NEWS AND VIEWS

Central Dogma Reversed

THE central dogma, enunciated by Crick in 1958 and the keystone of molecular biology ever since, is likely to prove a considerable over-simplification. That is the heretical but inescapable conclusion stemming from experiments done in the past few months in two laboratories in the United States. For the past twenty years the cardinal tenet of molecular biology has been that the flow or transcription of genetic information from DNA to messenger RNA and then its translation to protein is strictly one way. But on pages 1209 and 1211 of this issue of *Nature*, Baltimore and Mizutani and Temin claim independently that RNA tumour viruses contain an enzyme which uses the viral RNA as a template for the synthesis of DNA and thus reverses the direction of genetic transcription. And at a meeting at the Royal Society last week Spiegelman announced that his group has confirmed the findings of Baltimore and Temin. In the past four weeks he and his colleagues have detected an RNA dependent DNA polymerase in six different RNA tumour viruses.

The discovery of this unprecedented enzyme, which obviously has profound implications for the whole of molecular biology, as well as for the mechanism of cancer induction by RNA viruses, is an extraordinary personal vindication for Dr Howard Temin. If ever a man was in a position to say I told you so, it is he. In 1964 he put forward a hypothesis to explain the induction of cancer by RNA viruses which demands the existence of an enzyme capable of making DNA off RNA templates. Ever since, and until a few weeks ago with only the flimsiest experimental evidence, he has stuck to his guns in the face of widespread scepticism. But his announcement at Houston (*Nature*, 226, 1003; 1970) and Baltimore's announcement at Cold Spring Harbor (*Nature*, **226**, 1093; 1970), which have been so promptly followed by Spiegelman's confirmatory report at the Royal Society, are likely to generate one of the largest bandwagons molecular biology has seen for many a year. That, of course, would be inevitable at any time, but it is especially the case today when cancer is one of the few remaining passwords to the dwindling coffers of the granting agencies in the United States.

But why did Temin ever seriously entertain the idea of inverted transcription? The concept was a crucial part of his attempt to explain the familiar observation that RNA tumour viruses can transform normal cells into genetically stable cancer cells, which for generations continue to yield a small amount of the transforming virus. It is hard to imagine how single stranded RNA molecules—the genomes of the tumour viruses—can be stably integrated into the DNA genomes of the transformed cells. On the other hand, it is equally difficult to imagine how a virus can permanently transform a cell without the viral genome being stably associated with the host cell genome such that every daughter cell receives not only a complete chromosome complement but also copies of the viral genome. Temin's solution to this paradox was to suggest that the viral RNA is first transcribed into a DNA provirus which is then integrated into the DNA of the chromosomes in the host cell.

Until a few weeks ago the evidence supporting this notion was at best circumstantial and equivocal. First, transformation by RNA tumour viruses can be inhibited by compounds which inhibit DNA synthesis. Second, Temin and others claimed, on the basis of inconclusive hybridization experiments, that transformed cells contain more DNA capable of hybridizing with viral RNA than untransformed cells. Finally, the generation of virions in cells transformed by RNA viruses is sensitive to actinomycin D, a drug which specifically inhibits the synthesis of RNA off DNA templates. Of course, none of these observations could be dismissed out of hand, but critics of Temin found it easy enough to offer plausible alternative explanations which obviate the heresy of contradicting the central dogma. The discovery that at least half a dozen RNA tumour viruses contain RNA dependent DNA polymerase, however, has changed all that.

The results reported by Baltimore, Mizutani and Temin and Spiegelman are clearly very preliminary. All that can be said is that the enzyme is associated with the virions and that it needs the RNA of the virus as a template to incorporate the four deoxynucleoside-triphosphates into DNA. It will not polymerize the ribonucleoside-triphosphates into RNA and it is not sensitive to actinomycin D. The few inconsistencies within and between the experiments of Baltimore and Mizutani and Temin are likely to prove trivial. For example, differences as to whether or not it is necessary to treat virions with nonionic detergents to expose the enzyme probably reflect nothing more significant than differences in the integrity of the virions in different batches of virus. The RNA tumour viruses are notoriously fragile and, as Spiegelman said, the requirement for detergent treatment varies from batch to batch of the same virus.

In the next few months the race will be to characterize the enzyme fully and that will depend on isolating it from the virions. Once that has been done it should be possible to answer the key questions. Does the enzyme make single or double stranded DNA or both and is the base sequence of the DNA complementary to that of the viral RNA? Will the enzyme transcribe the whole of the viral RNA? Does it transcribe any RNA molecule or is it specifically dependent on viral RNA templates? Is the enzyme itself coded for by a viral gene and do uninfected eukaryotic cells or bacteria contain similar RNA dependent DNA polymerases?

The answers, whatever they prove to be, are bound to have great significance for cancer research, molecular genetics and even in the long run genetic engineering. If, for example, RNA dependent DNA polymerases prove to be unique to tumour viruses a new approach to cancer chemotherapy will emerge. For it should be possible to find compounds which specifically inhibit the viral enzyme without having any effect on the nucleic acid metabolism of the host cells. On the other hand, if similar enzymes occur in uninfected cells the current shibboleths of molecular biology stemming from the central dogma, which like all dogmas has had a blinkering as well as an inspiring effect, will be due for critical reappraisal.

Advantages of an Antagonist

STUDENTS of nervous transmission should be stimulated to a new burst of enthusiasm by the report by Curtis, Duggan, Felix and Johnston (this issue, page 1222) on antagonism to γ-aminobutyric acid (GABA). This compound, which has emerged during the past few years as an important but puzzling component of the mammalian brain, has turned out to be antagonized by bicuculline, an alkaloid already known for its convulsant effects. This should at last make it possible to find out where and how GABA is working.

The transmission of electrical activity from one neurone to another occurs only at the specialized contact zones known as synapses, and usually involves the release of an intermediary chemical messenger, or transmitter, rather than a simple transfer of electrical current. Electrical activity in the presynaptic nerve evokes an explosive release of chemical transmitter which diffuses across the synaptic gap separating the two neurones, and interacts with specific receptor sites on the surface of the postsynaptic neurone. The released transmitter may either excite the postsynaptic neurone into activity, or inhibit the ability of the postsynaptic neurone to respond to other excitatory inputs.

Most of the transmitters utilized by neurones in the mammalian central nervous system (CNS) remain unidentified, although a small proportion of the synapses in the brain seem to involve noradrenaline and acetylcholine, which are well known as transmitters in the peripheral nervous system.

In particular there has been considerable doubt about the identity of the major inhibitory transmitter substances involved in brain function. Inhibitory transmission plays a crucial part in the information processing functions of the CNS, for it is the continual interplay of inhibitory and excitatory inputs which determines whether an individual neurone is activated. In invertebrates, in which inhibitory transmission may occur in the peripheral nervous system, at least one inhibitory transmitter has been firmly established. Crustacean muscles are innervated by both excitatory and inhibitory nerve fibres, and the transmitter released at inhibitory neuromuscular junctions is γ-aminobutyric acid (GABA) (Otsuka *et al.*, *Proc. US Nat. Acad. Sci.*, **56**, 1110; 1966).

In mammals inhibitory transmission between nerves is largely confined to the CNS, and GABA occurs in relative abundance in the mammalian brain but not in other tissues. During the past few years evidence has accumulated which favours the view that GABA functions as an important inhibitory transmitter in the mammalian CNS. At the same time it has become apparent that GABA is unlikely to be the only inhibitory transmitter. In the spinal cord, for example, inhibitory transmission seems to involve chiefly the amino-acid glycine as the transmitter (Werman *et al.*, *Life Sci.*, **4**, 2075; 1965). The effects of glycine on spinal neurones are blocked by the convulsant alkaloid strychnine, which also blocks various spinal inhibitory nervous pathways (Curtis *et al.*, *Exp. Brain Res.*, **5**, 238; 1968). In other areas of the CNS, notably the cerebral cortex and cerebellum, however, inhibitory synaptic transmission is not sensitive to the actions of strychnine, and GABA has been found to mimic rather precisely the actions of the naturally occurring inhibitory transmitter (Krnjevic and Schwartz, *Exp. Brain Res.*, **3**, 320; 1967; Obata *et al.*, *Exp. Brain Res.*, **3**, 320; 1967). Radioactively labelled GABA and the endogenous amino-acid can be released from the cerebral cortex in response to electrical stimulation of inhibitory synaptic pathways (Mitchell and Srinivasan, *Nature*, **224**, 663; 1969; Iversen *et al.*, *Brit. J. Pharmacol.*, **38**, 452; 1970).

Another important piece of evidence in favour of the GABA hypothesis is presented by Curtis *et al.* on page 1222 of this issue. Until now, no drug has been known which would specifically antagonize the effects of GABA on mammalian neurones. Now the team of neurophysiologists and chemists at Canberra report that the alkaloid bicuculline may be such a tool. Bicuculline, when administered to anaesthetized cats either by local micro-application (electrophoretically discharged from glass microelectrodes) or by intravenous injection, potently blocked the normal inhibitory effects of locally applied GABA on neurones in the cerebral cortex and cerebellum. This effect seems to be quite specific, for the responses of the same neurones to the inhibitory actions of glycine or noradrenaline were unimpaired. Curtis *et al.* were able to show that bicuculline also blocked the effects of stimulating the normal inhibitory synaptic inputs to such neurones, thus adding considerable weight to the view that the inhibitory effects of such pathways are normally produced by a release of GABA. On the other hand, bicuculline failed to block strychnine-sensitive inhibitory synaptic transmission in the spinal cord, where glycine rather than GABA is thought to be the transmitter.