recognizably different strains of such viruses, many of the multiple genome progeny particles are heteropolyploid (**Fig. 1D**) and they may also have phenotypically mixed envelope antigens.

Mapping Viral Genomes

Viral genomes can be mapped several ways. Genetic maps can be constructed on the basis of recombination or complementation tests, and physical maps may show the cut sites of various restriction enzymes or may be obtained by determining the sequence of nucleotides that constitute the viral genome.

Recombination maps

Among viruses that undergo intramolecular recombination, the probability of recombination occurring between two markers reflects the distance between them and recombination frequencies in adjacent intervals are approximately additive. Two-factor crosses are used to determine recombination frequencies between pairs of mutants; for very close or distant markers three-factor crosses are used to resolve ambiguities. Recombination maps have been made for several large DNA viruses, notably herpes simplex virus, and for poliovirus. With the determination of nucleotide sequences the genetic markers of a number of viruses have been located on the relevant physical maps.

For viruses that have segmented genomes, reassortant maps can be constructed by crossing mutants of different serotypes that have electrophoretic polymorphisms for each of the genome segments. They have confirmed that mutations able to recombine reside on different genome segments.

Restriction maps

During the 1960s it was shown that a phenomenon that had been called 'restriction' in bacteriophages, whereby certain bacteriophages failed to replicate in particular species of bacteria, was due to the rapid degradation of the bacteriophage DNA by specific bacterial endonucleases, which were therefore called restriction endonucleases. Subsequently several hundred of these enzymes have been identified and purified from various bacteria. Each recognizes a unique short, palindromic sequence of nucleotides (a sequence that reads the same backwards as forwards), generally four to eight nucleotide pairs long. Depending upon the location and frequency of the specific unique sequence in a particular DNA molecule, a particular restriction endonuclease cleaves the DNA into a precise number of fragments of precise sizes. Other endonucleases, recognizing different sequences, cleave the same DNA into different numbers and sizes of fragments. These DNA fragments, produced by a panel of endonucleases, may be separated by gel electrophoresis. Different viruses, often even very closely related strains of the same virus, yield characteristically different restriction endonuclease fragment patterns, sometimes called fingerprints or restriction fragment length polymorphisms (RFPL). These have been invaluable for distinguishing between different species of viruses with large genomes, such as the various poxviruses. Restriction enzymes can also be used to analyze the molecularly cloned cDNA copies of genes or genomes from RNA viruses. Once the restriction maps of a virus have been determined, the location of many genetic markers, such as temperature-sensitive mutations, at specific locations on the viral genome can be determined by rescue of the deleterious mutation by the corresponding wildtype restriction fragment.

Complementation groups

Complementation tests are used to divide collections of mutants into functional groups, and are particularly useful with virus groups in which recombination mapping is not possible because of the absence of recombination, such as togaviruses and paramyxoviruses.

Molecular Genetics

Since about 1970 there has been an explosive development of techniques that have revolutionized viral genetics, namely methods for molecular cloning, DNA and RNA sequencing, fine mapping of transcripts and expressing genes in procaryotic and eucaryotic cells. In conjunction with site-directed mutagenesis, these techniques have made it possible to introduce into viral genomes virtually any selected mutation and to remove genes and *cis*-acting elements from viral genomes and study their activities in isolation from the rest of the genome. The basic strategy is molecular cloning by the use of recombinant DNA techniques.

Recombinant DNA

The identification of the cleavage sites of the restriction endonucleases and the development of knowledge of the enzymes involved in DNA synthesis opened up the possibility of deliberately introducing specific foreign DNA sequences into DNA molecules. When these recombinant molecules replicate, there is a corresponding amplification of the foreign DNA.

The process is called molecular cloning. When the replicating recombinant molecules are placed in a situation where their genetic information can be expressed, the polypeptide specified by the foreign DNA is produced. These results are usually achieved by incorporating the foreign DNA into a bacteriophage or a plasmid, which serves as a cloning vector for introducing the foreign DNA into bacterial or other cells. Vectors are available that replicate in bacteria, yeasts, animal cells and intact animals. For animal cells, a variety of animal viruses can be used as vectors, SV40, retroviruses and vaccinia virus being popular choices. The development of recombinant DNA methodology has been facilitated by great improvements in the techniques of sequencing DNA and the perfection of methods for making complementary DNA (cDNA) from either viral RNA or mRNA. The cluster of techniques used is often called 'genetic engineering'.

Sequence analysis

A great deal of information can be gleaned from knowledge of the sequence of part or all of a viral genome. Open reading frames (ORF), which are long translational sequences uninterrupted by stop codons (UAA, UAG, UGA), suggest the presence of protein coding sequences, especially if the codons for given amino acids occur with the frequency found in known protein coding sequences of the virus. The function of the predicted protein can sometimes be surmised by the similarity of its imputed sequence to that of a protein of known function. Such comparisons are carried out by searching international computer-based databases of nucleotide and amino acid sequences. Thus the existence of vaccinia growth factor was predicted when sequence analysis of the vaccinia virus genome revealed an ORF similar to that of known mammalian growth factors. It is also possible from an examination of the sequence to predict that specific portions of a predicted protein will have particular functions, such as signal sequences for targeting proteins to the endoplasmic reticulum or the plasma membrane, transmembrane sequences, glycosylation sites and nucleotide binding sites. Short sequence motifs can be identified which serve as signals in gene expression. Methionine codons (AUG) used to initiate translation are found at the beginning of ORFs, embedded in a consensus sequence GCCGCC/GCCAUGG. Sites of mRNA cleavage and polyadenylation are usually signaled by AAUAAA followed by certain other signals. The start sites for transcription by RNA polymerase II are usually about 30 bp downstream from TATA boxes.

Uses of Genetic Engineering of Animal Viruses

Practical applications of genetic engineering of viruses include the development of nucleic acid probes for diagnosis and novel methods for the production of vaccines, such as the use of vaccinia virus as a vector. Combined with the availability of simple and fast methods of sequencing nucleic acids, genetic engineering has also led to studies of animal virus genomes that could not be contemplated before it became possible to produce large quantities of selected fragments of viral nucleic acid by the use of the polymerase chain reaction. Among the achievements so far are:

1. Complete sequencing of the genome of DNA viruses of several families, including the 230 kbp genome of cytomegalovirus (a herpesvirus) and the 192 kbp genome of vaccinia virus.
2. Complete sequencing of cDNA corresponding to the entire genome of several RNA viruses.
3. Recognition of the number and sequence of viral or proviral DNAs that are integrated into the DNA of transformed cells.
4. Development of diagnostic probes for use in *in vitro* assays, including *in situ* hybridization.
5. The polymerase chain reaction can be used to detect low copy number of a viral genome.
6. Marker rescue by transfection with gene fragments, as a method of genetic mapping.
7. Production of proteins coded by specific viral genes, using bacterial, yeast, baculovirus and animal cell expression systems or by cell-free translation.
8. Synthesis of peptides based on DNA sequence data.

Transgenic Mice

Transgenic mice provide a new tool for investigating many problems in virology, immunology and developmental biology. They are produced by injecting selected fragments of DNA into the nuclei of fertilized eggs washed out of the mouse oviduct. After replacement, some ova develop normally to form the base of a colony of transgenic mice. The technique provides insights into the potential role in viral pathogenesis of individual viral gene products in the context of the intact animal. For example, transgenic mice have been produced in which every cell contained greater than genome length hepatitis B DNA sequences. All mice had HBs antigen in plasma, but the viral sequences were selectively expressed only in cells of the liver, kidney and pancreas. Likewise, transgenic mice

containing the DNA for the early region of bovine papilloma virus developed skin tumors at 8–9 months of age. Extrachromosomal viral DNA was detected in tumor cells and integrated viral DNA in normal tissues.

See also: **Defective interfering viruses; Herpesviruses 6 and 7 – human (*Herpesviridae*); Interference; Reoviruses (*Reoviridae*): General features; Vaccinia virus (*Poxviridae*); Vectors: Plant viruses.**

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GIARDIAVIRUSES (*TOTIVIRIDAE*)

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History

In 1986, a 6.3 kb linear double-stranded (ds) RNA molecule was observed in nucleic acid extracts of *Giardia lamblia* Portland I trophozoites. *G. lamblia* is an anaerobic parasitic flagellate that inhabits the upper gastrointestinal tracts of humans as well as many other mammals. When infecting humans, the parasitic protozoan causes giardiasis, an acute diarrhea that often progresses to chronic, carrier-stage for adults and severe malnutrition for children. Further examination of this 6.3 kb dsRNA isolated from the extract of *G. lamblia* revealed it to be the genome of a small isometric virus, hence named giardiavirus (GLV), that specifically infects this protozoan.

It is interesting to note that although GLV was first detected in an isolate of *G. lamblia* Portland I (P1) obtained from Dr D. G. Lindmark of Cleveland State University, the same P1 isolate from the American Type Culture Center is virus-free. It therefore remains a mystery as to how the Cleveland P1 became exposed to the virus. All the information included here has been derived from studies of the virus originally isolated from this Cleveland P1 strain.

Taxonomy and Classification

GLV belongs to the family *Totiviridae*, genus *Giardiavirus*, of RNA viruses. This family is characterized by the nonsegmented dsRNA genome and simple virion structure. Members of *Totiviridae* include the yeast dsRNA virus (ScV-L). Many of the viruses recently discovered from protozoa, such as the *Trichomonas vaginalis* virus (TVV), *Leishmania braziliensis* virus (LBV), and *Eimeria stiedae* virus (ESV), also belong to this family.

Host Range and Geographic Distribution

Purified GLV readily infects many virus-free isolates of *G. lamblia* trophozoites, but not any other parasitic protozoa tested, including *Tritrichomonas foetus, Trichomonas vaginalis, Trypanosoma brucei brucei, Entamoeba histolytica* and *Eimeria stiedae.* The virus has also been shown not to infect two transformed human intestinal cell lines. It is therefore believed that giardiavirus has a rather narrow host range. It probably infects only *G. lamblia* in nature.