TUMOUR BIOLOGY

Chemical Transformants

from our Cell Biology Correspondent

THE properties of chick embryo fibroblasts infected and transformed by certain temperature sensitive mutants of Rous sarcoma viruses have established that the maintenance of the transformed cell phenotype depends upon the continuous expression of one or more viral functions. Similar experiments with a solitary temperature sensitive mutant of polyoma virus lead to the same conclusion. Both the RNA and the DNA tumour viruses have small genomes and it seems unlikely, to say the least, that each and every change in the phenotype of the cell transformed by one of these viruses results directly from the action of a distinct viral protein. The viruses simply do not have enough genetic information to be so profligate. It follows therefore that the limited number of transforming proteins specified by the tumour viruses must induce either pleiotropic effects or they must interact with various cellular molecules and indirectly bring about transformation.

At present the nature of the transforming viral proteins is not known, neither is anything known about the putative cellular molecules which may be targets for the viral proteins. But just as it is possible to inactivate by mutation the viral genes involved in transformation, it ought to be possible to mutate the cellular genes specifying the target molecules, and Di Mayorca *et al.* (*Proc. US Nat. Acad. Sci.*, **70**, 46 ; 1973) may have done this; certainly the data they present can be so interpreted.

Di Mayorca *et al.* exposed BHK_{21} clone 13 cells to dimethylnitrosoamine or nitrosomethylurea and then plated the cells in liquid media to measure their plating efficiency and in soft agar to measure their ability to grow in suspension into colonies. These BHK cells do not grow in agar suspension unless they have been transformed and this technique of suspension plating has been widely used as a method of selecting and assaying for transformation of BHK cells. Using these procedures Di Mayorca *et al.* detected transformants at a frequency of 6×10^{-6} in populations of BHK cells exposed to appropriate doses of either dimethylnitrosamine or nitrosomethylurea. That transformants arise after exposure to these chemical carcinogens/mutagens is not in itself particularly surprising, but the phenotype of the transformed cells is remarkable. Apparently all the transformants isolated after the cells have been exposed to the carcinogens as well as a single clone of spontaneously transformed BHK cells prove to be temperature sensitive for the two markers of the transformed state that were assayed—the ability to grow in agar suspension and the morphology of the colonies. In all cases these transformants failed to grow in agar at 32° C and in liquid media grew to form typical monolayers, but at 38.5° C they behaved as transformed cells growing to form colonies in agar and to form multilayered foci in liquid medium.

These results are apparently fully reproducible and the properties of these chemical transformants are in marked contrast to the properties of BHK cells transformed by polyoma virus which exhibit the transformed phenotype at 32° C and 38.5° C. These temperature sensitive chemical transformants also differ from cells transformed by temperature sensitive tumour viruses, the latter having a transformed phenotype at lower temperatures and an untransformed phenotype at higher temperatures.

The obvious interpretation of these data which Di Mayorca *et al.* offer is that the cells they have isolated carry a temperature sensitive lesion in a cell gene which specifies a product required for the maintenance of the normal or untransformed phenotype. They suggest that at 32° C the putative cellular gene product is functional whereas at 38.5° C it is inactivated because of a temperature sensitive mutation and as a result the cells acquire the transformed phenotype as their temperature is raised. Clearly if such a cellular gene product, that is required to maintain a cell in the untransformed state, exists it must be inactivated when a cell is transformed by a tumour virus; it must presumably be inactivated, for example, when BHK cells are transformed by polyoma virus and this putative cellular protein may be a target of the viral transforming gene proteins.

No doubt such speculations are not to everybody's taste, but surely few would deny that the cells which Di Mayorca and his colleagues have isolated have remarkable properties.

TEMPERATE VIRUSES

Infection in Humans

from our Medical Virology Correspondent

SUBACUTE sclerosing panencephalitis (SSPE), a fatal disease of children and young adults, is a chronic progressive infection of the central nervous system by a measles-like virus (M. Katz *et al.*, *Nature*, **222**, 888 ; 1969 ; and V. Ter Meulen *et al.*, *Curr. Topics Microbiol. Immunol.*, **57**, 1 ; 1972). The evolution of the disease can be divided into clinical stages characterized by mental and behavioural changes, disorders of the locomotor system and loss of cerebral-cortex function. The SSPE agent has many of the biological properties of measles virus, but unlike conventional strains of measles it remains intimately bound to cells and it has a different pattern of replication and intracellular behaviour.

The chronic nature of this viral infection, the possibility that the formation of immune complexes may be important in the aetiology of this disease and perhaps in causing some of its damage to nervous tissue (A. D. Dayan and M. I. Stokes, *Brit. Med. J.*, **2**, 374 ; 1972) suggest that SSPE may be a model of temperate viral infections in humans, possibly an important group

Transformation by Herpesvirus

SOME herpesviruses of mammals are known to possess an oncogenic potential, and Duff and Rapp (*Nature New Biology*, **233**, 48 ; 1971) believe that herpes simplex virus, a human virus, can transform hamster cells. Whether herpes simplex virus or any of the other herpesviruses which are indigenous to man have any role in the development of cancer in human beings is, however, another and a vexed question. Obviously human herpesviruses, which can be shown to transform human cells in culture, must be seriously considered as potential human carcinogens and for this reason numerous groups are carrying out the appropriate experiments. Darai and Munk in Heidelberg, for example, have been investigating the infection of human embryonic lung cells with *Herpesvirus hominis* type 2, and they report in next Wednesday's *Nature New Biology* (February 28) that when cultured in appropriate conditions the infected cells have some of the properties characteristic of transformed cells.

The complete replication of *Herpesvirus hominis* in cultures of human lung cells results in cell lysis, and to prevent this Darai and Munk raised the temperature of the culture 1 hour after infection from 37° to 42° C. Cells which survived infection for eight days at 42° C were then returned to 37° C and repeatedly subcultured. Several cell lines were established and these cells were tested in a variety of ways.

The cells all proved to contain a *Herpesvirus hominis* antigen in the cytoplasm ; to tend to form syncytia as a result of cell fusion apparently promoted by a viral-coded cell surface like a protein ; to resist superinfection and to survive longer in culture than control uninfected cells. These properties suggest that the cells may have been transformed.

of disorders in man including such divergent diseases as viral hepatitis (A. J. Zuckerman, *Immunopathology* VI, edit. by P. A. Meischer, Schwabe, Basel, 1971, p. 436) and multiple sclerosis (F. Wolfgram *et al., Multiple Sclerosis, Immunology, Virology and Ultrastructure,* Academic Press, 1972). This is now the subject of intensive investigation in many laboratories.

Traditionally the tissue damage caused by viral infections has been explained by the ability of viruses to multiply in cells, thereby leading to cell injury or destruction. It is now becoming clear, however, that other mechanisms may be operating and that lesions may be caused by the immune response of the host to viral antigens and that the immune system itself may be disturbed by some viruses. Thus, certain viruses depress the humoral and cellular immune responses of the host, whereas other viruses may enhance the antibody response. Several viral antigens persist in the circulation and combine with specific antibody resulting in antigen-antibody complexes. These complexes may lodge in various sites, and in other instances combination with complement releases substances which cause tissue damage. Immunological injury mediated by antibodies may also result when new antigens produced by viruses on infected cell surfaces interact with specific antiviral antibody and complement (Memorandum: Virus-associated Immunopathology, *Bull. Wld Hlth Org.*, **47**, 25 ; 1972).

This concept of virus-associated immunopathology has recently been extended to temperate viral infections and it is possible that the ability to produce infectious immune complexes may be important in establishing chronic infections such as SSPE and hepatitis B in man and lymphocytic choriomeningitis, aleutian mink disease and murine sarcoma and leukaemia in animals. Chronicity may be attributable to differences in the affinity of the antibodies produced, a factor which is partly under genetic control, perhaps on mechanisms linked to histocompatibility genes (W. F. Bodmer, *Nature,* **237**, 139 ; 1972). It is difficult, however, to explain the relative rarity of SSPE, if it is caused by measles virus, in the face of the ubiquitous distribution of measles. What is needed is an animal model, but experimental inoculations of animals with different strains of SSPE agent have generally yielded divergent results. P. Albrecht and colleagues (*J. Infect. Dis.*, **126**, 154 ; 1972) have reported recently that a hamster-brain-adapted strain of measles virus produced encephalitis in rhesus monkeys with morphological findings similar to those described in SSPE and quite unlike acute measles encephalitis in man. The principal property responsible for this potential of measles virus seems to be an aberrant intracellular replicative cycle of the virus which affects the maturation process at the cell membrane. Further understanding of temperate viral infections in humans thus seems likely in the near future.

PHOTOPHOSPHORYLATION

Without Membranes

from our Photosynthesis Correspondent

IN 1954 it was discovered that isolated chloroplasts could utilize light energy to make adenosine triphosphate (D. Arnon *et al., Nature,* **174**, 394). This finding had important implications because it meant that chloroplasts could produce ATP as well as $NADPH_2$ for driving CO_2 fixation. Photophosphorylation is now a well documented phenomenon which can readily be detected with lamellae fragments obtained from chloroplasts which have been broken osmotically. It has even been shown to occur in small subchloroplast vesicles obtained either by sonication (R. E. McCarty, *J. Biol. Chem.*, **244**, 4292 ; 1969) or by treatment of inner lamellae with digitonin (N. Nelson *et al., ibid.*, **245**, 143 ; 1970). The common feature of these phosphorylating systems was that they were dependent on the presence of an intact membrane and also they required the addition of electron carriers or acceptors. It now seems possible, however, that photophosphorylation can occur in the absence of both intact membranes and artificially supplied electron carriers.

McPhee and Brody (*Proc. US Nat. Acad. Sci.*, **70**, 50 ; 1973) report the striking observation that an acetone extract obtained from chloroplasts when layered on an aqueous surface will exhibit light-induced phosphorylation. The extract was obtained from particles derived by sonicating spinach chloroplasts, and was used to produce a monolayer at an air-water interface by compression to a surface tension of about 20 dynes cm^{-1}. Electron microscopy indicated the films to be partly composed of small structured particles of about 200 Å diameter at a concentration of one particle per 2×10^8 Å^2 of compressed surface. On illumination McPhee and Brody detected ATP production with quantum yields between 0.1 and 1. The ability of the monolayer to catalyse photophosphorylation was dependent on maintaining a compression of at least 10 dynes cm^{-1} and could be doubled by adding phenazine methosulphate.

The artificial system was also found to induce additional esterification of inorganic phosphate not directly correlated with ATP formation. To explain their results McPhee and Brody argue that the phosphorylation observed may take place by a two-step reaction, one involving transphorylation between two ADP molecules to yield AMP and ATP followed by esterification of AMP to ADP.

Although McPhee and Brody have not used their data to discuss the chemical (E. C. Slayter, *Eur. J. Biochem.*, **1**, 31 ; 1967) or chemiosmotic (P. Mitchell, *Biol. Rev.*, **41**, 445 ; 1966) hypotheses their observations will undoubtedly be used by the "anti-chemiosmotic school" in attempts to dislodge Mitchell's elegant hypothesis.

Poly(A) Tract and Messenger RNA Ageing

THE function of the tracts of adenylic acid residues that are known to occur at the 3′ ends of many messenger RNA molecules remains a mystery, but experiments reported by Sheiness and Darnell in *Nature New Biology* next Wednesday (February 28) indicate that as messengers age in the cytoplasm of HeLa cells the length of the poly(A) tracts decreases ; this may be a clue to their function.

Sheiness and Darnell analysed the poly(A) liberated by ribonuclease from populations of messenger RNAs in cytoplasmic extracts of HeLa cells fed pulses of ^{3}H-adenosine and then actinomycin D. They conclude from such experiments, first, that the tracts of poly(A) in cytoplasmic messengers shorten as a result of metabolic ageing and, second, that this shortening occurs in the cytoplasm after the messengers have been transported from the nucleus. Furthermore, this time-dependent shortening of the poly(A) tracts occurs when protein synthesis is blocked. It seems plausible to suggest therefore that the lifetime of a messenger may be determined by the length of the 3′ poly(A) tract and the rate at which the messenger loses adenylic acid residues. In other words, the function of poly(A) tracts of messengers may be to programme the lifetime of the molecules.

Also in *Nature New Biology* next week Gross *et al.* report the isolation from the polysomes of sea urchin embryos of a "9S" RNA fraction which stimulates a cell-free system from Krebs II mouse ascites cells to incorporate amino-acid. Fingerprint analysis of the product of *in vitro* translation indicates that it includes histones, and by comparing the stimulation of incorporation of tyrosine and tryptophan by 9S RNA Gross *et al.* conclude that their 9S RNA seems to be relatively free of contamination by messengers for proteins other than histones.