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The Mechanism of Interferon Production [and Discussion]

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The mechanism of interferon production

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Interferons are formed when most cells are treated with viruses or double-stranded RNA (to form IFN-α or β, or both) or when lymphoid cells are treated with mitogens or the appropriate antigen (to form IFN-γ). Interferon-α and β are formed as a result of transcription of cellular genes – probably in response to double-stranded RNA in the cytoplasm. The process can be controlled at three levels. (1) In mouse teratocarcinoma stem cells or early mouse embryos the interferon system is inaccessible and only becomes inducible as differentiation proceeds. (2) The target(s) responding to double-stranded RNA probably involve sequences upstream from the 5′ end of the interferon genes, sequences now becoming accessible by gene cloning. (3) Levels of interferon mRNA can be regulated either by an increased rate of transcription or by an increased half-life of the mRNA.

Introduction

Interferon (IFN) was discovered when Isaacs & Lindenmann (1957) treated chick chorioallantoic membranes with heat-inactivated influenza virus. Cells of other species, for example mouse and human, were also found to produce interferons, and since these interferons were often only active on the homologous cells, it was clear that they must be different from each other. There is thus a species diversity. Moreover, cells of one species can produce more than one type of interferon and I shall refer to this as cellular diversity. For example, human cells can produce either IFN-α (formerly known as leucocyte interferon), IFN-β (formerly known as fibroblast interferon) or IFN-γ (formerly known as type II or immune interferon) depending on the cell treated and the inducing agent used. Finally there is a sequence diversity, for it has recently been found that there is not one, but a substantial number (18 at the present count) of human α genes, which have related but distinct DNA sequences.

All interferons are proteins formed as a result of the activation of cellular genes but, when human IFN-α, β and γ are compared, it is evident that IFN-α and IFN-β are more similar to each other than they are to IFN-γ (see table 1).

IFN-α and IFN-β are produced by most human cells either *in vivo* or in tissue culture, while IFN-γ is only produced by cells of the lymphoid system. The molecular biology of the production of IFN-α and IFN-β has been studied for some years, while that of IFN-γ has scarcely begun. This article will therefore be limited to the mechanism of production of IFN-α and IFN-β.

When cells are treated with viruses or double-stranded RNA they produce either IFN-α or IFN-β, or a mixture of the two. With a few exceptions, no interferon is produced until the cells are treated with the inducer, and interferon production ceases a few hours after it has started. The system can therefore be turned on and off, and it is natural to ask about the mechanisms involved, since they may be of general applicability to the control of gene expression. A number of questions suggest themselves:

(1) What is the nature of the proximal inducer?
(2) How are the interferon genes switched on and off?
(3) Is there any transcriptional control?
(4) Is there any translational control?

Generation of the proximal inducer

The basic question is how the treatment of cells with agents like viruses or double-stranded RNAs elicits the transcription of a silent cellular gene. Three lines of evidence suggest that they operate through a common mechanism involving double-stranded RNA as an essential intermediate.

Table 1. Similarities and differences between IFN-α, β, γ

	IFN-α	IFN-β	IFN-γ
treatment at pH 2	stable	stable	unstable
induced by viruses or double-stranded RNA	yes	yes	no
induced by mitogens	no	no	yes
related gene sequences	yes	yes	no
introns in the gene	no	no	yes
same cellular receptor	yes	yes	no

First, a number of non-infective viruses can induce interferon, and all of them can induce the formation of double-stranded RNA. Early studies showed that heat and u.v.-inactivated myxo- and paramyxoviruses could induce IFN, even though they were non-infectious (Burke & Isaacs 1958). However, these observations were uninterpretable until it was discovered that these viruses contained a transcriptase, that non-infectious virus was capable of directing RNA synthesis and that the ability to make interferon correlated with such synthesis (Clavell & Bratt 1971; Meager & Burke 1972; Sheaff *et al.* 1972). This suggested that some RNA synthesis was needed for induction. Temperature-sensitive (ts) mutants of the alphaviruses, Sindbis and Semliki Forest viruses, were also used as interferon inducers. These viruses do not contain a transcriptase, and inactivation causes a loss of IFN-producing activity (Skehel & Burke 1968*a*), while the use of a temperature-shift system showed a requirement for RNA synthesis (Skehel & Burke 1968*b*). Use of ts mutants as inducers showed that RNA^+ mutants induced IFN formation at the non-permissive temperature, while RNA^- mutants did not, again pointing to a correlation between RNA synthesis and IFN induction (Lomniczi & Burke 1970). Some studies have failed to detect such a correlation between interferon production and double-stranded RNA formation (Lockart *et al.* 1968; Atkins & Lancashire 1976), possibly because of the difficulty of detecting very small amounts of double-stranded RNA, or because of leak or reversion of the ts mutants.

Second, both natural and synthetic double-stranded RNA can induce IFN. Reovirus, which contains ten pieces of double-stranded RNA as the genome, is an effective IFN inducer, even in the absence of virus RNA synthesis (Long & Burke 1971). In addition, a wide range of synthetic (e.g. poly(rI)·poly(rC)) or natural (e.g. reovirus) double-stranded RNAs are effective inducers. However, despite considerable effort, it has not so far been proved that double-stranded RNA has to enter the cell to induce (for a review see Pitha & Hutchinson 1977).

Third, the evidence for double-stranded (ds) RNA as the inducer has been considerably

strengthened by a series of papers by Marcus and his colleagues. They showed first that a preparation of DI particles of vesicular stomatatis virus (VSV) which contained covalently linked complementary [+] message and [−] anti-message RNA as a single-stranded ribonucleoprotein complex within the particle, was an extremely efficient inducer of IFN (Marcus & Sekelleck 1977). Conventional DI particles with the same polypeptide composition but containing [−] strand RNA failed to induce, and it was concluded that the inducer was a molecule of ds RNA formed by snap-back of the DI RNA. Since one particle of VSV DI, and hence one molecule of ds RNA, is sufficient to produce IFN, it is not surprising that earlier studies had encountered considerable difficulties when biochemical methods were used to decide whether ds RNA was formed or not. Marcus & Sekelleck (1980) later showed by use of ts mutants of VSV that primary transcription was necessary, and by use of u.v.-irradiation that about 10% of the genome must be intact, such partial transcription leading to the production of ds RNA. Using ts mutants of Sindbis virus, the same group showed that IFN production could be initiated by three RNA^+ and two RNA^- complementation groups at the non-permissive temperature but not by two other RNA^- complementation groups (Marcus & Fuller 1979). They concluded that these two genes had to be functional to synthesize the proximal inducer, and argued that these genes coded for a transcriptase necessary to form ds RNA from the virus RNA. A similar conclusion was reached by use of early passage DI virus (Fuller & Marcus 1980). Finally, an avian reovirus proved to be a very potent IFN inducer, especially after a small dose of u.v. irradiation (Winship & Marcus 1980). They concluded that the inducer was ds RNA and that the critical event was its release into the cytoplasm.

But is ds RNA the proximal inducer? That is, does it interact with the genome directly or does it produce some other substance, as yet unidentified, that interacts with the genome? We know that ds RNA can produce such substances (2,5A) in interferon-treated cells, but what about in normal cells? We do not know, and this is a neglected area of research.

Stewart (1979, pp. 74–76) has distinguished three possible mechanisms by which the proximal inducer could interact with the genome: (1) that induction is due to the proximal inducer directly activating the promoters of the IFN genes; (2) that, as suggested by Tan & Berthold (1977), all inducers owe their activity to their capacity to inhibit the synthesis of a rapidly turning-over repressor that normally represses the IFN genes; (iii) that the repressor is IFN itself, which will certainly bind to polynucleotides (De Mayer-Guignard *et al.* 1977). We have no experimental evidence to decide between these and other ideas.

How are the interferon genes switched on and off?

Either directly or indirectly, ds RNA initiates transcription of the IFN-α and β genes, while some analogous process leads to transcription of the IFN-γ gene. Some hours later transcription ceases, the interferon mRNA decays and no more interferon is made. The cell is then refractory to further induction for some time. What is the molecular basis of these processes?

First it is useful to distinguish three distinct operational states of the interferon gene system:

(1) the system may be switched off or inaccessible to interferon inducers;
(2) the system may be inducible;
(3) the system may be transcribing actively.

The interferon system is uninducible in mouse teratocarcinoma stem cells, early mouse embryos and in cells that have ceased interferon production, although we do not know whether

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the control mechanisms are the same in all three cases. Burke *et al.* (1978) found that mouse teratocarcinoma stem cells, which are transformed stem cells, neither produced nor responded to interferon. However, as the cells differentiate they become capable of making interferon when treated with an inducer, and also become susceptible to its effect. This effect has been found with several lines of mouse teratocarcinoma cells (Wood & Hovanessian 1979; Nilsen *et al.* 1980), and the increase in interferon yield per culture as the cells differentiate has been shown to be because an increasing proportion of the cell populations produce about the same amount of interferon rather than because a very small proportion of the cells produce increasing amounts of interferon (Barlow *et al.* 1982).

The differentiation of teratocarcinoma cells in culture has been widely studied as a model for development of the early embryo, and we have therefore developed a single-cell assay for interferon so as to be able to measure whether or not very small pieces of early mouse embryos could produce interferon (Barlow *et al.* 1982). The result was clearcut: mouse embryos did not produce interferon after exposure to an inducer until 7 days after fertilization, and then it was seen first in the outermost cell layers. As the embryos developed further, inner layers of cells developed this capacity, all tissues of the embryo becoming positive by 8 days. We do not know the biological significance of this change. Is there any connection between this change about one-third of the way through mouse pregnancy, and the well known susceptibility of humans to rubella in the first trimester? Possibly interferon fails to protect the embryo from infection by rubella, or, more speculatively, interferon is not produced because of damaging effects on the course of differentiation?

However, most mammalian and avian cells can produce interferon when treated with an appropriate inducer, and the few that cannot, such as Chinese hamster ovary cells, do so when fused with a different cell to form a cell hybrid, showing that the gene is present but repressed. The genes for human IFN-α, β and γ have been cloned, first as copy DNA (cDNA) clones from the mRNA, and then as genomic clones, by using the cDNA clones to scan a genomic library. Eukaryotic genes all possess a number of common sequences upstream from the 5′ end of the gene, and the interferon genes also have these. In addition they are expected to have an inducible promoter sequence. Such control sequences have been found by Brinster *et al.* (1982) to lie between 50 and 90 nucleotides upstream from the inducible metallothionein gene, and by R. Axel *et al.* (personal communication) to lie within 150 nucleotides of the 5′ end of the heat shock gene. Several groups are now searching for similar control sequences for the interferon genes. There are presumably similar but distinct sequences for the α and β genes because human cells may produce one or the other, or a mixture, depending on the inducer. There must be some link between the two gene systems since J. Shuttleworth & J. Morser (unpublished) found that, in Namalwa lymphoblastoid cells, the α and β gene systems are coordinately controlled. There may also be separate control sequences for the members of the α gene family whose products make up the mixtures known as leucocyte or lymphoblastoid interferon.

Finally the gene may be 'on', producing IFN mRNA for translation and secretion. There have been no investigations of the changes accompanying this initiation of transcription nor do we know how the gene is switched off after interferon production ceases. However, it is possible to measure the amount of IFN mRNA present in a cell either by injection of the cellular RNA into *Xenopus* oocytes, when interferon is formed and secreted from the oocytes, or by hybridization with cloned IFN genes. Both these procedures can be used to show the presence of transcriptional control.

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Is there any transcriptional control?

Transcription leads to the production of IFN mRNA, which is transported into the cytoplasm (Burke & Veomett 1977) before translation. The level of IFN mRNA may be modulated in several ways.

First, there is kinetic control of IFN mRNA. Shuttleworth & Morser (unpublished) have measured the amount of IFN and also of IFN-α and IFN-β mRNA at different times after induction of Namalwa cells with Sendai virus. They found that the amount of both α and β mRNA increased in the cells with very similar kinetics, and then started to fall just before the time of maximum interferon yield, also with similar kinetics. Clearly there is some coordinate temporal control of the transcription of the two IFN genes.

Second, the rate of transcription of the IFN gene can be increased, so that more mRNA is produced, although it has the same half-life. This happens when Namalwa cells are pretreated with butyrate or 5-bromodeoxyuridine (Morser *et al.* 1980; Shuttleworth *et al.* 1982) and leads to a real increase in the amount of IFN-α produced as measured by the immunoradiometric assay (Secher 1981). However, it is not possible, by using either injection into *Xenopus* oocytes or nucleic acid hybridization, to distinguish between an increase in IFN mRNA due to an increase in polymerase molecules per gene, or to an increase in the number of IFN-α genes that are transcribing.

Third, the half-life of the IFN mRNA may be increased. This appears to be the explanation of the increase in yield observed when induced Namalwa cells are incubated at lower temperatures some hours after induction (Morser & Shuttleworth 1981), and also of the superinduction phenomenon (B. Raj & P. M. Pitha, personal communication 1982). Use of cell hybrids has shown that both priming and superinduction are under dominant control in mouse–human somatic cell hybrids (Graves & Meager 1980), although neither the nature of the control nor the chromosomal location of the gene(s) responsible has been elucidated.

Is there any translational control?

There is some evidence for translational control, for when Namalwa cells are treated with butyrate or 5-bromodeoxyuridine the increase in interferon yields is greater than the increase in mRNA levels (Shuttleworth *et al.* 1982). The evidence suggested, but did not prove, that the increased efficiency is due to an increased rate of elongation or termination or both, or to a larger number of ribosomes on each IFN mRNA molecule. The use of metabolic inhibitors has produced evidence for intracellular proteolytic processing, presumably the removal of the signal sequence (Morser & Colman 1980).

Interferon mRNA may be translated in heterologous cells, cell-free translation systems or best by injection into *Xenopus* oocytes (for a review see Stewart 1979, pp. 90–96). Cell-free systems translate human IFN mRNA poorly, if at all, and this may be because of the need for subsequent processing. When IFN mRNA is translated in *Xenopus* oocytes, the product is secreted (Colman & Morser 1979). Further work showed that all secretable proteins are secreted from the oocyte after translation of the injected mRNA, and the oocyte has been used as a surrogate system to study the secretion of newly synthesized proteins.

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Discussion

It was agreed that the failure of embryonic and carcinoma cells to produce interferon could be due to the failure to produce its proximal inducer, although some RNA viruses can multiply satisfactorily in these cells, and can presumably produce double-stranded RNA. Thus, if the proximal inducer was not double-stranded RNA but some further product, embryonic cells may be unable to produce this substance. In discussion, it was pointed out that the fact that interferon did not inhibit its own synthesis, but often stimulated it as in the priming reaction, implied that the switch-off of interferon mRNA synthesis was probably not due to an effect of the interferon produced by the cells.

In reply to another question, Professor Burke said that there was no evidence to show that interferon production was dependent on the stages of the cell cycle.

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