BIOTECHNOLOGY, VIRAL ONCOGENESIS AND JAAGSIEKTE

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ABSTRACT

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A brief description is given of the discovery of retroviral and cellular oncogenes and of their putative role in oncogenesis. Attempts to apply the biotechnological techniques that were so successful in the study of other retroviruses to the newly-discovered jaagsiekte retrovirus are briefly reviewed.

Jaagsiekte was first described as a disease entity in South Africa more than a century ago. It is a cancer involving the epithelial lining of the lung alveolus, causing the cells to proliferate until the lung consists of a solid mass of tumour cells, and the animal dies of asphyxia (Tustin, 1969).

From the outset and long before its neoplastic nature was appreciated, it was known as a contagious disease. Nevertheless, despite the efforts of many scientists in various countries around the world, its cause remained unknown until very recently. Some 3 years ago, our group at Onderstepoort obtained conclusive evidence that jaagsiekte is caused by a retrovirus, which is shown in Fig. 3 (Verwoerd, Williamson & De Villiers, 1980; Verwoerd, Payne, York & Myer, 1983).

This was an exciting discovery, and not only because the aetiology of the disease had been elucidated at last. It came at a time when the study of retroviruses associated with leukaemias and lymphomas in chickens, mice and other mammals, including man, yielded dramatic new insights into the ways in which cancers arise.

Our research on the jaagsiekte retrovirus is of necessity still at a very early stage. I should therefore like first to illustrate the current concepts of the role played by retroviruses in the transformation of normal cells to the malignant state, using, as an example, Rous sarcoma virus (RSV), which was the first retrovirus discovered. I shall also mention the important role that modern biotechnological techniques have played in elucidating the complex virus-cell interactions involved. At the end of my talk I shall return to the jaagsiekte virus, briefly discuss some of our results and problems and indicate where we are going in our research. Retroviruses have been popular objects for research, not only because of their association with cancer, but also because of their unique replication cycle. After infecting the host cell, the RNA genome of the virus is converted into a DNA copy be means of the enzyme reverse transcriptase. The DNA copy, or provirus, is then integrated into the cellular DNA. The provirus can remain either inactive or latent, or be transcribed into RNA, which then functions both as messenger RNA for the synthesis of viral proteins and as genome for progeny viral particles. These mature during budding from the cell surface.

Two groups of retroviruses can be distinguished. The first, of which Rous sarcoma virus is an example, is highly oncogenic, transforms cells in culture, and produces neoplasms within days or weeks. During a study of RSV, mutants were found that lost the ability to transform cells but could replicate in a perfectly normal way.

It also lost a part of its genome. This led to the discovery that the transforming retroviruses contain genes which are not involved in viral replication, but are responsible for tranformation and were therefore called oncogenes (or onc genes). Because it produces a sarcoma, the RSV oncogene is called src (Bishop, 1982).

To investigate the origin, structure and function of the src gene, much larger quantities were needed than could be isolated from the virus. Using DNA recombinant techniques, it was therefore cloned by splicing into the DNA of a bacterial plasmid which can by produced in large quantities in *Escherichia coli* cultures. Using cloned src, it was shown unambiguously that the oncogene could transform cells and produce cancers without the help of the virus. By radio-active labelling of src, a probe was obtained which could be used to compare the nucleotide sequence in src with other DNA's by means of molecular hydridization. The most important problem to be solved was: if the src gene is not a viral gene, did it originate in the cell? By hybridizing a src probe with fragmented cellular DNA, it was indeed found that there are homologous genes in normal chicken cells. These sequences, called cellular or c-oncogenes in contradistinction to viral or v-oncogenes (or c-src to v-src), represent highly conserved normal genes, homologous but not identical with the viral genes, which have been shown to be transcribed and therefore to fulfil a physiological function in normal cells.

The next important discovery was the identification of the product of v-src as a protein kinase, specific for the amino acid tyrosine. It was also shown that the c-src gene codes for a closely related protein kinase. In an attempt to further elucidate the mechanism of oncogenesis, the protein kinase was found to be localized at the plasma membrane, a site which is known to play a role in the regulation of cell division. Phosphorylation of proteins is known to play a central role in many regulatory reactions in the cell. It thus seems logical that increased kinase activity could be the trigger changing a normal to a cancer cell. This hypothesis was supported by the demonstration that the amount of phosphorylated tyrosine in a cell increases tenfold after transformation with src. It soon became clear that the mechanism of viral carcinogenesis is much more complicated, however. Other retroviruses were found to contain different oncogenes, some coding for kinases, others not. Twenty different oncogenes are known at present, suggesting that many normal cellular genes with oncogenic potential may exist (Bishop, 1983).

FIG. 1 Macroscopic appearance of a typical experimentally-produced jaagsiekte lung. The ventral parts of both lungs consist of solid tumour tissue; dorsally normal tissue is interspersed with small adenomatous nodules.
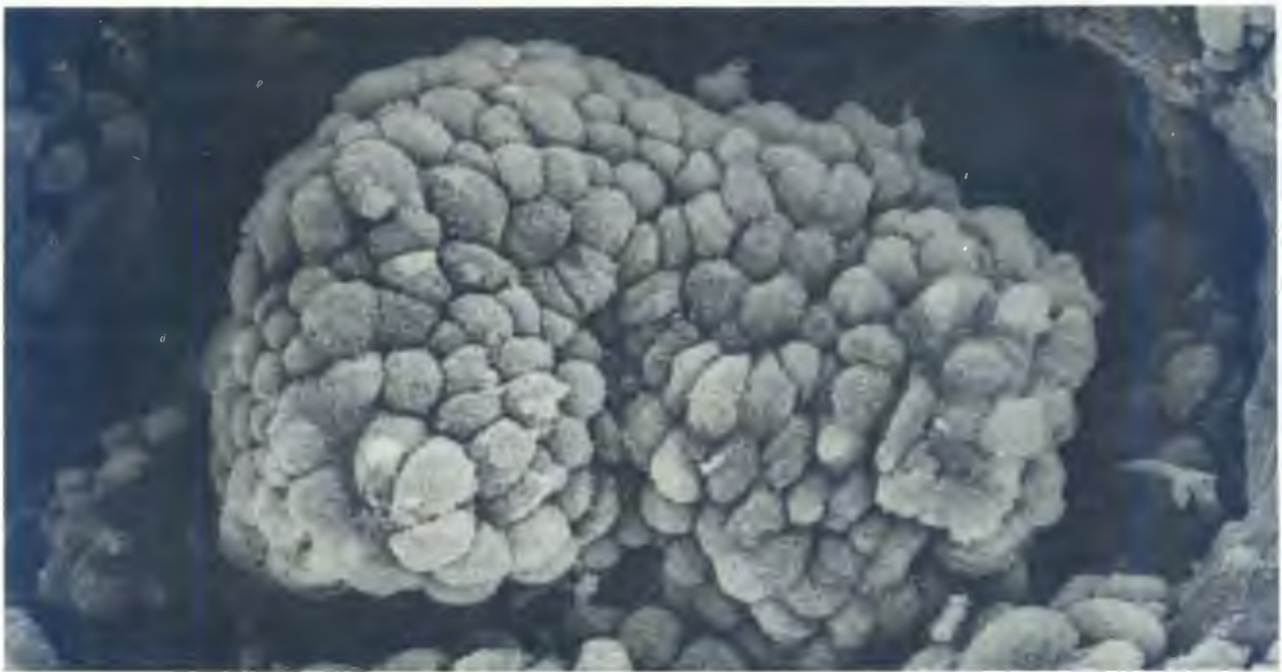
FIG. 2 Scanning electronmicrograph of the primary lesion in jaag-siekte: a cluster of cancerous epithelial cells proliferating into and filling up the alveolar space.
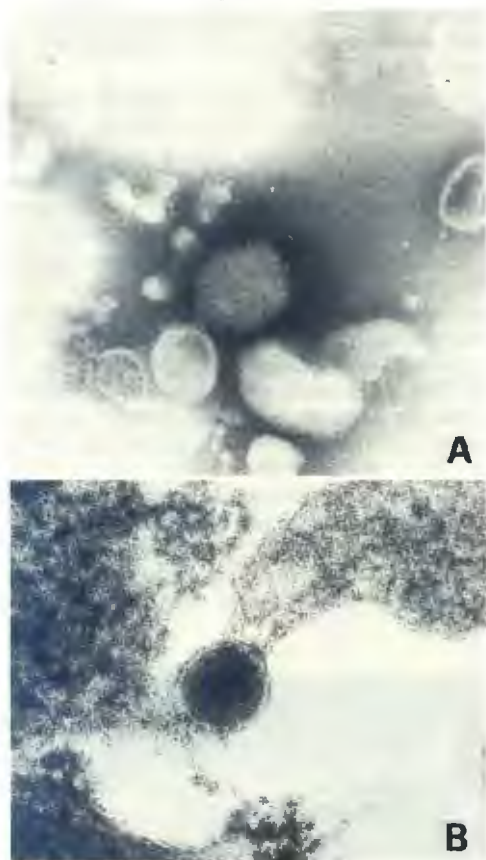

FIG. 3 Electron microscopic appearance of the jaagsiekte retrovirus. A. Negatively stained virion, purified from lungrinse material, showing the spiked surface structure typical of many retroviruses. B. Maturing viral particle budding from the surface of a tumour cell.

From these results the concept emerged that the acutely transforming retroviruses are recombinants containing a transforming gene, which is derived from a normal cellular gene. The virus thus acts as a vector, carrying the gene from one cell to another. Nature has therefore been doing genetic engineering ages before we had coined the term!

The second group of retroviruses represented by the majority of leukaemia associated viruses, does not carry an oncogene. They do not transform cells *in vitro*, and latent periods of months or years are the rule.

In contrast to the first group, these viruses appear to act in an indirect way by activating a normally suppressed cellular oncogene. This, by the way, is also the mechanism by which chemical and physical carcinogens are thought to act. One way in which the virus could activate a cellular gene is by being integrated into the cell DNA in such a way that the viral genes control the expression of the cellular gene. This was proved experimentally by isolating a non-transforming cellular oncogene, splicing it to a viral promoter and then showing that it has been converted to a transforming gene.

Another technique for the demonstration of cellular oncogenes, which I can mention only briefly, is the direct transfection assay. In this technique, DNA is isolated from cancer cells, fragmented, and used to transform other cells *in vitro*. In this way, a large number of DNA isolates with transforming potential have been obtained, mainly from human tumours. The reason for mentioning this is the fact that most of these preparations contained DNA sequences homolgous to oncogenes derived from retroviruses of animal origin. The study of animal retroviruses has thus also contributed significantly to our understanding of the basic mechanisms of oncogenesis in man (Cooper, 1982).

But let me return now to some biotechnological aspects of our studies on the jaagsiekte retrovirus. A few words on the economic importance of the disease and the aims of our research might be in place here. Apart from Australasia the disease is worldwide in its distribution. In this country, as in most other countries with substantial sheep populations, it is endemic with an incidence of between 1 and 20 %, which seems to be increasing. It is therefore of considerable economic importance, and the ultimate goal of our work is obviously to control or eradicate the disease. There are basically 2 possiblities to achieve this in practice:
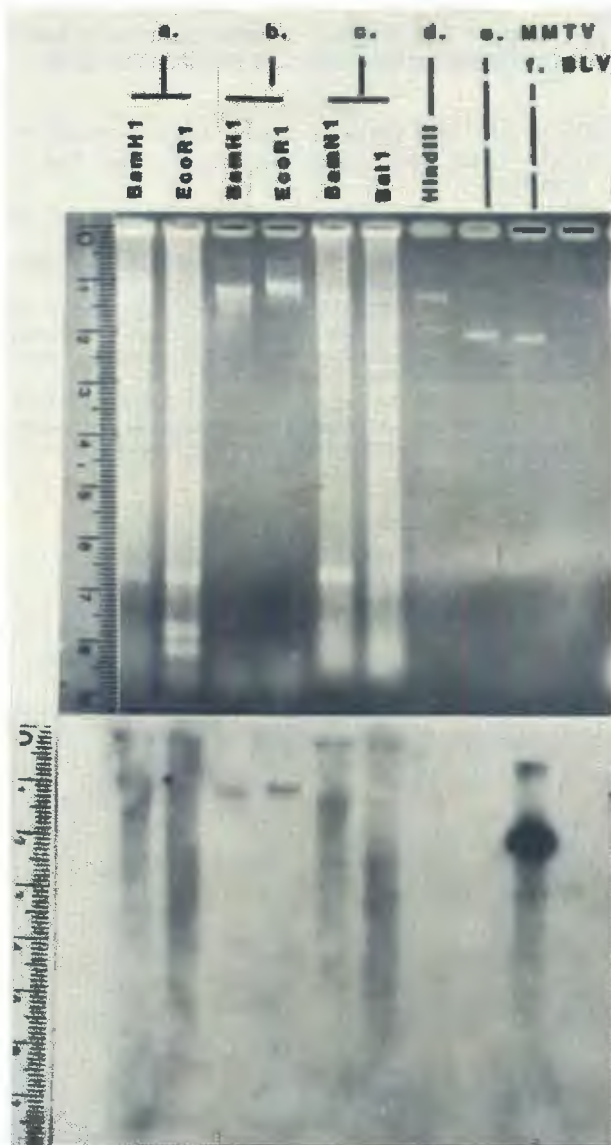
FIG. 4 The result of a molecular hybridization experiment in which a ^{32}P-labelled probe prepared by nick translation of the BLV genome cloned in pBR322 was hybridized to DNA isolated from: (a) normal sheep lung, (b) cultured 15.4 jaagsiekte tumour cells, (c) an advanced jaagsiekte lesion, (d) λ-phage as marker, (e) cloned mouse mammary tumour provirus (MMTV) and (f) cloned bovine leukaemia provirus (BLV). Preparations (a)–(d) were digested with the retriction endonucleases indicated before electrophoresis in agarose gels. The top part of the figure shows the electrophoretic pattern, the lower part represents the autoradiograph obtained after Southern blotting and hybridization under non-stringent conditions. Cloned BLV was kindly provided by Dr A. Burny, Brussels, Belgium.

1. a vaccine
2. a sensitive serological test to detect and eliminate carriers of the virus.

For both of these options it is essential to know whether the virus is endogenous, i.e. integrated into the DNA of all sheep cells, or not. It would also be very useful to know whether JSRV carries an oncogene, and, if it does, whether it is related to any of the known oncogenes. To do the relevant experiments, it is essential to have a radioactive probe, that is, a DNA copy of the viral RNA, for molecular hybridization.

At present, the main obstacle in our research is our inability to grow the virus *in vitro*. Our only source of virus is the lung tumour, which we can fortunately produce experimentally. Yields of virus are low and we have not been able to purify it completely from contami-
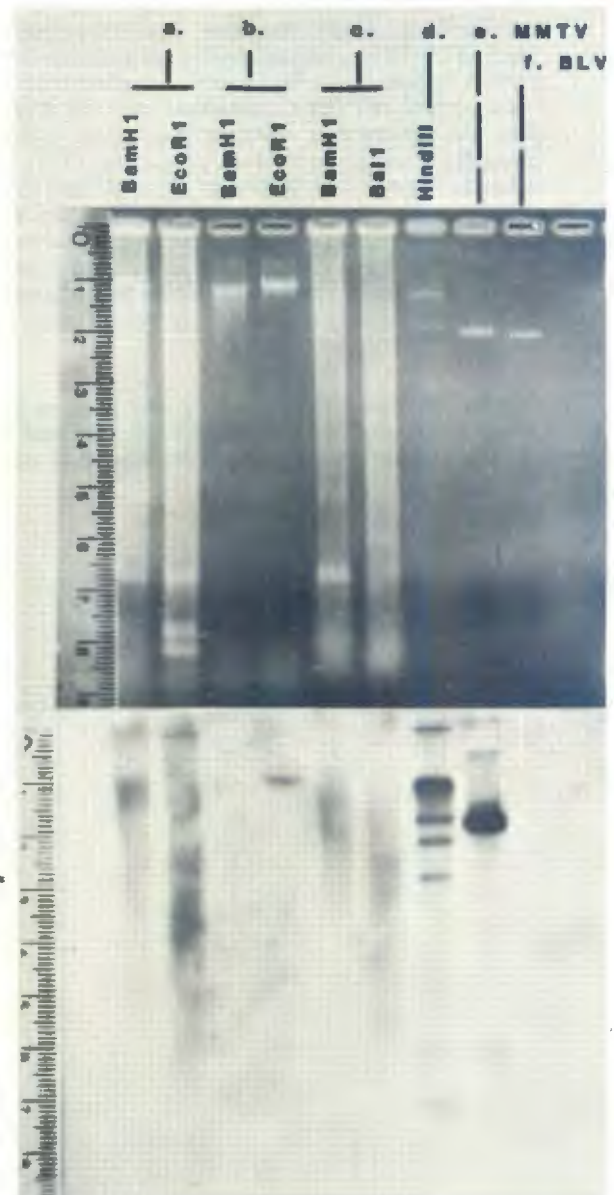
FIG. 5 The result of molecular hybridization of a ^{32}P-labelled MMTV probe to the same set of DNA preparations illustrated in Fig. 4, under identical experimental conditions. Cloned MMTV was kindly provided by Dr H. Diggelmann, Lausanne, Switzerland.

nating lung material. The limited amounts of virus were sufficient for a serological comparison of our isolate with other retroviruses and for a preliminary investigation of the host animal's immune reponse—but that is another story. For the preparation of a probe, larger amounts of viral RNA or cDNA were needed, and therefore we decided to attempt cloning of the JSRV provirus. We had 3 options to obtain the provirus, or cDNA, for cloning.

1. To copy the viral RNA in intact particles, utilizing the endogenous reverse transcriptase. For some unknown reason, this reaction was very inefficient in our hands.
2. To isolate the viral RNA and reverse transcribe it with exogenous enzyme. This reaction was successful, but produced only small fragments of DNA unsuitable for cloning.
3. To rescue provirus from tumour tissue or tumour cells grown *in vitro*. No unintegrated viral DNA was found in these cells, therefore we had to resort again to the use of probes to identify integrated viral sequences in fragments of cell DNA gene-

rated with restriction enzymes. A probe prepared form JSRV RNA, isolated from a lung rinse pellet, picked up numerous bands but also cross-reacted with normal cell DNA, however, and was therefore too non-specific to be of much use. A last possibility we explored was to use the DNA of other retroviruses as probes in the hope that they might have common sequences which would detect JSRV proviruses. We have so far tested cloned bovine leukaemia virus (BLV) and cloned mouse mammary tumour virus (MMTV) for this purpose, but in both cases no cross-reaction was detected, as illustrated in Fig. 4 and 5.

To summarize, we still have a long way to go in our attempts to clone and study the JSRV genome and to elucidate the way in which it causes the tumour. It may even happen that progress on other fronts may lead to practical control measures before we reach this goal.

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