Cell-free Protein Synthesis and Interferon Action: Protein Kinase(s) and an Oligonucleotide Effector, pppA2′p5′A2′p5′A

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Interferon, or more correctly the interferons, for it is now clear that there are several antigenic types of interferon molecule even within a species, are glycoproteins which are produced by cells in response to virus infection. On exposure to interferon, cells develop an antiviral state in which the replication of a wide spectrum of viruses is inhibited: which is, of course, the basis for our interest in interferon. (A comprehensive account of the interferons is given in [4].)

Human interferon was shown some years ago to alleviate the effects of influenza and the common cold and more recent human clinical trials have shown it to have a marked beneficial effect in the treatment of osteogenic sarcoma, chronic hepatitis and herpetic infections in immunosuppressed patients. The first full double-blind, placebo-controlled human clinical trial in immunosuppressed patients has just been completed and the results are positive: interferon does work [11]. Interferon, therefore, is being and will increasingly be used clinically. It would be good to know a little more about what it does and how it does it.

Interferon is not directly antiviral. Interferon treatment is always of the cell. It is active in very small amounts, probably only a few molecules per cell are required to trigger the response. After interferon treatment it takes several hours for the antiviral state to develop during which time there is a requirement for RNA and protein synthesis. The simplest interpretation of the data is that interferon induces the cell to produce a protein or proteins which are the actual antiviral agents. Over the years the work of a number of groups has indicated that in different cell-virus systems the antiviral affect of interferon may be expressed at the level of viral RNA synthesis, viral protein synthesis, uncoating of the virus, or, in the case of the RNA tumour viruses, at the level of virus maturation or release. Moreover interferon is not an exclusively antiviral agent. It induces changes in the cell membrane and inhibits the growth of many cell lines. It was this antigrowth property, incidentally, rather than any putative viral aetiology that provided the rationale for the osteogenic sarcoma trial ([5, 12] for recent reviews of interferon action).

Against this rather complex background we chose to analyse the clear cut inhibition of protein synthesis seen in certain cell-free protein synthesising systems from interferon–treated mouse L-cells. More particularly, we first became involved with double-stranded RNA (dsRNA) in the interferon system when it seemed possible to us that the inhibition of protein synthesis

that we and others had observed in interferon-treated, vaccinia-virus infected L-cells and cell-free systems was triggered by viral dsRNA. There was, therefore, some sort of logical progression into the work on dsRNA and most important a possible link between the results with dsRNA and events in the interferon-treated, virus-infected cell and cell-free system.

In accord with this, some years ago, we observed that protein synthesis in cell-free systems from interferon-treated cells shows an enhanced sensitivity to inhibition by dsRNA [8]. Most importantly, this enhanced sensitivity has exactly the same dose response curve as the antiviral effect in the intact cell. In other words as soon as you begin to pick up an antiviral effect in the intact cell you can detect the enhanced sensitivity to dsRNA in the cell-free system [8]. It was of interest, therefore, to determine the basis for this latter phenomenon.

To cut a long story short when one incubates an extract from interferon-treated cells with dsRNA and ATP one activates a protein kinase (s) which, by analogy with the work of a number of groups with rabbit reticulocyte lysates ([3] for example), is thought to phosphorylate one of the initiation factors (elF-2) involved in the initiation of protein synthesis. This interferon mediated kinase has been observed by a number of groups working in mouse, human and chick cell systems ([10, 14, 15] L. A. Ball personal communication) and it is impossible to overemphasize its potential significance in mediating the multiplicity of effects attributed to interferon. The difficulty, as always with a kinase, is to know which phosphorylations to look for and the significance of those observed. For example, the interferon mediated kinase also phosphorylates histones [14] and it is obviously tempting to speculate that this may have some physiological significance in the effects of interferon on transcription and cell growth. Meanwhile it is reasonable to assume that the kinase does indeed phosphorylate elF2 and that this plays a part in the inhibition of protein synthesis observed. It may not, however, be the only or even the major factor involved, for when one incubates interferon-treated cell extracts with dsRNA and ATP one also activates an enzyme (2–5 A synthetase) responsible for the synthesis of the unusual oligonucleotide pppA2′p5′A2′p5′A (which for convenience will be referred to as 2–5 A) which is effective at subnanomolar concentrations in the inhibition of protein synthesis in cell-free systems.

Interestingly both the 2–5 A synthetase and the kinase will bind to a column of dsRNA itself bound to sepharose. Accordingly we've used this property to purify and fractionate these enzymes and the way is now open for an investigation of the effect of each individually on protein synthesis in the cell-free system. Meanwhile we have used the 2–5 A synthetase in its stable column-bound state to synthesize and radioactively label the 2–5 A with α- or γ^{32}P- or ^{3}H-ATP [6, 9]. An analysis of the radioactive inhibitor yielded the structure pppA2′p5′A2′p5′A in which the 2′–5′ linkage is unusual not having been reported previously for biologically synthesized material [9].

Here, therefore, we have an inhibitor of a type not previously described. Furthermore, it is not unique to the interferon-treated mouse L-cell-free system. What is almost certainly the same inhibitor is also synthesized by an

enzyme fraction from interferon-treated chick cells and, interestingly in this system the 2–5A synthetase has also been detected in smaller amounts in control cell extracts [1]. In addition, from our own work we know that the 2–5A system also operates in rabbit reticulocyte lysates [7]. The 2–5A preparations from rabbit reticulocyte lysates and interferon-treated mouse L-cell systems have the same specific biological activity in the inhibition of protein synthesis in cell-free systems [6,7]. Moreover, preparations from both sources have now been subjected in parallel to the same detailed analysis as we have already published for the 2–5A from interferon-treated cell extracts and it is beyond reasonable doubt that they are the same.

The availability of reticulocyte derived material on a larger scale has enabled us to carry out proton and phosphorus NMR analyses of the purified 2–5A. Although these studies are not yet complete sufficient has been done to confirm the major aspects of the structure proposed. Ultimate proof of this structure will come from chemical synthesis. This is in process and preliminary results suggest that chemically synthesized 2–5A shows the same specific biological activity as the biologically synthesized material.

How then does 2–5A work? Its high potency (it is effective at subnanomolar concentrations in the cell-free system) combined with the time lag in the development of the response to it, suggests that it is not directly inhibitory but that it, in turn, sets in train a sequence of events leading to the observed inhibition [6,7,9]. Indeed it is now clear from our own work and that of a number of other groups that at least one of the things that it does is to activate a nuclease ([2,13] C. Baglioni, L.A. Ball, M. Revel, C.E. Samuel and their respective collaborators, personal communication).

Interestingly the 2–5A, the nuclease and the inhibition of protein synthesis are all unstable. On addition of exogenous 2–5A to cell-free systems a nuclease is activated and protein synthesis is inhibited. Meanwhile the 2–5A is degraded, nuclease activity diminishes in parallel and, in the absence of a 2–5A regenerating system, protein synthesis will resume on freshly added mRNA as efficiently as in corresponding controls. The enzyme which degrades 2–5A appears to have activity similar to that of snake venom phosphodiesterase yielding AMP or ATP as the products of digestion (L.A. Ball, B.R.G.W. and I.M.K., unpublished data, M. Revel personal communication). It is possible that it is the induced nuclease which carries out this degradation providing a self-regulatory feed-back mechanism whereby the 2–5A activates the nuclease responsible for its own destruction, but this remains to be established. What is clear, however, is that cells have a mechanism (s) for coping with 2–5A and the components interacting with 2–5A all appear subject to cyclic activation and deactivation, which is exactly what one might expect for a delicately balanced control system. Interferon pretreatment of the cell, incidentally, appears mainly, if not exclusively, to enhance the synthesis of 2–5A, its activity and fate are essentially identical in interferon-treated and control cell extracts.

We routinely synthesize and assay 2–5A in the cell-free system. It is also active, however, in intact cells. Although one requires more of it than in the cell-free system, the 2–5A is still potent, a concentration of just over 10 nM

being sufficient to bring about a 50% inhibition of protein synthesis in suitably permeabilised cells.

Finally, I would like to emphasize that the interferon mediated sensitivity to dsRNA is an enhanced sensitivity. This, the potency of the 2–5 A and the fact that the 2–5 A synthetase is found in a variety of both interferon-treated and control systems makes it very tempting to speculate that, in addition to any role that it may play in the antiviral action of interferon, the 2–5 A system (with or without enhancement by interferon) may be involved in the regulation of normal cell growth or development.

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