has suggested that loss of the cell chromosome results in loss of transformation, indicating that the viral nucleic acid is possibly integrated with the host cell chromosome.

It seems that the events occurring in a transformed cell are similar to the early stages of vegetative growth of viruses, but that the late functions such as viral replication and synthesis of viral protein do not take place. Why should this be so, and why are the transformed cells not killed? Two alternative explanations have been offered: either the virus is defective when it enters the cell, or else it may be that the transformed cell is unable to synthesize the late products of the virus. By fusing a cell transformed with *SV40* with a susceptible cell, research workers at the Salk Institute at La Jolla and at the Wistar Institute in Philadelphia have shown that virus synthesis begins and the cycle is completed. This suggests that there is no defect, at least not in the *SV40* virus.

Certain requirements for transformation can now be defined. These are a functional virus particle, the absence of cell death, integration of the viral DNA so that it is perpetuated, and absence of the late gene functions of the virus. But do the physiological changes in the transformed cell depend on viral DNA being permanently integrated into the host chromosome, thereby creating the switch that alters regulation, or is the change caused by a viral product which functions irrespective of the position in the cell of the virus DNA? To determine which of these is correct, Professor Stoker has made use of a phenomenon called anchorage dependence in *BHK21* cells infected with polyoma virus. When these cells were put into agar with transforming virus, only 1–5 per cent were transformed. When, however, a viscous solution of 'Methacel' was used, a third to a half of the cells appeared to have been transformed—that is, these cells could now form colonies in the 'Methacel'. When removed and cultured further, it was found that the majority reverted to normal.

From this Professor Stoker concludes that transformation can either be temporary (in which case the cells become normal again when the virus is lost) or else permanent transformation can occur, in which the viral DNA is integrated. He suggests that although the physiology of the transformed cell does not depend on integration, transformation can only be perpetuated if the viral DNA is integrated. When polyoma virus enters any cell in which it functions, the cell is probably transformed into a tumour cell. Subsequently, the cell may die accompanied by growth of the virus, or else it may continue for a short time as a transformed cell until the virus is lost and is not therefore tagged on to cell replication. Finally, the virus DNA may be integrated, and thereby the transformed characteristics are perpetuated indefinitely, resulting in cancer.

5′ Terminus of R17 RNA

from our Cell Biology Correspondent

MESSENGER RNA is translated in the 5′ to 3′ direction, and the nucleotide sequence of the 5′ terminal region of *m*RNA molecules probably specifies both the binding site for ribosomes and the initiation of in-phase translation. The simplest expectation would be that an initiator codon, either AUG or GUG, is 5′ terminal and fulfils both these functions. Unfortunately, determination of the 5′ sequence of *m*RNA, or of any other RNA molecule, is in practice extremely difficult. First, the only *m*RNA molecules which can readily be isolated in sufficient purity and quantity to warrant such analysis are RNA phage genomes. Second, and more generally important, the ideal method for determining 5′ termini is by specifically labelling the 5′ terminal nucleotide of non-radioactive RNA and then isolating the labelled nucleotide. But, as Takanami (1966, 1967) has discovered, this is difficult without at the same time causing the degradation of the RNA molecules—and every breakage generates a spurious 5′ terminus.

Takanami found that *f2* phage RNA had to be treated with alkaline phosphatase before it could be labelled with ^{32}P phosphate by the agency of the enzyme polynucleotide kinase, and it appears to be impossible completely to free these enzymes of ribonuclease so that some molecules are always degraded. Takanami's results have, however, one important implication—the 5′—OH group of the terminal nucleoside of *f2* RNA made *in vivo* is phosphorylated and probably triphosphorylated, *pppXp*. . . . Such termini might have been expected, because RNA chains grow 5′ to 3′ by the nucleophilic attack of the 3′—OH group on the growing polynucleotide on the 5′ *α* phosphate of the incoming nucleoside triphosphate. Not all RNA molecules possess 5′ triphosphate termini, however; Takanami claims that *E. coli* ribosomal RNA has 5′ phosphoryl termini, *pXpXp*. . . .

The occurrence of the 5′ triphosphate group suggests an alternative approach to the determination of the 5′ nucleotide of RNA. If ^{32}P-labelled phage RNA grown *in vivo* has a 5′ triphosphate, alkaline hydrolysis of such RNA should uniquely liberate the 5′ terminal base as the tetraphosphate *pppXp*. Roblin (*J. Mol. Biol.*, **31**, 51; 1968) in some very carefully executed experiments has shown that alkaline hydrolysis of R17 RNA in fact yields *pppGp*, so that the 5′ terminus must be *pppGpX.pY*. . . . He has also shown (doctoral thesis, Harvard University) that the second base is a purine and the third a pyrimidine. This means that neither AUG nor GUG initiating codons are 5′ terminal, and so at least the first three bases must have some function other than initiating polypeptide synthesis. Likewise the last eleven bases at the 3′ terminus of phage RNA do not include a chain-terminating codon (see *Nature*, **217**, 311; 1968). In collaboration with J. T. August, Roblin (doctoral thesis, Harvard University) has also shown that the 5′ terminus of *Qβ* RNA made *in vitro* is *pppGpGpXpYp* . . . where *X* is a purine and *Y* is a pyrimidine.

It now seems likely that all the RNA phage genomes have *pppGp* at the 5′ terminus. Dahlberg (quoted in Roblin's thesis) has apparently found this for *f2*, and both *Qβ* minus and plus strands begin *pppGp* (Banerjee *et al.*, 1967; Bishop, Pace and Spiegelman, 1967). Moreover, Maitra and Hurwitz (1965) showed the predominant 5′ termini of RNA made *in vitro* by *E. coli* RNA polymerase to begin either with *pppAp* or *pppGp*.

Why do purines predominate at the 5′ terminus of *m*RNA, and what is the effect of the 5′ triphosphate group on ribosome binding and the coding properties of these molecules? It should be possible to answer these questions as more and longer 5′ terminal sequences are determined.