REVIEW ARTICLE

Regulation of cell proliferation and differentiation by interferons

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Introduction

The interferons are a widely studied group of proteins which were first identified by their ability to protect cells against virus infections (Stewart, 1979). They are synthesized and secreted by a variety of cell types in response to several classes of inducers (notably virus infections) and exert their effects *in vivo* as a result of interaction with other cells in distant parts of the body. In this sense the interferons are analogous to the polypeptide hormones. A functional similarity with hormones is further emphasized by the recent identification in target cells of specific membrane receptors for interferons and by the multiplicity of biological effects which result from the interactions of the interferons with these receptors.

In recent years it has become clear that the interferons are capable of influencing cellular physiology and behaviour in a number of ways. Their effects include inhibition of cell growth and proliferation, regulation of the expression of specific genes, modulation of cell differentiation, and activation of certain cell types in the immune system (e.g. macrophages and natural killer cells). The mechanisms underlying the antiviral actions of the interferons have been widely studied and frequently reviewed (Baglioni, 1979; Hovanessian, 1979; Lengyel, 1982; Sen, 1982). In contrast, information concerning the effects of interferons on cell proliferation and differentiation has only been obtained during the last decade, as our understanding of mechanisms of growth regulation and gene expression in eukaryotes in general has increased. This review aims to summarize the current situation regarding these actions of the interferons and suggests areas in which our perception of such control of cell function may be expected to increase.

Abbreviations used: IFN, interferon; Hu-IFN, human interferon; Mu-IFN, mouse interferon; dsRNA, double-stranded RNA; 2'5' (A)_n, pppA(2'p5'A)_n where *n* = 2 or more; EBV, Epstein-Barr virus; SV40, simian virus 40; MEL, murine erythroleukaemia.

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There are multiple types of interferon which can be distinguished biochemically, immunologically and, in some cases, functionally. They represent a family of more or less related gene products with molecular masses in the range of 15000–30000 Da; amino acid sequences and the structures of the genes coding for the different proteins have been best characterized in the case of the human interferons. The current system of nomenclature distinguishes three classes: α , β and γ interferons. The α and β types are both synthesized in response to virus infections and correspond to the older classifications of 'leucocyte' and 'fibroblast' interferons respectively, defining the major cell types which produce them. These classes were formerly also known as 'Type I' interferons. In contrast, IFN- γ is produced by T lymphocytes following mitogenic or antigenic stimulation. It was thus formerly known as 'immune' or 'Type II' interferon. In humans there are many species of IFN- α (more than 12), but probably only one IFN- β and one IFN- γ . The IFN- α species and IFN- β are more closely related to each other, structurally and functionally, than they are to IFN- γ .

Antiviral mechanisms

It is now recognized that there is no single mechanism which is responsible for impairment of viral replication in all interferon-treated cells. The relative importance of the different pathways which have been identified depends on the nature of the virus and probably also on the cell type infected. Viral protein synthesis is a major target for inhibition and this can be affected in several ways (Table 1). Interferon treatment of cells induces two enzymes of importance in the regulation of protein synthesis: a protein kinase (Sen *et al.*, 1978; Kimchi *et al.*, 1979a,b; West & Baglioni, 1979; Ohtsuki & Baron, 1979) and 2',5'-oligo(adenylate) synthetase (Kimchi *et al.*, 1979a; Hovanessian & Kerr, 1979; Ball, 1979; Dougherty *et al.*, 1980). Although both these enzymes are synthesized in uninfected cells, they are dependent on double-stranded RNA for their activity (Farrell *et al.*, 1978); it is likely that infection triggers their

activation by virtue of the dsRNA synthesized as an intermediate in the replication of many RNA and DNA viruses or present in the virions of others (e.g. reovirus) (Fig. 1). The protein kinase phosphorylates the smallest (α) subunit of protein synthesis initiation factor eIF-2 (Samuel, 1979) and thereby prevents this factor from recycling between successive rounds of translation (Siekierka *et al.*, 1982, 1984; Pain & Clemens, 1983). The 2',5' (A)_n

synthetase polymerizes ATP into a series of 2',5' phosphodiester bond-linked oligomers with a triphosphate group at the 5' end. These oligoadenylates are allosteric activators of an endogenous endoribonuclease (RNAase L) which can degrade both mRNAs and rRNAs (Kerr & Brown, 1978; Clemens & Williams, 1978; Slattery *et al.*, 1979; Wreschner *et al.*, 1981).

In addition to these antiviral mechanisms, other

Fig. 1. Two mechanisms for the inhibition of protein synthesis in interferon-treated cells

Interferons bind to cell surface receptor(s), as a result of which intracellular signals (of unknown nature) are generated. The effect of these signals is to induce within a few hours the synthesis of two enzymes, 2',5'-oligoadenylate synthetase and a protein kinase, probably by stimulation of transcription of their respective genes. Both enzymes require dsRNA for their activity. Large amounts of dsRNA can be generated during the replicative cycle of several types of RNA and DNA viruses and small regions of double-stranded structure may also be present in cellular RNA molecules. When activated, 2',5'-oligoadenylate synthetase converts ATP into compounds of the 2',5'(A)_n series which in turn activate ribonuclease L to degrade mRNA and rRNA in ribosomes. The protein kinase phosphorylates the α subunit of initiation factor eIF-2, thereby inhibiting the ability of the factor to catalyse the initiation of protein synthesis.

possible modes of inhibition in some infected cell types include interferon-induced impairment of 5'-methylation of newly synthesized mRNA (Sen *et al.*, 1977; de Ferra & Baglioni, 1983) and inactivation of the amino acid accepting ability of certain tRNA species (Zilberstein *et al.*, 1976) (Table 1). These effects are not dependent on dsRNA. In the case of productive infections by retroviruses a late step in the maturation of the viral particles, perhaps involving the cell surface, is blocked (Chang *et al.*, 1977) (Table 1). The interferons are also inhibitors of the replication of some DNA tumour viruses. In SV40-infected cells there is a clear discrimination between viral and cellular translation (Yakobson *et al.*, 1977), the basis for which has not been established.

Interferon receptors

The best characterized interferon receptor is that for IFN- α 2 in the human lymphoblastoid cell line, Daudi. This cell type is very sensitive to the growth inhibitory effects of IFN- α and IFN- β , but is not sensitive to IFN- γ (Tomita *et al.*, 1982). The receptor is a membrane protein of M_r 95 000–120 000 (Faltynek *et al.*, 1983) with a dissociation constant for IFN- α 2 in the range of 2.6×10^{-12} – 1×10^{-11} M (corresponding to concentrations of about 10–40 International Reference Units of interferon/ml) (Mogensen & Bandu, 1983). The latter values are identical with the physiological

concentrations of IFN- α needed to inhibit the proliferation of Daudi cells.

The absence of an effect of IFN- γ on Daudi cells suggests that IFN- γ requires a receptor which is different from that binding IFN- α or β . This conclusion is supported by the results of competition binding experiments (Branca & Baglioni, 1981). On the other hand, it appears that the IFN- α and - β species recognize a common receptor. This is consistent with the greater structural similarities between IFN- α and IFN- β than between these molecules and IFN- γ .

The immediate molecular events following interactions of interferons with their receptors are not known in detail. There have been reports of transient elevation of the concentration of cyclic GMP, occurring within 1–5 min of interferon addition to L1210 or Daudi cells (Tovey & Rochette-Egly, 1981; Rochette-Egly & Tovey, 1982), and of changes in protein phosphorylation within 2 h in human bladder carcinoma cells (Soslau *et al.*, 1984). The intracellular concentration of cyclic AMP is elevated by interferon treatment of several cell types (Fuse & Kuwata, 1978; Degré & Glasgow, 1981; Panniers & Clemens, 1981; Schneck *et al.*, 1982), although the opposite effect (with an inhibition of adenylate cyclase activity) has been reported in one study (Banerjee *et al.*, 1982). Interferon-receptor complexes may be internalized and the bound interferon degraded over the subsequent few hours

Table 1. *Major mechanisms of antiviral actions of interferons*

Pathway and typical cell types	Dependence on dsRNA	Effect	Typical viruses affected
2',5'-Oligo(adenylate) synthetase/RNAase L (L cells, Ehrlich ascites, chick embryo, HeLa cells, fibroblasts)	Yes	Degradation of mRNA and rRNA	Encephalomyocarditis virus Mengo virus Reovirus Vesicular stomatitis virus
dsRNA-dependent protein kinase (L cells, Ehrlich ascites, human amnion, HeLa cells, fibroblasts)	Yes	Phosphorylation of initiation factor eIF-2	Mengo virus Reovirus Vesicular stomatitis virus
Inhibition of methylation of new mRNA (HeLa cells, Ehrlich ascites)	No	Reduced efficiency for initiation of translation	Reovirus Vesicular stomatitis virus
Phosphodiesterase (L cells)	No	Impairment of amino acid accepting ability of -CCA terminus of tRNA	(Significance <i>in vivo</i> uncertain)
Translation of viral mRNAs (monkey cells)	No	Specific inhibition of viral (but not cellular) mRNA translation	Simian virus 40
Virus maturation and budding (AKR mouse leukaemia)	No	Inhibition of virus assembly and release	Murine leukaemia virus

(Branca *et al.*, 1983). However, the significance of these effects in signalling the antiviral, antiproliferative or other actions of the interferons remains to be determined. It is to be expected that this will be an area of rapid growth in the interferon field in the near future.

Cell growth regulation by interferons

Soon after the characterization of 'interferon' as an agent with antiviral activity it was recognized that the proliferation of some cell types in culture is inhibited following their exposure to crude preparations of this material (Paucker *et al.*, 1962). Furthermore, mice inoculated with tumour cells showed a slower rate of tumour growth and increased survival if they were simultaneously given mouse interferon (Gresser & Bourali, 1970). Several reviews covering the earlier literature on antitumour effects of interferons have been published (Gresser, 1972; Gresser & Tovey, 1978; Balkwill, 1979; Taylor-Papadimitriou, 1980).

These experimental findings led to the exciting prospect of clinical use of human interferons in cancer therapy. Data from clinical trials using IFN- α or IFN- β treatment of a limited range of types of tumour are incomplete, although the preliminary results are not as encouraging as might have been expected (Priestman, 1983). Nevertheless, some human tumours appear to respond quite well to interferon therapy (see, for example, Quesada *et al.*, 1984).

Contrary to earlier worries that the antiproliferative effects of interferon preparations were due to impurities, it is now clear that these properties are characteristics of the same molecules that promote antiviral activity. Interferons purified to homogeneity by standard procedures of protein fractionation (Knight, 1976; Iwakura *et al.*, 1978) or synthesized in bacteria by recombinant DNA methods (Evinger *et al.*, 1981) have pronounced cell growth inhibitory effects. Genetically engineered interferon species can be comparable in their activity with mixtures of the naturally produced proteins (Taylor-Papadimitriou *et al.*, 1982). It should be noted, however, that the relative ratios of antiproliferative/antiviral activity for different bacterially synthesized interferon species can vary over a wide range (Rehberg *et al.*, 1982) suggesting different molecular mechanisms for the two types of effect.

There are several experimental models for studying the cell growth inhibitory effects of interferons, each with its own advantages and disadvantages. In addition to the treatment of animals carrying a variety of tumours (spontaneous, chemically induced, virally induced or arising from the growth of cells inoculated into the host),

administration of interferons to immunodeficient nude mice carrying tumours from other animal species has provided valuable information (Taylor-Papadimitriou *et al.*, 1982). Most studies on cell growth regulation by the interferons, however, have been carried out on cells grown in tissue culture. A wide range of cell types has been used, including normal cells, permanent lines of non-tumourigenic cells such as 3T3 mouse fibroblasts and transformed tumour cells of a variety of histological types.

Cells in culture show widely varying degrees of sensitivity to the growth-inhibiting properties of interferons. Furthermore, from sensitive cell types lines can be selected which are resistant to the effect of interferons (e.g. Brouty-Boyé *et al.*, 1973; Kuwata *et al.*, 1976; Dron & Tovey, 1983). Experimental conditions may also influence sensitivity. Inadvertent growth suppression due to other factors, such as in cells which have grown to confluence, cells which have differentiated or cells lacking nutrients, may limit the apparent ability of interferons to inhibit growth.

There is no absolute tissue specificity of the different interferon classes. Hu-IFN- β is somewhat more effective than Hu-IFN- α in inhibiting the growth of osteosarcoma cell lines (Einhorn & Strander, 1977) whereas lymphoid and leukaemic cell lines are relatively more sensitive to Hu-IFN- α (Einhorn & Strander, 1977; Chadha & Srivastava, 1981). The Daudi line of Burkitt's lymphoma-derived B lymphoblastoid cells is particularly sensitive to Hu-IFN- α (Hilfenhaus *et al.*, 1976; Borden *et al.*, 1982). It is of interest that IFN- γ is a more potent inhibitor of cell growth (relative to its antiviral effects) than either IFN- α or - β (Crane *et al.*, 1978; Rubin & Gupta, 1980b; Fleischmann, 1982). Furthermore, there is evidence for a synergistic potentiation of anticellular activity between Mu-IFN- γ and Mu-IFN- α or - β (Fleischmann, 1982), suggesting that these classes of interferon may have different mechanisms of action.

A number of studies have attempted to determine the relative sensitivities of normal versus tumour cells to the antiproliferative effects of interferons. Cultured normal human T cells and B cells proved resistant to high concentrations of Hu-IFN, in contrast to lines of myeloma, Burkitt's lymphoma and leukaemic T cells (Attallah *et al.*, 1980). Similarly, normal bone marrow myeloid progenitor cells growing in soft agar were more resistant to recombinant Hu-IFN- α species than were two leukaemic cell lines (Grant *et al.*, 1982). It is clear, however, that interferons can inhibit the proliferative activity of some normal cells, such as human breast fibroblasts and epithelial cells (Balkwill *et al.*, 1978) and lymphocytes stimulated

by mitogens (Lindahl-Magnusson *et al.*, 1972; Weinstein *et al.*, 1977; Gurari-Rotman *et al.*, 1978). Equally, some tumour cells are totally resistant to high concentrations of interferons (Adams *et al.*, 1975; Hilfenhaus *et al.*, 1977).

Cell cycle changes in interferon-treated cells

It appears that no single stage in the cell cycle is uniquely sensitive to interferon action. The results summarized in Table 2 are more likely to reflect differences in the responses of different cell types to conditions which restrict growth and proliferation than they are to reveal the specific biochemical basis of interferon action.

In cells which have the ability to enter a quiescent G_0 state under adverse growth conditions, interferon treatment lowers their probability of exiting from this phase into the cell cycle (Sokawa *et al.*, 1977; Fuse & Kuwata, 1977; Lundgren *et al.*, 1979; Creasey *et al.*, 1980, 1981). The G_1 phase is also prolonged in actively proliferating cells following interferon treatment (Table 2). In other types of cells, particularly transformed cells, other phases of the cycle are prolonged. It has been observed that the time required for cells to carry out DNA replication (i.e. the length of S phase) can be increased (Creasey *et al.*, 1980; Panniers & Clemens, 1981) and some cell types even accumulate in S phase, having failed to complete DNA synthesis after interferon treatment (Lundblad & Lundgren, 1981). Delay in progression through G_2 has also been noted and, in some cases, all phases of the cell cycle appear to be

prolonged in response to interferons (Table 2). Some cell types are able to replicate their DNA and subsequently undergo nuclear division but cannot carry out cytokinesis. This results in accumulation of large binucleate (or even multinucleate) cells with elevated DNA and protein contents (Pfeffer *et al.*, 1979, 1982; Panniers & Clemens, 1981). Cells are sensitive to interferon treatment in all phases of the cycle and can respond whether they are quiescent or growing exponentially at the time of initiation of treatment (Leanderson & Lundgren, 1982).

It is valid to regard the interferons as naturally occurring negative growth factors which may be important in restricting cell proliferation *in vivo*. In accord with this view, interferons can antagonize cellular responses to positive effectors of cell proliferation. The stimulation of quiescent cells into growth by serum is inhibited by interferon treatment (Sokawa *et al.*, 1977; Balkwill & Taylor-Papadimitriou, 1978; Lundgren *et al.*, 1979) and the effects of individual growth factors can also be blocked (Lin *et al.*, 1980; Taylor-Papadimitriou *et al.*, 1981). In the case of 3T3 cells, changes were observed in the dose-response relationship for inhibition of entry into S phase by interferons, depending on the number and concentrations of the growth factors which were added to the cells. This suggests inhibition by interferons either of the binding of growth factors to their receptors or of a subsequent metabolic event associated with growth factor action. In human fibroblasts, interferon treatment has been reported to stimulate production of prostaglandin E (Yaron *et al.*, 1977).

Table 2. *Effects of interferon treatment on phases of the cell cycle*

Cell cycle phase	Cell types	Effect of interferon	References
$G_0 \rightarrow S$ transition	Melanoma	Inhibition	Creasey <i>et al.</i> (1980)
	3T3 fibroblasts		Sokawa <i>et al.</i> (1977)
	P ₃ HR-1 lymphoblasts		Balkwill & Taylor-Papadimitriou (1978) Lundgren <i>et al.</i> (1979) Taylor-Papadimitriou <i>et al.</i> (1981) Leanderson & Lundgren (1982)
G_1	Erythroleukaemia	Prolongation	Matarese & Rossi (1977)
	Fibroblasts	Prolongation	Mallucci <i>et al.</i> (1983)
	Ehrlich ascites	Shortening	Panniers & Clemens (1981)
S	Melanoma	Prolongation	Creasey <i>et al.</i> (1980)
	Ehrlich ascites	Prolongation	Panniers & Clemens (1981)
	Glioma	Failure to complete	Lundblad & Lundgren (1981, 1982)
G_2	Erythroleukaemia	Prolongation	Matarese & Rossi (1977)
	3T3 fibroblasts		Balkwill & Taylor-Papadimitriou (1978)
	Ehrlich ascites		Panniers & Clemens (1981)
	Fibroblasts		Mallucci <i>et al.</i> (1983)
Mitosis	Ehrlich ascites	Prolongation	Panniers & Clemens (1981)
All phases	Fibroblasts	Prolongation	Balkwill <i>et al.</i> (1978)
	Breast epithelial		

This agent could cause inhibition of cell proliferation. However, in other cell types inhibitors of fatty acid cyclo-oxygenase (an enzyme required for prostaglandin biosynthesis) only partially inhibited, or completely failed to prevent, the anticellular effects of interferons, suggesting that involvement of prostaglandins is not essential (Brideau & Stringfellow, 1983; Forti *et al.*, 1984).

Although the interferons are regarded as cytostatic agents, there are a number of reports which show that prolonged treatment of some cell types can result in cell death (Rubin & Gupta, 1980b; Ito & Buffett, 1981; Gewert *et al.*, 1984). Significantly, tumour cells are susceptible to this cytotoxic effect of interferons whereas normal human fibroblasts are completely resistant (even though they do show a growth inhibition). This difference may reflect the relative abilities of tumour cells and normal cells to survive conditions under which they cannot proliferate, but the molecular basis for it is not known.

DNA replication in interferon-treated cells

Because cell proliferation is inhibited it has been assumed that interferon treatment of susceptible cells leads to cessation of DNA replication. This conclusion appears consistent with the marked inhibition of incorporation of radioactive thymidine into DNA which is observed within a few hours of adding interferons to cells such as L1210 mouse leukaemia (Tovey *et al.*, 1975) or human lymphoblastoid (Daudi) cells (Gewert *et al.*, 1981). Furthermore, as described above, the ability of interferons to prevent the transition of certain cell types from a G_0 'resting' state into S phase means that, in the overall cell population, DNA synthesis is inhibited, at least indirectly.

The simple view that interferons inhibit cell proliferation by preventing DNA replication is, however, complicated by several considerations. Firstly, there is no rigorous evidence to show that interferon treatment has any direct effect on the rate of DNA synthesis. Very few studies of the molecular basis for the inhibition of cell proliferation have been carried out, but in the small number of existing reports the conclusions have been unexpected. Our own studies on Daudi cells (Gewert *et al.*, 1983, 1984; Moore *et al.*, 1984) indicated that DNA synthesis is in fact inhibited far less than might be predicted from the extent of impairment of cell proliferation. These findings suggest a loss of co-ordination between DNA replication and subsequent cell division, resulting in over-replication of at least some of the cellular genome. The fate of the newly synthesized DNA in these circumstances is discussed below.

Another reason why the effects of interferon treatment on DNA synthesis are sometimes misin-

terpreted is that the membrane transport of thymidine and/or its subsequent intracellular phosphorylation by thymidine kinase are inhibited in several cell types. This has been observed in L1210 leukaemia cells (Brouty-Boyé & Tovey, 1978), Daudi cells (Gewert *et al.*, 1981), glioma cells (Lundblad & Lundgren, 1982) virus-transformed embryonic cells (Fuse & Kuwata, 1978) and normal mouse embryo fibroblasts (Mallucci *et al.*, 1983). Uptake of some other nucleosides is not affected by interferon treatment. Thus, for example, labelling of newly synthesized DNA by [3H]thymidine is inhibited within a few hours of interferon treatment, whereas the incorporation of [3H]deoxyadenosine into DNA is scarcely altered (Brouty-Boyé & Tovey, 1978; Gewert *et al.*, 1983). Other cell types, such as human fibroblasts, which show a clear slowing of their rate of proliferation after 3 days of interferon treatment, continue to incorporate thymidine at as much as 86% of the control rate (Pfeffer *et al.*, 1979). This indicates that there is no major impairment of either thymidine uptake or DNA synthesis in these cells.

On the other hand, there is a report of lower DNA polymerase activity after interferon treatment in glioma cells which showed a tendency to accumulate in S phase (Lundblad & Lundgren, 1981, 1982). These cells apparently have an impairment in their ability to complete DNA replication, rather than a failure to enter into S phase. It is also clear that, in interferon-treated cells, there are additional subtle disruptions to the complex series of events required for DNA replication. We have shown that short Okazaki fragments, which are the initial products of RNA-primed discontinuous DNA synthesis, tend to accumulate and are delayed in their ligation to larger size classes of DNA in interferon-treated Daudi cells (Moore *et al.*, 1984) (Fig. 2). Moreover, a significant fraction of newly replicated DNA is unstable in these cells and is degraded within a few hours of its synthesis (Fig. 2). This may be a corollary of the loss of co-ordination between DNA replication and cell proliferation. The results suggest that the organization of newly synthesized DNA, perhaps involving its packaging into chromatin, is different in interferon-treated and control cells. Indeed, small but consistent changes in chromatin structure have been detected in interferon-treated HeLa cells and Daudi cells by measurement of circular dichroism spectra (Suhadolnik *et al.*, 1984) and sensitivity to digestion by micrococcal nuclease (R. Exley & M. J. Clemens, unpublished work) respectively. In view of the role of poly(ADP-ribose) polymerase in DNA ligation and processing in eukaryotic cells (Creissen & Shall, 1982) these changes in chromatin structure may be a result of a decrease in the

Fig. 2. Regulation of DNA replication and chromatin assembly in interferon-treated cells

Semi-conservative, discontinuous DNA replication occurs via the synthesis by DNA polymerase α of short Okazaki fragments on RNA primers. These fragments are then extended and ligated to each other and to larger DNA molecules in a complex series of reactions involving DNA polymerase α , exonuclease(s) (to remove the RNA primers), DNA ligase(s) and other nuclear and cytoplasmic proteins. The newly replicated DNA is rapidly packaged into nucleosomes during these processes, by combining with newly synthesized histones. The newly assembled chromatin undergoes further modifications, requiring protein synthesis *de novo*, to give rise to mature chromatin. Interferon treatment of susceptible cells results in impairment of DNA polymerase activity, DNA ligation and assembly of chromatin (depicted by heavy arrows). There is also evidence for degradation of newly replicated DNA and/or chromatin in interferon-treated cells. For details see the text. Abbreviations: dT, thymidine; dNMPs, deoxyribonucleoside monophosphates; dNTPs, deoxyribonucleoside triphosphates; NTPs, ribonucleoside triphosphates.

(poly) ADP-ribosylation of nuclear proteins observed after interferon treatment (Butt & Sreevalsan, 1983; Suhadolnik *et al.*, 1984). In none of these studies, however, has it yet been clearly established whether these nuclear changes are causes or consequences of the inhibition of cell proliferation.

Regulation of protein synthesis in interferon-treated cells

Although the role of protein synthesis inhibition in the antiviral effects of interferons has been widely studied, there are surprisingly few detailed reports on changes in translational activity during the regulation of proliferation of uninfected cells. Some studies have shown decreased incorporation of radioactive amino acids into total cell protein in interferon-treated fibroblasts and Daudi cells (Fuse & Kawata, 1976; Pfeffer *et al.*, 1979;

Clemens *et al.*, 1984). On the other hand, there appears to be little or no inhibition of protein synthesis during impairment of growth in other cell types (Tovey *et al.*, 1975; Fuse & Kuwata, 1977; Panniers & Clemens, 1981). The interpretation of some of these results is, however, complicated by technical considerations. Inhibition of protein synthesis in uninfected cells appears to be a relatively late response to interferon treatment, often requiring 24–48 h to develop (Clemens *et al.*, 1984). Furthermore, the conditions of culture used during the measurements may influence the rates of protein synthesis observed. Conditions which improve the incorporation of a labelled amino acid are not always the most suitable for maintaining maximal rates of synthesis. A frequently used approach has been to increase the amount of radioactivity incorporated by lowering the concen-

tration of the unlabelled form of the precursor amino acid (or even leaving it out of the culture medium altogether). This raises the specific radioactivity of the labelled precursor but can have serious consequences for the rate of synthesis which is measured.

Care must also be exercised that the measured rate of incorporation of label is an accurate reflection of the true rate of protein synthesis (for review see Waterlow *et al.*, 1978). Since incorporation is a function of both the rate of synthesis and the specific radioactivity of the precursor, spurious results can be obtained if the latter is different in two populations of cells which are to be compared. Differences in the specific radioactivity of the labelled amino acid in two cell populations can be minimized by raising the extracellular amino acid concentration to 2.5 mM (Henshaw *et al.*, 1971; Garlick *et al.*, 1980). Using this approach in our own work, we found that in Daudi cells the specific radioactivity of [^3H]phenylalanine was the same in the medium, in the intracellular free pool and in phenylalanine bound to tRNA following incubation for 15 min (M. A. McNurlan, unpublished work). When assayed under these conditions, interferon treatment of Daudi cells resulted in a progressive decrease in the rate of protein synthesis, whether expressed on the basis of per cell or per mg of total protein (Clemens *et al.*, 1984).

Very few attempts have been made to compare the rates of protein synthesis with the rates of protein accumulation (i.e. growth) in interferon-treated cells. The latter represents the balance between synthesis and degradation. In Daudi cells which are growing exponentially we found that the rate of synthesis is not much higher than the rate of growth, implying that there is little turnover. As might be expected in transformed cells with these characteristics, the initial interferon-induced growth inhibition of Daudi cells is similar in magnitude to the reduction in the rate of protein synthesis. The effect of interferons on protein degradation appears to depend on the length of treatment of the cells. In Daudi cells, protein degradation is initially reduced but with prolonged treatment the rate of degradation rises (M. A. McNurlan, unpublished work). The nature of the regulation of protein breakdown needs to be more fully understood before the processes which co-ordinate synthesis and degradation during cellular growth control by interferons can be elucidated.

The role of 2',5'-oligoadenylates

Since relatively little attention has been paid to changes in protein synthesis in relation to cell growth regulation by interferons, other than with Daudi cells, firm evidence for the molecular basis of these changes is scarce. Some authors have

suggested a role for the 2',5'-oligoadenylate synthetase/ribonuclease L system (Fig. 1) in the control of cell proliferation, but such an idea is mostly an extrapolation from the situation in virus-infected cells. In some studies, not involving interferon treatment, the levels of 2'5'(A)_n synthetase, 2'5'(A)_n-dependent ribonuclease or the 2'5'(A)_n compounds themselves have been found to correlate inversely with rates of cell proliferation or the percentage of cells in S phase. This has been observed, for example, in glucocorticoid-treated or serum-starved lymphoblastoid cells (Krishnan & Baglioni, 1980, 1981), in regenerating liver (Etienne-Smekens *et al.*, 1983; Smekens-Etienne *et al.*, 1983), in cells at different densities and growth states in culture (Kimchi *et al.*, 1981a; Jacobson *et al.*, 1983; Creasey *et al.*, 1983) and in a range of normal and hormonally-sensitive tissues (Stark *et al.*, 1979). However, there are many cases in which the effects of interferon treatment on cell proliferation do not correlate well with the extent of induction or activation of 2'5'(A)_n synthetase. Some cell lines have high constitutive levels of this enzyme yet grow perfectly well in the presence or absence of interferons (Verhaegen *et al.*, 1980); others show an induction of 2'5'(A)_n synthetase by interferons which bears no obvious relationship to the extent of growth inhibition (Hovanessian *et al.*, 1980; Vandenbussche *et al.*, 1981; Verhaegen-Lewalle *et al.*, 1982; Tomita *et al.*, 1982; Tovey *et al.*, 1983; Chapekar & Glazer, 1983). In Daudi cells and colon carcinoma cells, where 2'5'(A)_n synthetase is rapidly induced, no evidence could be found for the accumulation of the 2',5'-oligoadenylate products (Silverman *et al.*, 1982; Chapekar & Glazer, 1984) or for any increased turnover of total mRNA during the decline in protein synthesis accompanying growth inhibition (Clemens *et al.*, 1984). In the latter study the data were more consistent with inhibition of polypeptide chain initiation and eIF-2 activity in the interferon-treated cells. However, there is no obvious correlation between the activity of the eIF-2-specific protein kinase and cell growth inhibition (Verhaegen *et al.*, 1980; Silverman *et al.*, 1982).

The absence of overall activation of either the 2'5'(A)_n synthetase/ribonuclease L or the protein kinase systems does not preclude the possibility of small localized effects of these enzymes on the synthesis of specific proteins of crucial importance for cell proliferation. Baglioni's group has shown that there can be compartmentalized activation of both the synthetase and the protein kinase in the vicinity of RNA species containing double-stranded regions, such as intermediates of viral RNA replication (Nilsen & Baglioni, 1979; De Benedetti & Baglioni, 1984). Certain cellular RNAs with a high degree of secondary structure could locally

activate the 2'5'(A)_n system and then be preferentially degraded. The high rate of turnover of the 2',5'-oligoadenylates, due to the action of 2',5'-phosphodiesterase (Schmidt *et al.*, 1979), ensures that activation of the 2'5'(A)_n-dependent ribonuclease is only transient, even in cells initially containing quite high concentrations of the nucleotides (Hovanessian *et al.*, 1979; Williams *et al.*, 1979). Thus 2'5'(A)_n may be able to exert effects on the expression of specific gene products without accumulating to detectable levels or activating gross mRNA breakdown.

An additional possibility is that 2',5'-oligoadenylates have a quite separate role in cellular growth regulation, unrelated to their effects on protein synthesis. Direct anti-mitogenic actions of 2'5'(A)_n have been observed when the 'core' oligonucleotides (lacking the 5'-terminal triphosphate) are added to lectin-stimulated lymphocytes (Kimchi *et al.*, 1979c, 1981a), transformed lymphoblastoid cells (Martin *et al.*, 1981; Leanderson *et al.*, 1982) or serum-stimulated 3T3 fibroblasts (Kimchi *et al.*, 1981b; Eppstein *et al.*, 1983). Although both DNA replication and cell proliferation are highly sensitive to the rate of translation, these anti-mitogenic effects cannot all be consequences of a decrease in protein synthesis caused by ribonuclease activation. A clone of NIH-3T3 cells, defective in 2'5'(A)_n-dependent nuclease activation, still showed an anti-mitogenic response to 2'5'(A)_n cores (Eppstein *et al.*, 1983), and in Daudi cells inhibition of cell proliferation and thymidine incorporation into DNA by 2'5'(A)_n were observed without much inhibition of protein synthesis (Martin *et al.*, 1981). 2'5'(A)_n cores or their derivatives may interact with other targets besides ribonuclease L, since several proteins which bind these oligonucleotides have been identified (Nilsen *et al.*, 1982; St Laurent *et al.*, 1983). The functions of these 2'5'(A)_n-binding proteins have not yet been determined. The ability of 2'5'(A)_n synthetase to add AMP residues in 2'-5' linkage to nucleotides such as NAD⁺, A5'p₄5'A and ADP-ribose (Cayley & Kerr, 1982), and the presence in cells under some conditions of unusual 2',5'-oligoadenylate species (Hersh *et al.*, 1984), raises the possibility that a range of cellular functions may be regulated by 2'5'(A)_n nucleotides.

Interferons and cell differentiation

Morphological changes

Several studies have revealed changes in cellular morphology following interferon treatment of a variety of cultured cells. Such changes are characterized by alterations in the organization of the cytoskeleton and in surface properties of the cells (reviewed by Pfeffer *et al.*, 1982). In many cases an

increase in cell volume and protein content occurs (Pfeffer *et al.*, 1979, 1980; Panniers & Clemens, 1981; Pfeffer & Tamm, 1983) and, in fibroblasts and some carcinoma cell lines, this is accompanied by increased cell spreading on tissue culture surfaces. Large arrays of actin-containing microfilaments become visible inside the cells and increased amounts of fibronectin are distributed over the cell surface (Pfeffer *et al.*, 1980; Wang *et al.*, 1981; Gerfaux *et al.*, 1981; Shibata & Taylor-Papadimitriou, 1981; Bourgeade *et al.*, 1981; Lin *et al.*, 1983). In lymphoblastoid cells, an increased tubulin mRNA content is observed after interferon treatment, resulting in an enhanced potential for tubulin synthesis and microtubule assembly (Fellous *et al.*, 1982a). These changes in cytoskeleton organization, together with increased rigidity of the plasma membrane (Wang *et al.*, 1981), may account for the increased cellular adhesion and decreased cellular mobility observed after interferon treatment. Several of these effects can also be produced by treating cells with other agents which inhibit cell proliferation, such as dibutyl cyclic AMP (Panniers & Clemens, 1981) or sodium butyrate (Bourgeade & Chany, 1979; Bourgeade *et al.*, 1981). This suggests that such phenotypic changes are part of a programme of events associated with decreased proliferative potential and, in the case of tumour cells, the disappearance of properties characteristic of the transformed state.

Models for differentiation

There are several experimental models of cell differentiation in which effects of interferon have been observed (Table 3). In some cases interferon inhibits the differentiation process. On the other hand, stimulation of myogenesis in human myoblast cultures (Fisher *et al.*, 1983) and enhancement of differentiation of mouse and human myeloid leukaemia cells by interferons in the presence of other inducing agents (Tomida *et al.*, 1980, 1982) have been observed. Such apparently conflicting responses to interferon may reflect the differential modulation of the genes required for differentiation in a range of systems. There is now good evidence that the expression of some genes is enhanced whilst that of others is suppressed by interferon treatment, as discussed below.

A particularly interesting case in which interferons modulate cell differentiation is the stimulation of haemoglobin synthesis by chemical inducers in mouse erythroleukaemia (MEL) cells (Table 3). It has been shown that the chemically induced differentiation of MEL cells is accompanied by the synthesis and secretion of a type I interferon which may play a part in the induction of haemoglobin synthesis and/or the cessation of cell proliferation

Table 3. *Effects of interferon treatment on cell differentiation*

Differentiation model	Effect of interferon	References
Insulin-dependent 3T3-L1 conversion to adipocytes	Inhibition of lipid synthesis	Keay & Grossberg (1980)
Spontaneous and melanotropin-stimulated melanogenesis	Inhibition; impaired activity of tyrosinase	Fisher <i>et al.</i> (1981)
Myogenesis (chick cells)	Inhibition	Lough <i>et al.</i> (1982)
Myogenesis (human myoblasts)	Accelerated myotube formation and creatine kinase isoenzyme transition	Fisher <i>et al.</i> (1983)
Mouse or human myeloid leukaemia cell conversion to macrophages and granulocytes	No effect alone, but enhancement of effects of differentiation inducers	Tomida <i>et al.</i> (1980, 1982)
Induction of haemoglobin synthesis in mouse erythroleukaemia cells	Inhibition by high concentrations; impaired accumulation of globin mRNA	Rossi <i>et al.</i> (1977a,b)
	Stimulation by low concentrations and by endogenously synthesized interferon	Lieberman <i>et al.</i> (1974), Kimchi (1981)
Lymphocyte maturation	Enhanced expression of histocompatibility antigens; increased concentrations of HLA and β_2 -microglobulin mRNAs	Lindahl <i>et al.</i> (1976), Fellous <i>et al.</i> (1982b), Rosa <i>et al.</i> (1983b)
Differentiation of macrophages and natural killer cells	Stimulation; enhancement of Ia expression on cell surface	Nakamura <i>et al.</i> (1984), Saksela <i>et al.</i> (1979)

(Friedman-Einat *et al.*, 1982). Interestingly, dimethyl sulphoxide induction of erythroid differentiation has been reported to be accompanied by changes in DNA sedimentation behaviour (Terada *et al.*, 1978), an increase in DNAase activity and a large decrease in DNA ligase activity in MEL cells (Scher *et al.*, 1982). These effects are reminiscent of the changes affecting newly replicated DNA in interferon-treated lymphoblastoid cells (Fig. 2) (Moore *et al.*, 1984).

As with the inhibition of cell proliferation, a possible role for 2',5'-oligoadenylate in the control of differentiation has been suggested by some studies, although the biochemical basis for such a phenomenon remains elusive. Treatment of Moloney sarcoma virus-transformed cells with chemical inducers of differentiation induces 2'5'(A)_n synthetase activity independently of any effect on interferon synthesis (Besancon *et al.*, 1981). The differentiation of thymocytes into peripheral T-lymphocytes *in vivo* and of MEL cells into haemoglobin-synthesizing cells in culture are also accompanied by the appearance of increased synthetase activity (Kimchi, 1981). These results do not necessarily prove a role for 2'5'(A)_n in cell differentiation, since the level of synthetase activity may be more related to the inhibition of cell proliferation which occurs simultaneously. Nevertheless exogenous 2'5'(A)_n core can stimulate differentiation of a human monocyte cell line, as

assessed by acquisition of new functional characteristics (Schmidt *et al.*, 1984).

Modulation of the expression of membrane antigens by interferon treatment has been observed in several systems and may represent a common cellular response. Enhanced synthesis and expression of histocompatibility antigens (Lindahl *et al.*, 1976; Basham *et al.*, 1982; Burrone & Milstein, 1982; Rosa *et al.*, 1983a) can be correlated with increased concentrations of the mRNAs coding for β_2 -microglobulin and HLA polypeptides (Fellous *et al.*, 1982b; Rosa *et al.*, 1983a,b). A stimulation of Ia antigen expression on the cell surface of macrophages also occurs, particularly in response to IFN- γ (Nakamura *et al.*, 1984). Changes in membrane properties and the state of differentiation of cells of the immune system will almost certainly prove to be involved in such effects of interferons as enhancement of antibody formation (Leibson *et al.*, 1984) and stimulation of activity of natural killer cells against tumour cell targets (Gidlund *et al.*, 1978; Lee *et al.*, 1982).

Regulation of expression of specific genes

It is now clear that within a few hours of exposure of cells to homologous interferon species there are changes in the concentrations of mRNAs coding for several discrete products (Table 4). It seems likely that the transcription of the gene

Table 4. *Changes in expression of specific genes in interferon-treated cells*

Gene product (cell type)	Stimulation (+) or inhibition (-) of expression	mRNA concentration	References
2',5'-Oligoadenylate synthetase (L cells)	+	Increased	Shulman & Revel (1980)
Ornithine decarboxylase (3T3 cells)	-	?	Sreevalsan <i>et al.</i> (1979)
Lysozyme (myeloid leukaemia)	+	?	Tomida <i>et al.</i> (1980)
Tyrosinase (melanocytes)	-	?	Fisher <i>et al.</i> (1981)
Poly(ADP-ribose) polymerase (3T3 cells, HeLa cells)	-	?	Butt & Sreevalsan (1983), Sreevalsan <i>et al.</i> (1982), Suhadolnik <i>et al.</i> (1984)
Creatine kinase MM (myoblasts)	+	?	Fisher <i>et al.</i> (1983)
Tyrosine aminotransferase (hepatoma cells)	-	?	Beck <i>et al.</i> (1974)
Glutamine synthetase (neural retina)	-	?	Matsuno & Shirasawa (1978)
Aryl hydrocarbon hydroxylase (foetal mouse liver)	+	?	Nebert & Friedman (1973)
Thymidine kinase (RSa cells, Daudi cells, fibroblasts)	-	?	Fuse & Kuwata (1978), Gewert <i>et al.</i> (1981, 1983), Otsuka <i>et al.</i> (1982), Dubois <i>et al.</i> (1983)
Globin (mouse erythroleukaemia)	-	Decreased	Rossi <i>et al.</i> (1977a)
HLA-A,B,C, β_2 -microglobulin (melanoma, fibroblasts, lymphoblastoid cells)	+	Increased	Basham <i>et al.</i> (1982), Burrone & Milstein (1982), Fellous <i>et al.</i> (1982b), Rosa <i>et al.</i> (1983a,b)
I-region antigens (macrophages)	+	Increased	Nakamura <i>et al.</i> (1984)
c-Ha-ras (Transformed 3T3 cells, human bladder carcinoma)	-	Decreased	Samid <i>et al.</i> (1984), Soslau <i>et al.</i> (1984)
pp60 ^{src} (RSV-transformed rat cells, human bladder carcinoma)	-	Decreased	Lin <i>et al.</i> (1983), Soslau <i>et al.</i> (1984)
c-myc (Daudi cells)	?	Decreased	Jonak & Knight (1984)
SV40 T antigen (monkey cells)	-	Decreased	Yamamoto <i>et al.</i> (1975), Metz <i>et al.</i> (1976)
14.5 kDa protein (Ehrlich ascites cells)	+	Increased	Farrell <i>et al.</i> (1979)
Four proteins (44 to approx 70 kDa) (fibroblasts)	+	?	Knight & Korant (1979)
Five proteins (56-120 kDa) (Ehrlich ascites cells, L cells, fibroblasts)	+	?	Gupta <i>et al.</i> (1979), Rubin & Gupta (1980a), Gupta <i>et al.</i> (1981)
Four proteins (56-68 kDa) (fibroblasts)	+	Increased	Colonna (1981), Colonna & Pang (1982)
24 proteins (28-90 kDa) (fibroblasts)	+	?	Weil <i>et al.</i> (1983)
Four proteins (31-50 kDa) (fibroblasts)	+	?	Sundstrom <i>et al.</i> (1983)

coding for 2'5'(A)_n synthetase is stimulated by interferons (Shulman & Revel, 1980), although effects on pre-mRNA processing or the turnover of cytoplasmic mRNA cannot be excluded as additional mechanisms for increasing the concentration of the mRNA for this enzyme. Similarly, the amounts (and probably the rates of synthesis) of mRNAs coding for several new proteins ranging in size from 14 500 to 120 000 Da are increased within 2-20 h of interferon treatment (see Table 4 for references). The functions of most of these newly induced proteins are unknown, but the synthesis of at least some of them appears to be transient even

when interferons are continuously present (Rubin & Gupta, 1980a; Gupta *et al.*, 1981). IFN- γ is able to induce some polypeptides in addition to those induced by IFN- α or IFN- β (Weil *et al.*, 1983); this may be related to the existence of distinct cell surface receptors for IFN- γ . There are also several genes which appear to be repressed in interferon-treated cells, notably certain cellular oncogenes.

The difficulty in interpreting data of this kind is to know whether changes in expression of particular genes are causes or effects of the modulation of cell proliferation or differentiation. This is particularly true, for example, for an enzyme such as

ornithine decarboxylase (Sreevalsan *et al.*, 1979; Gazitt, 1981) which is regulated by many conditions affecting cell growth. However, in one study (Sundstrom *et al.*, 1983), the induction of new proteins by interferon treatment occurred independently of whether the cells were growing or quiescent, and it seems certain that at least some of the gene products affected by interferons play an important role in mediating the anticellular and/or antiviral effects.

It was originally reported that cells require the presence of a functional thymidine kinase gene in order to exhibit sensitivity to interferons (Lewis *et al.*, 1983). Subsequent studies now indicate that it is not the activity of this enzyme *per se* which is important but the presence of DNA sequences within the thymidine kinase gene (Mengheri *et al.*, 1983; Lewis & Esteban, 1984). These regions of DNA presumably exert some control function on other genetic activities, but the relationship to the mechanism of induction of interferon-sensitive genes remains to be discovered.

Interferons and cell transformation

Transformed cells in culture exhibit a number of features which distinguish them from their normal counterparts. These include the ability to grow to higher cell densities, the ability to grow in soft agar, loss of contact inhibition in culture, and various morphological characteristics. Reversal of these changes can be observed in interferon-treated Ehrlich ascites tumour cells (Panniers & Clemens, 1981), sarcoma virus-transformed mouse or rat cells (Hicks *et al.*, 1981; Gerfaux *et al.*, 1981; Lin *et al.*, 1983), bovine papilloma virus-transformed mouse cells (Turek *et al.*, 1982) and 3T3 cells containing the human c-Ha-ras oncogene (Samid *et al.*, 1984). As with the morphological changes described above, these effects are reminiscent of those brought about by treatment of transformed cells with cyclic AMP or its analogues. Experiments in which the effects of interferons and exogenous cyclic AMP analogues have been compared, and analysis of the reactions of cyclic AMP-unresponsive or adenylate cyclase-deficient cell variants to interferons, have led to the conclusion that cyclic AMP may be involved in some, but not all, of the effects of interferons.

Using labelled DNA hybridization probes specific for cellular oncogenes it has been shown that interferon treatment lowers the steady state concentrations of RNAs transcribed from transfected c-Ha-ras genes in 3T3 cells (Samid *et al.*, 1984), endogenous c-Ha-ras and *src* genes in human bladder carcinoma cells (Soslau *et al.*, 1984) and the translocated c-myc gene of Daudi cells

(Jonak & Knight, 1984). In the case of at least the *ras* and *src* genes, the rate of synthesis of their protein products (p21 and pp60^{src} respectively) is also inhibited (Samid *et al.*, 1984; Lin *et al.*, 1983). It is not clear whether these changes in oncogene expression represent causes or effects of cellular growth inhibition (c-myc expression, for example, is cell cycle-dependent; Kelly *et al.*, 1983) but answers to this question may be expected in the near future.

The regulation of DNA tumour virus functions by interferons is another area of research in which rapid advances are likely. Because of the sensitivity to interferons of certain human lymphoblastoid lines of the B-cell lineage, which contain multiple copies of the Epstein-Barr viral genome, it is of interest to examine effects on the functions of this large DNA virus. In general, viral functions associated with both productive infection and transformation of B cells with exogenous EBV are suppressed by treatment of cells with interferons (Adams *et al.*, 1975; Menezes *et al.*, 1976; Doetsch *et al.*, 1981) whereas expression of endogenous viral antigens and activities in established EBV-transformed lines is apparently not inhibited. The appearance *de novo* of EBV nuclear antigen, a DNA-binding protein normally found in all EBV-infected and transformed cells, is inhibited if interferon treatment is begun simultaneously with addition of exogenous virus (Menezes *et al.*, 1976; Henderson *et al.*, 1982). In contrast, the spontaneous production of EBV early antigen in transformed cell lines is either not affected or is actually enhanced by interferon treatment (Adams *et al.*, 1975; Tovey *et al.*, 1982; Zeng *et al.*, 1982). The basis for these differential effects on newly infected and transformed cells is not known, but in the latter case it is tempting to speculate that, as with the effects of some tumour promoters (zur Hausen *et al.*, 1979; Lin *et al.*, 1982), interferon treatment alters the lymphoblastoid cellular phenotype from a state which is nonpermissive for viral replication to a more differentiated 'normal' phenotype. Paradoxically, this may permit some of the early events involved in virus production to occur. However, there is no evidence for increased production of complete EBV particles in interferon-treated cells.

A similar difference in response of Simian Virus 40 (SV40) functions is observed after interferon treatment of virus infected compared with transformed cells. Expression of the viral T antigen, a nuclear protein required for both viral replication and cellular transformation, is not inhibited by interferons in SV40-transformed 3T3 cells (Oxman *et al.*, 1967) but is impaired when non-transformed 3T3 cells are treated with interferons before or during an exogenous infection by SV40 (Yama-

moto *et al.*, 1975; Metz *et al.*, 1976; Yakobson *et al.*, 1977).

An interesting report has been published documenting the elimination of episomal viral genomes in cell clones obtained after prolonged interferon treatment of bovine papillomavirus-transformed mouse cells (Turek *et al.*, 1982). This result suggests either a selective inhibition of viral DNA replication over several cell generations or degradation of the multiple copies of the viral genome originally present in the cells.

An important factor in determining the sensitivity of virally induced transformation to interferon action may be whether there is covalent integration of viral genes into host cell DNA. Integration clearly does occur in the case of small DNA viruses such as SV40, as well as the RNA retroviruses, but there is scant evidence for it yet in cells transformed by EBV or bovine papilloma virus. The incorporation of new genetic material into cellular DNA can be inhibited by interferon treatment, as shown by impairment of formation of stable transformants after transfer into cells of plasmids containing the thymidine kinase or dihydrofolate reductase genes (Otsuka *et al.*, 1982; Dubois *et al.*, 1983). However, once integrated, the new DNA sequences probably become interferon-resistant in their expression. The biochemical basis for the effect on integration is not known but may be related to the inhibition of DNA ligation (Fig. 2), and the impairment of poly(ADP-ribose) polymerase activity (Table 4). The latter enzyme is known to be involved in DNA ligation in eukaryotic cells (Creissen & Shall, 1982). Further work will be required to address these questions as well as to fill the many other gaps in our knowledge of how the interferons regulate cell transformation, proliferation and differentiation.

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