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Lecture title: **Genetic Recombination between Viruses**

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Summary: ***Genetic Recombination between Viruses***

When two different viruses simultaneously infect the same cell, genetic recombination may occur between the nucleic acid molecules during or after their synthesis; this may take the form of intramolecular recombination, reassortment, or reactivation (the latter if one of the viruses had been inactivated)

Intramolecular Recombination

Intramolecular recombination involves the exchange of nucleotide sequences between different but usually closely related viruses during replication , It occurs with all double-stranded DNA viruses, presumably because of template switching by the polymerase. Intramolecular recombination also occurs among RNA viruses (e.g., picornaviruses, coronaviruses and toga viruses); western equine encephalitis virus arose as a result of intramolecular recombination between an ancient Sind bis-like virus and eastern equine encephalitis virus. Such phenomena are likely more widespread among RNA viruses than has been appreciated because the detection of recombinants has been difficult. Now, use of the reverse-transcriptase polymerase Chain reaction has overcome earlier technical problems.

Recombination between viral and cellular genetic information has been established and, for at least some viruses, is also important in virus evolution. After all, viruses have access to the almost unlimited gene pool of their host cells and certainly have the capacity to incorporate and exploit genes that favor their growth and survival. The presence of cellular genes or pseudo genes within the genomes of retroviruses is well established, and the same has now been found for other RNA viruses. For example, in influenza virus infections, proteolytic cleavage of the viral hemagglutinin by cellular proteases is essential for the production of infectious progeny. During the adaptation of non virulent influenza virus strains to chicken cells (which are nonpermissive for hemagglutinin cleavage), a pathogenic variant was isolated that contained an insertion of 54 nucleotides that was complementary to a region of host cell 28S ribosomal RNA. This suggests template switching by the viral polymerase during viral RNA replication. This insertion seems to have changed the conformation of the viral gene product, the hemagglutinin, rendering it accessible to cellular proteases and thereby producing infectious virions in previously nonpermissive cells. The pathogenetic consequences of cellular information being inserted into viruses by intramolecular recombination can be dramatic. The discovery that Marek's disease virus, an oncogenic herpesvirus of chickens, had been misclassified because it carries extra genes was particularly surprising. This virus had been considered a gammaherpesvirus, partly

because all other oncogenic herpesviruses are members of this subfamily. Subsequently, as the genome of the virus was partially sequenced

Equally surprising was the discovery of the molecular basis for the progression of bovine viral diarrhea to mucosal disease. When a cellular ubiquitin gene is inserted into a nonstructural gene of noncytopathic bovine viral diarrhea virus strains they become cytopathic and gain the capacity to cause persistent infection. Severe disease, i.e., mucosal disease, occurs when such mutant viruses infect bovine fetuses previously infected with noncytopathic, nonpersistent virus strains during the first 80 to 100 days of gestation.

Reassortment

Reassortment is a form of genetic recombination observed in RNA viruses with segmented genomes, whether these be single or double stranded and whether these involve few or many segments. Reassortment has been documented in families with 2 (Arenaviridae and Birnaviridae), 3 (Bunyaviridae), 6, 7, or 8 (Orthomyxoviridae), or 10, 11, or 12 (family Reoviridae) genome segments

In a cell infected with two related viruses within each of these families, an exchange of segments may occur, with the production of viable and

stable reassortants. Such reassortment occurs in nature and is an important source of genetic variability; for example, bluetongue virus isolates have been found in cattle that are reassortants of two parental viruses.

Reactivation

The term multiplicity reactivation is applied to the production of infectious virus by a cell infected with two or more virus particles of the same strain, each of which had suffered a lethal mutation in a different gene. Multiplicity reactivation could theoretically lead to the emergence of infectious virus if animals were inoculated with vaccines produced by UV-irradiation or chemical mutagenesis; these methods are therefore not used for vaccine production. Cross-reactivation, genome rescue, and DNA fragment rescue are terms used to describe genetic recombination between infectious and inactivated viruses of related but distinguishable genotypes (or a DNA fragment from such a virus). Appreciation of such phenomena was important in the development of viral vectors as vaccines.

Recombinant DNA Technology

The discovery of restriction endonucleases and the recognition of other enzymes involved in DNA synthesis (polymerases, ligases, transferases)

opened up the possibility of deliberately introducing foreign genetic information into the DNA of viruses, bacteria, yeast, and vertebrate cells. The discovery that such foreign DNA can be amplified greatly by coreplicating along with the DNA of its host opened up many other possibilities, including novel vaccines (e.g., DNA vaccines) and diagnostic reagents (e.g., hybridization probes). Overall, the technology is called molecular cloning or, in lay terms, genetic engineering. When a foreign DNA segment is inserted into an appropriate vector in frame and with appropriate upstream and downstream regulatory sequences and the recombinant plasmid is introduced into a host cell, the foreign DNA may be expressed, i.e., the protein it specifies may be produced in large amounts. This technology is called expression. The vector used may be a bacteriophage (a virus of bacteria) or a bacterial plasmid so that the protein is expressed in bacteria

Similarly, the foreign DNA may be incorporated into a yeast plasmid or virus so that it may be expressed in yeast, insect cells, or mammalian cells. The bacteriophage or plasmid or virus serve as cloning vectors (to amplify the foreign DNA) and/or expression vectors (to produce gene products). Some vectors have been engineered to replicate in different host cells and are called shuttle vectors. Some eukaryotic vectors allow the introduction and expression of foreign genes in intact animals these are called transgenic vectors

Role of Vectors in Recombinant DNA Technology

The use of animal viruses and vertebrate and insect cells for the expression of foreign genes was developed early in the evolution of molecular cloning techniques. Papovaviruses were first utilized to introduce foreign genes into mammalian cells: early understanding of the nature of the genomes of these viruses and their capacity to incorporate rather large amounts of foreign DNA underpinned this breakthrough research. Success in developing full-length infectious clones of DNA viruses used as vectors and full-length complementary DNA clones from RNA viruses, used as vectors, has opened the door for many new ways to produce valuable proteins (for vaccines and diagnostic reagents) and nucleic acids (for diagnostic probes and DNA vaccines).

Poxviruses as Vectors Among the DNA viruses

poxviruses have been prototypes as cloning and expression vectors, delivering foreign genes of interest into many kinds of cultured cells and into mammals and birds. The large genomes of the poxviruses have allowed the insertion of large amounts of foreign DNA into nonessential genome regions. Vaccinia virus has become a workhorse of recombinant DNA technology; hundreds of foreign proteins of biological and medical importance have been vectored by the vaccinia virus system. When engineered properly, the proteins vectored by vaccinia virus and

expressed in mammalian cells or animals have had the predicted molecular mass, have undergone proper posttranslational modifications required for biological activity (proteolytic cleavage, glycosylation, phosphorylation, myristylation), and have been transported to the proper intracellular or extracellular compartment. Because of the broad host range of vaccinia virus, many cell-type specific products have been vectored successfully. Vaccinia virus recombinant vectors are generated by incorporating the foreign DNA into the intact viral genome via homologous recombination. This is facilitated by adding vaccinia DNA flanking sequences to the foreign DNA. In infected cells, recombinant, chimeric vaccinia genomes are packaged faithfully into infectious progeny. In contrast to procedures used with other DNA viruses, the transfected foreign DNA can only be rescued by infectious vaccinia virus. This is due to the noninfectious nature of isolated poxvirus DNA, which replicates in the cytoplasm and requires many virion-associated enzymatic functions. One particular advantage of vaccinia virus recombinants is their very large carrying capacity has been estimated that the vaccinia virus can carry over 25 kbp of foreign DNA without its infectivity being affected. This allows the simultaneous expression of multiple foreign proteins and offers promise for the development of multivalent vaccines. Vaccinia virus-vector technology has been extended to other poxviruses, providing vectors and candidate vectored vaccines with desired limited host ranges (avipoxviruses, capripoxvirus,

raccoonpoxvirus, suipoxvirus, etc.). The practical application of poxvirus-vectored vaccine candidates has been demonstrated for the control of several veterinary and zoonotic diseases.

Transgenic Mice and Recombinant DNA Technology

Transgenic mice provide a valuable tool for investigating many problems in virology, immunology, and developmental biology. They are produced by injecting selected cloned 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 genes and gene products in the context of the intact animal. For example, transgenic mice have been produced in which every cell contained genome length hepatitis B DNA sequences. All of these mice had viral antigen in their plasma, but the viral genes were selectively expressed only in cells of the liver, kidney, and pancreas. Likewise, transgenic mice containing the DNA for the early region of bovine papillomavirus developed skin tumors at 8-9 months of age. In these mice, episomal viral DNA was detected in tumor cells and integrated viral DNA in normal tissues.

Practical Achievements of Recombinant DNA Technology

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 genomes of representative viruses of all the families of DNA viruses containing animal pathogens, including the 230-kbp genome of a cytomegalovirus and the 192-kbp genome of vaccinia virus.
2. Complete sequencing of the complementary DNA corresponding to the genomes of representative viruses of most of the families of RNA viruses containing animal pathogens.
3. Characterization of the viral or proviral DNAs that are integrated into the DNA of transformed cells and animals infected with retroviruses and papillomaviruses.
4. Development of probes for use in rapid diagnostic assays, including hybridization assays (dot-blot assays).
5. Application of the polymerase chain reaction to amplify specific viral genome sequences for diagnostic and research purposes.
6. Development of marker rescue by transfection with gene fragments as a method for genetic mapping.

7. Production of proteins coded by specific viral genes, using bacterial, yeast, baculovirus, and animal cell expression systems and by cell-flee translation systems.

8. Production of peptides based on DNA sequence data for use in diagnostics and immunotherapeutic.

Genetic Shift and Drift and the Evolution of Influenza A Viruses

Influenza A viruses produce important diseases in birds, horses, swine, mink, marine mammals, and humans. Because of the importance of human influenza, detailed long-term studies of the evolution of the viruses have been carried out, often in the vain hope of predicting future epidemics. Since the first isolation of an influenza virus in 1933, many strains have been obtained from all parts of the world and their antigenic properties studied in detail. In recent years their genetic properties have been studied in parallel, thus revealing the molecular bases for their remarkable evolutionary progression. Influenza A viruses are classified according to epitopes on their two envelope proteins, the hemagglutinin (H) and neuraminidase (N). All 15 subtypes of the hemagglutinin have been found in birds, 3 of them also in humans, 2 each in pigs, horses, seals, and whales, and 1 in mink. The 9 N subtypes show a similar distribution. An outstanding feature of influenza A viruses is the antigenic variability of these two proteins as a result of two types of changes, genetic or antigenic drift and shift. Antigenic drift occurs within a subtype and involves a gradual accumulation of point

mutations; those affecting neutralizing epitopes produce strains each antigenically slightly different from its predecessor. In contrast, antigenic shift involves the sudden acquisition of the gene for a completely new hemagglutinin or neuraminidase, giving rise to a novel subtype that may spread rapidly around the world, unencumbered by any herd immunity.

Genetic/Antigenic Shift

During the past century there have been five pandemics of human influenza: in 1890, 1900, 1918, 1957, and 1968. The pandemic at the end of the first World War killed over 20 million people more than the war itself. In 1957 the HIN1 subtype was suddenly replaced by a new subtype, A/H2N2, known as "Asian flu" because it originated in China. Within a year over a billion people had been infected

Clear evidence that distinct mechanisms are involved in the processes of antigenic shift and drift have come from sequencing of hemagglutinin genes of representative isolates; sequencing has shown relatively close relationships between strains within each of the three human subtypes, H1, H2, and H3, but major differences between subtypes, indicating that a sharp discontinuity in the evolutionary pattern had occurred with the emergence of H2 viruses in 1957 and H3 viruses in 1968. As data from sequencing all eight gene segments of many strains of influenza viruses isolated from several species of animals and birds became available, it became clear that all of the influenza viruses of mammals, including

humans, originated from the avian influenza gene pool, which itself presumably evolved from a common ancestral avian influenza virus. In 1957, five of the eight gene segments of the prevalent human HIN1 subtype were replaced by Eurasian avian influenza genes to produce the human H2N2 subtype; then, in 1968 the human H2N2 subtype acquired two gene segments, including the hemagglutinin gene from another avian influenza virus to produce the human H3N2 subtype. Moreover, retrospective serological studies have indicated that the 1890 human pandemic subtype was H2NS, the 1900 subtype H3NS, and the 1918 subtype HIN1, suggesting a pattern of recycling of the three human H subtypes (H1, H2, H3) and suggesting the origin of the virus that caused the 1918 pandemic in swine. Influenza A viruses from birds grow very poorly in humans, and vice versa; indeed, reassortants containing avian genes have been tested as experimental vaccines because of their a virulence and their inability to spread from human to human. However, both avian and human influenza viruses can replicate in swine, and genetic reassortment between them can be demonstrated experimentally in that host. It has now been shown quite certainly that an antigenic shift in nature occurs when the prevailing human strain of influenza A virus and an avian influenza virus concurrently infect a pig, which serves as a "mixing vessel." Every 10-20 years a reassortant virus from the pig, containing genes encoding replicative functions from a

human virus and a hemagglutinin gene derived from an avian virus, emerges

Genetic/Antigenic Drift

After a new pandemic influenza virus strain has emerged as a consequence of genetic reassortment, antigenic drift begins when point mutations accumulate in all of its RNA segments , Mutations in the gene encoding the hemagglutinin sometimes alter its antigenic sites. When antiserum against the formerly prevalent strain no longer neutralizes the variant, a new strain has emerged. Changes in the hemagglutinin are clustered in five regions of the molecule, which correspond to important antigenic sites. Substitution of a single amino acid in a critical antigenic site may abolish the capacity of the antibody to bind to that site. However, some regions of the hemagglutinin protein are conserved in all human and avian strains, presumably because they are essential for the maintenance of the structure and function of the molecule. The important feature of antigenic drift in human influenza viruses is that in immune populations the new strains have a selective advantage over their predecessors and tend to displace them. Although minor variants may cocirculate, a novel strain usually supplants previous strains of that subtype in a particular region.