Strand Switching during SV40 Transcription

THE two DNA tumour viruses, simian virus 40 and polyoma virus, are small and simple and have thus attracted the attention of tumour virologists intent upon understanding the viral transformation of animal cells from the malignant to the non-malignant state. The genomes of SV40 and polyoma virus are double stranded, covalently circular DNA molecules, with molecular weights of about 3×10^6 and are capable of coding only a mere 200,000 Daltons of polypeptide; in other words, these genomes almost certainly comprise less than ten and perhaps as few as six genes, one or more of which presumably specify the protein(s) responsible for maintaining cells in a transformed state.

The ultimate goal, therefore, of many tumour virologists is to identify and characterize this gene and its products, and, given the sophisticated technology of years, seemed entirely feasible. Nevertheless, progress towards it has been painfully slow. Experiments carried out recently in three American laboratories are certain, however, greatly to revitalize flagging enthusiasm for these viruses.

The three groups in question—Khoury and Martin of the National Institutes of Health, who have just published the first of their new results in *Nature New Biology* (238, 4; 1972), Lindstrom and Dulbecco of the Salk Institute, who reported their work in the *Proceedings of the National Academy of Sciences* in June (69, 1517; 1972), and Sambrook, Sharp and Keller of Cold Spring Harbor Laboratories, whose results have yet to appear in print—have all shown that as SV40 replicates in permissive cells stretches of both of the two strands of the viral DNA are transcribed. In short, transcription of SV40 DNA *in vivo* involves strand switching, a device hitherto known to occur only during the replication of some DNA phages with much larger genomes.

Since the late nineteen-sixties many groups, including the three which have made this surprising observation, have used various nucleic acid hybridization procedures in efforts to measure the extent and patterns of transcription of SV40 DNA during its replication and in transformed cells. And in spite of the obvious and formidable technical difficulties it has been shown that as this virus replicates between a third and a half of the genome is transcribed throughout the replication cycle into so-called early RNA while the remainder of the genome is transcribed into so-called late RNA only after the initiation of replication of the viral DNA.

What happens in transformed cells is more obscure and controversial; depending on the hybridization techniques used and the particular transformed cell line studied it has been variously estimated that transcription involves from much less than half to the complete genome. There is, however, general agreement that the viral RNA in transformed cells includes most if not all of the sequences present in the early RNA of lytic infections.

To gain further insight into the transcription of SV40 DNA first in permissive cells and subsequently in transformed cells, the three groups have exploited Westphal's (1970) observation that *in vitro* *Escherichia coli* DNA dependent RNA polymerase transcribes superhelical

SV40 DNA asymmetrically to yield an RNA molecule complementary to only one of the two DNA strands. This RNA made *in vitro* can be obtained in comparatively large amounts and can be labelled to high specific activities. It has now been used to effect the isolation of the separated strands of SV40 DNA and, in conjunction with appropriate hybridization procedures, the three groups have used this RNA to sort out which DNA strands serve as template for the synthesis of early and late RNA made *in vivo*.

The groups all find that early RNA isolated from infected permissive cells before the onset of viral DNA replication is transcribed from the strand that is used at template by *E. coli* RNA polymerase *in vitro*. By contrast late RNA, which cannot be detected at early times during infection, is transcribed off the other SV40 strand, that which is never used by the *E. coli* polymerase. In short the appearance of these late RNA sequences depends on switching the strand which is being transcribed.

These findings, which are a major step forward in understanding of the transcription of SV40, are for several reasons most unexpected. For one thing, in many quarters it had been assumed that the transcription of SV40 DNA *in vitro* by the *E. coli* enzyme, a highly asymmetric reaction, faithfully reflected the situation *in vivo*, but in the event that reaction has proved to be a useful red herring. For another thing, the SV40 genome is very much smaller than the genomes of those DNA phages, the transcription of which involves similar strand switching, and it had been generally assumed that such a simple virus might well regulate its affairs without resorting to the complexity of transcriptional strand switching. The fact that the transcription of the replicative form DNA of phage ϕ X174, which is almost exactly the same size as SV40 DNA, is totally asymmetric bolstered this assumption.

Experimental observations, however, have overtaken prejudice and raise a host of intriguing questions. First, how is this switch from early to late transcription, which presumably demands the existence in SV40 DNA of two promoter sites at which transcription begins and two terminators, effected? Why is the late promoter inactive until the replication of the viral DNA begins or if it is active at early times how is the late RNA specifically degraded? And what happens when the SV40 DNA is covalently integrated into the DNA of a transformed cell?

It is known that viral RNA in transformed cells includes sequences also present in the early RNA made during virus replication in permissive cells. Does this mean that integration involves a break in the SV40 DNA molecule somewhere in the late genes and the consequent synthesis of RNA molecules in which early RNA sequences are linked by a stretch of anti-late viral RNA to host RNA sequences? These and many more questions still have to be answered, but that they are now even being asked, which was not the case only a few months ago, gives some indication of the importance of the discovery of strand switching during the transcription of the SV40 genome.—From our Cell Biology Correspondent.