news and views

Interferon, dsRNA and the pleiotypic effector

from Tim Hunt

WHY can't viruses grow in cells that have been exposed to interferon? This deceptively simple question has received almost as many and varied answers as there are workers in the field (see Friedman *Bact. Rev.* **41,** 543; 1977 for a recent account) but it looks as though a consensus is at last emerging. In the past two years viral protein synthesis has been identified as one of the major targets of the antiviral system(s) activated in interferon-treated cells, and while effects on transcription of viral genomes, or on the uncoating or assembly of virions are by no means ruled out (indeed almost certainly occur in some systems), concentration on the defect in protein synthesis has yielded helpful, and rather surprising insights into how interferon works.

Studies of the translation of viral mRNA in cell-free extracts of interferon-treated cells have shown that these cells have at least two modes of defence against the invading organisms, both of them activated by double-stranded RNA (dsRNA), which is probably one substance that informs a cell that it is being attacked by a virus. The dsRNA seems to activate a protein kinase which phosphorylates two polypeptides of molecular weights 67,000 and 35,000 (Lebleu *et al. Proc. natn. Acad. Sci. U.S.A.* **73,** 3107; 1976; Zilberstein *et al. FEBS Lett.* **68,** 119; 1976). Neither of these polypeptides has been identified, but it is likely that the smaller one belongs to the initiation factor eIF-2, that phosphorylation inactivates it leading to an impairment of initiation of protein synthesis. The argument here is not strong, and rests heavily on the results obtained in the reticulocyte lysate, where the evidence for such a defect in eIF-2 being caused by dsRNA is rather compelling (Farrell *et al. Cell* **11,** 187; 1977).

The second thing that happens in a cell extract from interferon-treated cells when dsRNA is added is an enhanced degradation of mRNA (Sen *et al. Nature* **264,** 370; 1976). Activation of this latent endonuclease requires the combined presence of dsRNA and ATP, and it looked as though phosphorylation of an inactive precursor was involved. Such is not the case. It turns out that besides switching on a protein kinase, dsRNA activates an enzyme which synthesises the trinucleotide pppA2′p5′A2′p5′A, and it is this substance which activates the nuclease, which in turn degrades mRNA which in turn stops protein synthesis. As little as 1–10 nanomolar trinucleotide is sufficient to inhibit protein synthesis. The discovery and identification of the substance was made by Ian Kerr and his colleagues at the National Institute of Medical Research, (Kerr & Brown *Proc. natn. Acad. Sci. U.S.A.* **75,** 256–260; 1978) and so far its structure has not been confirmed by others. However, Kerr's characterisation is extremely thorough, including the pattern of degradation by various enzymes, proton and phosphorus NMR spectroscopy, and chemical synthesis. Higher homologues, particularly the tetramer and pentamer are also found and are also active as inhibitors of protein synthesis, but the dimer and the non-triphosphorylated core, ApApA are without activity. Interestingly, both Kerr and Revel's group find that the nuclease which is activated by 'two-five A' (as Kerr calls it) is capable of degrading two-five A, so that the system seems to be self-limiting, in principle.

Synthesis of two-five A is much better in extracts of interferon-treated cells than in control extracts, making it likely that interferon induces the synthesis of 'two-five A synthetase'. Reticulocyte extracts can also make this substance, and respond to it (Hovanessian & Kerr *Eur. J. Biochem.* **84,** 149; 1978; Clemens & Williams, *Cell,* in the press) by degrading globin mRNA. (Reticulocyte lysates so strongly resemble interferon-treated cell extracts in their sensitivity and response to dsRNA that one wonders if the erythrocyte pathway of differentiation depends on exposure to a hormone like interferon at some stage); but it is clear that the phosphorylation of eIF-2 in response to dsRNA is the major reason for inhibition of protein synthesis in reticulocyte lysates. Two-five A takes much longer than dsRNA to inhibit protein synthesis, and does not lead to any defect in initiation factors. Which of these pathways is of greater significance in other cell-free systems, and more pertinently, in intact cells is an open question. It may be that different cells will prove to have different reliances on these two systems, which seem to be quite independent of one another. In both cases it would seem that interferon actually induces a new enzyme, in system (1) a protein kinase, in system (2) the trinucleotide synthetase—one could call these the long-lost 'antiviral proteins' although the question of their specificity remains less than clear.

The other quest that this fascinating finding recalls is Gordon Tomkins's search for the 'pleiotypic effector'. Could it be that two-five A is just that? If it is its discovery and identification is a most important advance in cellular physiology at least as significant as that of cyclic AMP; its astounding potency is going to make its detection very difficult, however, since it is roughly 1,000 times as active! It is too early to say whether there is any truth in this idea, and one does not know whether any other substances besides dsRNA can switch on its synthesis; might other environmental shocks lead to its formation? And might it have other effects besides activation of the nuclease? These are questions which are receiving attention.

Returning to interferon; it seems

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Erratum

In the article '10 nm filaments' (*News and Views* **272,** 577; 1978) line 16 paragraph 2 on page 577 should read '. . . the brain preparation contains a mixture of immunologically distinct neurofilament *and glial filament* chains, both of molecular weight ~50 K . . .'.

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