

Introduction to Oncogenesis by RNA Tumor Viruses

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A. RNA Tumor Viruses

RNA tumor viruses, or retroviruses as they are now called, are known to induce a variety of neoplasms in their natural host species. Thus the lymphoid leukosis prevalent in cattle, cats and chickens, and the thymic lymphomas and mammary carcinomas of mice are typically caused by retroviruses. Rare, extremely acute neoplasms, such as sarcomas, and erythroid and myeloid leukemias, are also recognized to result from retrovirus infection, as well as non-malignant diseases, such as osteopetrosis in chickens, anemia in cats, autoimmune and paralytic diseases in mice. The problem of identifying retroviruses with neoplastic potential in humans remains equivocal, although tantalising items of evidence continue to be thrown up, as described by several contributors to this volume. While much of the impetus and funding for research in viral oncology is based on the search for human tumor viruses, retrovirus research today is proving most useful for providing conceptual models of oncogenesis and experimental systems for probing the molecular and cell biology of neoplasia.

B. Transmission of Retroviruses

Although the genetic information of retrovirus particles is contained in RNA molecules, upon infection of the host cell this information is transcribed by the viral enzyme, RNA-directed DNA polymerase (reverse transcriptase) into a double-stranded DNA provirus. This provirus, like the genomes of DNA tumor viruses, becomes inserted into host chromosomal DNA, so that the "integrated" viral genes become adopted by the host as extra genetic information. Integration is probably not the oncogenic event itself, although the insertion of new DNA sequences at inappropriate sites could very conceivably cause disruption of cellular regulatory mechanisms. Nevertheless, integration is the means by which viral genes may be heritably transmitted to daughter cells. Furthermore, during the evolution of retrovirus-host relations, retrovirus genomes have on occasion become integrated into cells of the host germ line, with the result that the viral genes are now inherited from one generation to the next as host Mendelian factors. Such stable, inherited viral genomes (called endogenous viruses), with the exception of certain inbred strains of mice, are not known to be oncogenic, but may give rise to oncogenic agents on reactivation to viral form, and may become recombined with other DNA sequences to form new genetic elements that are potentially oncogenic.

Thus retroviruses can exist in latent form by masquerading as host genetic information. These endogenous viral genes may be unexpressed for many host generations, or some viral antigens may be synthesized in certain types of host cell. On occasion, complete virus may be activated, either spontaneously, or by treatment of the host cell with ionizing radiation or chemical carcinogens and mutagens. The reactivation phenomenon led to a hypothesis that all cases of oncogenesis by diverse agents might be accounted for by activation of endogenous viruses. This now seems unlikely, and the most efficient virus inducing agents, such as halogenated pyrimidines, have little carcinogenetic potential. The latency and inheritance of retrovirus genomes considerably complicates any analysis of epidemiology, not least because some of the newly activated viruses frequently cannot reinfect cells of the species in which they are inherited, but may be infectious for foreign species, a phenomenon called xenotropism.

Leukemogenic retroviruses, with the important exception of murine leukemia viruses, are typically transmitted as infectious agents. Thus leukosis is a contagious disease in cats and cattle which is spread horizontally by close contact with infected individuals. Horizontal infection of chickens results most frequently in effective viral immunity, but 'vertical' infection of eggs leads to immunological tolerance and as a consequence of, perhaps, a persistently high viral load, such congenital infection typically causes lymphoid leukemia. Congenital retrovirus infection also occurs in several mammalian species via either the placenta or milk, and activation of endogenous virus in mouse embryos can even lead to a 'reverse vertical' infection of a non-viremic mother.

The evolutionary origin of retroviruses is often obscure. The exogenous retrovirus causing bovine leukemia appeared as a new enzootic agent in Danish cattle some years ago and might have been transmitted from another species. The endogenous virus of cats has been acquired from primates related to baboons, whereas the exogenous viruses of the gibbon-woolly monkey group are related to endogenous rodent viruses. Clearly there has been much hopping in and out of host genomes of different species in the evolution and spread of retroviruses.

C. Retrovirus Genes

The proteins of retroviruses are, of course, antigenic, and the preparation of specific antisera for radioimmunoassays and other immunological techniques has been of great use for studying viral gene expression and virus relationships. The other major analytical tool in molecular virology is nucleic acid hybridisation. With the preparation of specific radioactive probes, the presence and expression of retroviruses and of single retrovirus genes can be accurately monitored, and the evolutionary relationships between retroviruses in different host species can be precisely assessed. Many of the papers presented in this volume therefore, describe studies employing refined immunological and molecular hybridization techniques.

Non-defective retroviruses have a simple unit genome comprising three well-defined genes coding for virion proteins. The *gag* gene encodes a large, precursor polypeptide which becomes proteolytically cleaved to generate the internal or core antigens of the virion. These proteins are named according to their estimated molecular weight, e.g. murine p30 denotes the major core protein of 30 000 daltons of murine leukemia virus (MuLV). Precursor polypeptides are similarly labeled pr65, or pr90, etc. The *env* gene encodes the proteins located in the envelope of the virion which is derived by budding from the plasma membrane of the host cell; thus murine gp70 denotes the glycosylated envelope protein of MuLV of approximately 70 000 daltons. The *pol* gene encodes the polymerase (reverse transcriptase).

The three genes are ordered in the genomic RNA molecular in the sequence 5'-gag-pol-env-3'; apart from some nucleotide sequences at the 3' end of the molecule, they appear to account for the entire genome. The genomic RNA can act as a messenger RNA for translation of *gag* and *pol* proteins, but the *env* proteins appear to be translated from a separately transcribed mRNA species.

D. Oncogenesis

Retroviruses can be roughly divided into three groups on the basis of oncogenicity. Those that cause acute neoplasms with short latent periods between infection and the appearance of the tumor are called 'strongly transforming' viruses. In most cases these viruses will also transform appropriate cells in culture. Those that cause tumors only after long latent periods (months rather than days in mice and chickens, the most closely studied host species) are called 'weakly transforming' viruses, and in vitro transformation systems have not to date been devised for these viruses. Some endogenous retroviruses such as those of cats and chickens, may be regarded as 'non-transforming' viruses, but this view may have to be modified when they are studied in more detail.

I. Strongly Transforming Viruses

These viruses occur only very rarely in nature, but their recognition, isolation and experimental use has led to major advances in our understanding of viral oncogenesis. The best known and most venerable example of a strongly transforming virus is the Rous sarcoma virus (RSV) of chickens; others are avian myeloblastosis and erythroblastosis viruses, and murine Friend erythroleukemia, Abelson lymphoma and Moloney, Kirsten and Harvey sarcoma viruses. RSV has a gene, designated *src* for sarcoma induction, in addition to the three genes essential for viral replication. Studies of deletion mutants and temperature-sensitive mutants have shown that the *src* gene is essential for fibroblast transformation and for sarcomagenesis, but is not required for viral replication. Recent data from Erikson and his colleagues (reported in this volume) indicate that the *src* gene product is a cytoplasmic protein of

60 000 daltons that possesses protein kinase activity. Precisely how this protein effects transformation and what are the crucial targets in the cell for phosphorylation remains to be determined. Nevertheless it is a remarkable advance in experimental oncogenesis that an enzyme has been identified with an oncogene.

In most strains of RSV, the *src* gene is carried as an extra gene to the viral genes, in the order 5'-gag-pol-env-*src*-3'. All other well studied strongly transforming viruses are defective for replication, that is, new genetic information specifying neoplastic transformation (*onc* genes) appears to be inserted in the viral genome in place of essential genes for replication. A part of the *gag* gene and the 3' end of the viral genome are maintained, giving a typical structure 5'-*ga-*onc**-3' (see Duesberg's paper, this volume). The infectivity of such defective viruses relies on the presence of replication-competent 'helper' viruses, and the disease spectrum caused by such viruses depends on the properties of this complex virus population. Transformed cells can be obtained in culture by cloning cells infected with the infectious virus complex at dilutions that prevent infection by the helper virus in addition to the defective virus. Such transformed, 'nonproducer' cells have been analysed for expression of virus related proteins, and recently several new proteins possibly carrying oncogenic functions have been detected, as reported in this volume. The polypeptide coded by the supposed *onc* region of the viral genome actually starts in what remains of the *gag* gene. Since the polypeptide therefore bears some *gag* antigens, it can be identified by immunoprecipitation with anti-*gag* antisera from lysates of transformed cells. Such *gag-*onc** 'polyproteins' have been detected in cells transformed by avian myelocytoma and erythroleukemia viruses (see Graf's paper in this volume) and murine Abelson leukemia cells. These proteins are not related to the *src* protein or to each other, and each may have individual functions resulting in neoplastic transformation. This would account for the high degree of specificity of the target cell for transformation, as each virus causes a specific type of cancer or leukemia.

Oncogenes appear to originate from the host, as genetic elements related by molecular hybridization to viral oncogenes are found in the host genome, though not linked to endogenous viral elements. Possibly the natural host sequences code for normal proteins important in the function or differentiation of particular cell types. When picked up and modified in viral genomes, and then re-inserted into appropriate target cells, they may cause disruption to regulatory cell functions blocking or even reversing the normal pathway of differentiation. One popular view is that some oncogene products may represent or mimic mitogenic hormones specific for the target tissues. Further analysis of the oncogenes of strongly transforming viruses should tell us much, both about differentiation and neoplasia, especially in hemopoietic cells.

II. Weakly Transforming Viruses

These viruses do not appear to carry oncogenes distinguishable from the three viral genes, *gag*, *pol* and *env*. Commonly occurring weakly transforming viruses are the murine mammary carcinoma virus, murine thymic lymphoma viruses, avian bursal leukosis viruses, and the leukemia viruses of cats and cattle. In contrast to the strongly transforming viruses, the tumors they cause appear after long latent periods, and only very few of the cells that become infected subsequently give rise to tumors. The tumors are probably clonal in origin, whereas with strongly transforming viruses such as RSV the tumors grow as quickly by infection and transformation of new target cells, as by mitosis of the originally transformed cell. There is growing evidence for the murine viruses inducing thymic lymphomas that genetic recombination involving the *env* gene takes place, often between xenotropic and mouse-tropic endogenous viruses, giving rise to new virus variants which may interact with and transform different cell types than those recognized by the parental viruses. The recombinant *env*-coded glycoproteins may play a dual role, both in allowing the virus to recognize and infect specific target cells bearing appropriate receptors for the glycoproteins and acting as a perpetual mitogenic stimulus to such cells.

E. Human Retroviruses

Homo sapiens is the only intensively studied vertebrate species from which C-type viruses cannot regularly be isolated. However, there are now a sufficient number of virus isolates and reports of virus footprints (see Gallo's contribution to this volume) to take the presence of retroviruses quite seriously. If that is so, the viruses must normally remain latent. Thus two basic questions really remain open: whether retroviruses are natural infections of man and whether they play a role in leukemogenesis. In the search for human retroviruses, more use might be made of marker-rescue techniques in using animal helper viruses for complementation or rescue of possible defective or fragmented viral genomes in human cells. Even if viral genomes do not operate in human leukemogenesis, the studies of viral leukemia in animals, and of the mechanism of action of oncogenes acquired by retroviruses, will prove to be of great importance in our understanding the nature of leukemia.