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Minireview

The control of interferon-inducible gene expression

Ian M. Kerr and George R. Stark

Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK

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The $\alpha\beta$ interferons (IFNs) transiently induce genes through an IFN-stimulable DNA response element (ISRE). IFN-cell surface receptor interaction triggers the cytoplasmic activation of the complex primary transcription factor E, which on translocation and interaction with the ISRE initiates transcription. Whether E is activated directly through the receptor(s) or through a more classical second message pathway(s) and the roles of additional factors in the $\alpha\beta$ and γ IFN responses remain to be established. Meanwhile analysis of mutants has revealed complexity and overlap in the α,β and γ IFN response pathways and the products of at least two viruses have been shown to inhibit IFN-inducible gene expression.

Gene expression; Interferon-inducible; Mutant; Viral inhibition

1. INTRODUCTION

At least three recent reviews on the control of interferon (IFN)-inducible gene expression are published or in press [1–3]. The most comprehensive is that from Bryan Williams whereas those from Levy and Darnell and Pfeffer and Tan present contrasting views as to the likely involvement of classical second message pathways in the response to the IFNs. Levy and Darnell favour the direct (although possibly multistep) activation of a transcription factor by the IFN–cell surface receptor complex without any requirement for classical second messengers, whereas Pfeffer and Tan review the not inconsiderable evidence for such a requirement. Here we briefly review what is known in these areas and suggest a way in which these contrasting views might be reconciled. In addition we discuss some of our recent work on mutants in the IFN response pathway and the inhibitory effects of virus infection on the IFN response.

The IFNs have profound effects on cells. They induce an antiviral state, can inhibit the growth of both normal and transformed cells and play an important role in the very complex cytokine network which regulates the immune system. The IFNs can be effective antiviral agents but their effectiveness may be limited by the ability of some viruses to inhibit different aspects of the IFN response. There are three major antigenic types of human IFN, α, β and γ in the new terminology, leucocyte, fibroblast and immune or Type I-α, β- and Type II-γ-IFNs in the old. The α, β IFNs appear to interact with the same receptor (but see below) and induce the same polypeptides with similar kinetics. γ-IFN interacts with a different receptor and induces an overlapping set of polypeptides with slower kinetics. For both human receptors, the IFN binding component has been cloned [4,5]. In the case of the γ receptor it is accepted that there must be an additional signal transduction subunit(s). For the α receptor(s) this is less clear-cut but also seems likely.

The IFNs are effective at very low levels; only one or a few molecules per cell are required to trigger the antiviral response. They induce upwards of 20 genes (the exact number is not known) and downregulate others (e.g. [6]). Induction is transient, apparently involving a switch-off as well as a switch-on mechanism and is followed to a variable extent by a refractory state [7,8]. Over the years, different known second message signal transduction pathways have been implicated in the IFN response (e.g. [3,9–11]), but as yet no consistent picture has emerged. (See [12] for a general review of IFN action.)

2. DNA ELEMENTS

Against this background we and others have cloned a number of IFN-inducible genes from human cells [14–17]. Classical deletion and construct analyses have shown that there is a highly homologous IFN-stimulable response element (ISRE) in the 5′ flanking promoter-enhancer region of these genes, for example, GGGAAAATGAAACT and AGGAAATAGAAACT for the 6–16 and 9–27 genes with which we have been

Correspondence address: I.M. Kerr, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK. Fax: (44) (71) 269 3581.

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working. This type of ISRE is both necessary and sufficient to confer IFN inducibility [18–21].

The early work was all carried out with α, β IFNs and it was generally accepted that there would be a separate γ IFN response element. On analysis of the 9-27 gene, however, it became clear that the same element is both necessary and sufficient to confer a γ as well as an α, β IFN response [22]. For the 9-27 gene the response to γ IFN is less than that to the α, β IFNs. Interestingly, in the case of a gene coding for an IFN-inducible guanylate-binding protein there is an additional response to γ IFN that is mediated by an element overlapping the ISRE [23]. In contrast, several of the genes that have ISREs perfectly capable of conferring both α and γ IFN responses upon a marker gene are poorly responsive to γ IFN when the ISRE is in its natural context. The basis for this is not yet clear (e.g. [24]). It is reasonable to conclude, however, that while an ISRE of this type is both necessary and sufficient to confer a response to α and γ IFN, the extent to which it does so can be influenced greatly by its context in the DNA. It remains possible that γ IFN in particular may also have effects post-transcriptionally on the accumulation of induced messenger RNA or indeed, for example in the case of Class I HLAs, on the correct assembly of the protein products [25]. The fact that the above type of ISRE responds to γ IFN does not, of course, mean there is not a different γ response element governing other genes. In fact, there is direct evidence for this from, for example, the work of Strominger's group on one of the Class II HLAs [26].

3. FACTORS INTERACTING WITH THE ISRE

There are three types of such factors: (1) those that are activated very rapidly without any requirement for protein synthesis, for example E in our terminology (ISGF3 in that of Levy and Darnell); (2) those that are induced more slowly and require protein synthesis, for example M (ISGF2) and the γ IFN-inducible factor G; (3) constitutive factors, upon which relatively little work has yet been done. E can be activated within 30 seconds in the cytoplasm of the cell in response to α, β IFNs and accumulates subsequently in the nucleus [27]. Activation of E can occur in cytoplasts excluding any possibility of nuclear involvement [27]. Highly purified E can stimulate ISRE-dependent transcription of a marker gene in cell-free transcription systems ([28], M.J. Guille, G.R. Stark and I.M. Kerr, unpublished) consistent with its role in initiating transcription. Levy and Darnell and their colleagues were the first to show that E is made up of at least two subunits, Eα (ISGF3α) and Eγ (ISGF3γ) [29]. Eα, which is itself made up of at least 3 polypeptides [28,30] is activated by α,β IFNs. Eγ is constitutive but its amount can be increased by γ IFN at least in some cell types. Treatment of cells with α,β IFN results in activation of Eα in the cytoplasm, where it combines with pre-existing Eγ to form active E, which on transfer to the nucleus initiates transcription of a family of IFN-inducible genes (Fig. 1). Eγ but not Eα is inactivated by treatment with *N*-ethylmaleimide. This provides a basis for a convenient reconstitution assay for each of these two subunits [29]. Extracts from α IFN-treated cells exposed to *N*-ethylmaleimide contain Eα but not Eγ, whereas extracts from control or γ IFN-treated cells contain Eγ but not active Eα. Combination of extracts containing Eα and Eγ reconstitutes E. This type of assay has been used extensively in analysing the mutants in the IFN response pathway and in studying the inhibitory effects of virus infection on the IFN response (see below).

It was suggested initially that ISGF2 (presumptive M) is a negative factor but on subsequent purification and sequencing [31] it proved to be identical to IRF1, a positively acting factor cloned by Tanaguchi et al. using an oligomerised oligonucleotide probe $(AAGTGA)_N$, based on a sequence present in the β IFN promoter-enhancer region [32]. The exact role of this factor in the induction of β IFN per se remains controversial. To the extent that it has a role, however, it is clearly positive. Our own data [33] are more consistent with a positive,

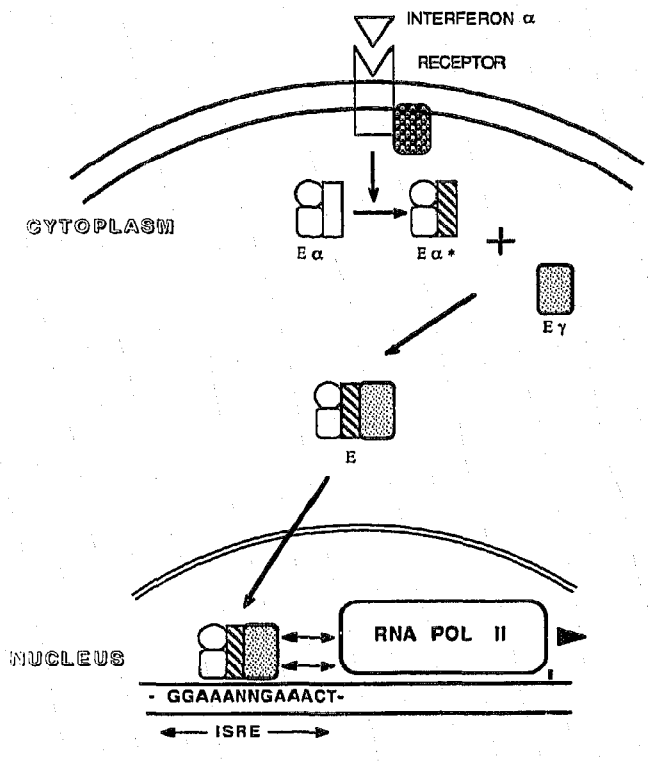

Fig. 1. Diagrammatic representation of events in the α IFN response pathway. Much of the detail is unknown: the structure of the receptor complex(es), the nature of the signal transduction subunit(s), whether there are alternative pathways, how Eα is activated and whether this activation is directly by the receptor complex or through an intermediary cascade, what governs transport of E to the nucleus and how transcription is activated on interaction of E with the ISRE, all remain to be established. Eα^*, activated Eα.

possibly maintain-on function for M (ISGF2). Interestingly, Pine et al. have shown that ISGF2 (IRF1) is multiply phosphorylated, but that its activity (ability to specifically bind an ISRE in a gel mobility shift assay) correlates with the absolute amount of polypeptide rather than its phosphorylation state [31]. In apparent contrast, mutants defective in M activity appear to have normal amounts of (IRF1) ISGF2 polypeptide, suggesting that some post-translational modification (not necessarily phosphorylation) or additional component may be required for activity (M.J. Guille, R. McKendry, G.R. Stark and I.M. Kerr, unpublished). Some of our own more recent data suggest that M is complex and does not simply equal IRF1.

So far we, and others, have defined a DNA element, the ISRE, that is both necessary and sufficient for the IFN response of many genes and have identified a number of factors through which activation of the ISRE is modulated by the IFNs. There is, however, much that we do not know. For example, how is E activated? Cytoplasmic activation of the transcription factor NF$\varkappa$B in response to a number of agents involves its release from a complex with an inhibitory subunit [34,35]. It is tempting to speculate that a similar mechanism might be involved in the activation of E, but to date no evidence has been obtained for the presence of such an inhibitory subunit. Activation could involve direct interaction of a component(s) of Eα with the receptor complex, or there could be a whole cascade of, for example, intermediate kinase reactions involved in the response pathway. It is not clear how to reconcile the relatively straightforward direct activation model with the apparent requirement for more classical second messengers [3]. One possibility that retains the attractive specificity of direct activation but allows for a classical second messenger requirement is that the latter is needed to maintain one or more components in the direct pathway in an IFN-responsive state. The availability of a cell-free system in which it is possible to obtain activation of E should greatly facilitate the further investigation of these early events.

While there is much still to be discovered concerning the nature of E and its activation, we know less about the roles of M (ISGF2) and G. Nor do we know how transcription is shut-off or how a refractory state is established. We also know a great deal less with respect to downregulated genes and the γ IFN response, despite the very recent report of a factor (GAF) which may function prior to G in the latter pathway [36].

4. MUTANTS IN THE IFN RESPONSE

Against this background much of our own recent effort has been devoted to isolating mutants in the α,β and γ IFN response pathways and to studying the effects of viral infection upon the IFN response. A number of different approaches are being employed to isolate mutants, but the most advanced involves the use of an IFN-inducible drug-selectable marker. The highly IFN-inducible 6–16 promoter was placed 5′ of a bacterial guanine phosphoribosyl transferase (*gpt*) gene in a suitable construct and transfected into hypoxanthine phosphoribosyl transferase negative human HT1080 cells. The isolation of a clone of cells in which expression of gpt is strictly regulated by IFN has permitted the selection, after mutagenesis, of mutants constitutive or defective in the IFN response [37]. The ability to select in both directions is facilitating downstream transfection experiments with cDNA and genomic libraries to rescue the mutants and isolate the genes responsible. We will be concerned here, however, only with two mutants defective in the IFN response.

The first, coded 11,1, is defective in α but not γ IFN-inducible gene expression. The defect is likely to be proximal to the α IFN receptor, possibly in a signal transduction subunit or in a factor or factors interacting with such a subunit. Consistent with this, mutant 11,1 is defective in activation of the Eα subunit of the early transcription factor E ([37], R. McKendry, J. John, I.M. Kerr and G.R. Stark, unpublished). The α and β IFNs share a common receptor and induce the same proteins with similar kinetics. It was unexpected therefore to find that 11,1 retains a partial response to β IFN [37]. This suggests either that there is a separate minor β IFN receptor or that β IFN interacts sufficiently differently with the α receptor(s) for the activity to be maintained in mutant cells.

A second mutant, U2 is also defective in the response to α IFN for all of the genes tested. In contrast to 11,1, however, activation of the Eα subunit of E is normal in U2, the defect is in the synthesis or activation of the Eγ subunit. Interestingly, again in contrast to 11,1, U2 is partially defective in the response to γ IFN. This is manifest in three ways. First, the response of the 9–27 gene to γ IFN is defective (although the response of several other genes to γ IFN is normal). Second, U2 has a markedly reduced antiviral response to γ IFN. Third, U2 is defective in the induction of the γ IFN-inducible factor G. Instead, in gel mobility shift assays, a novel γ IFN-inducible complex that migrates more rapidly than that containing G is observed (J. John, R. McKendry, I.M. Kerr and G.R. Stark, unpublished).

Overall therefore, these and additional mutants are proving valuable, not only as tools with which ultimately to clone genes in the signal transduction pathways, but also in revealing complexity in and unexpected overlap between the α, β and γ IFN response pathways.

5. EFFECTS OF VIRUS INFECTION

It has become increasingly clear that hardly surprisingly, viruses fight back. They do so at a number of levels and one of these, for hepatitis B virus and adenoviruses, is to inhibit IFN-inducible gene expres-

sion. When each of the known open reading frames of hepatitis B virus was assayed for the ability to inhibit IFN-inducible expression of a 6-16 promoter-enhancer driven marker construct, only the viral polymerase construct was found to be inhibitory. Consistent with this, cells stably transfected with the polymerase gene showed a much diminished response of the endogenous 6-16 and 9-27 genes to α and γ IFNs, assayed at the level of inducible messenger RNAs. So far, the inhibitory effect of the polymerase has been narrowed down to the terminal protein domain. The detailed mechanism of the inhibition is not known but the defect is in the production or activation of the Eα subunit of the IFN-inducible transcription factor E [38]. The induction of Class I and II HLAs by the IFNs is also inhibited in these cells. It is possible that such inhibition, particularly of the Class I HLAs by the expression of polymerase or terminal protein might be of clinical importance in establishing and maintaining chronic infection. In addition the inhibitory effect of constitutive expression of terminal protein operates on the production of the IFNs as well as the response to them. There is no induction of the β IFN gene in response to double-stranded RNA in cells expressing terminal protein [38].

The adenoviruses are amongst the more IFN-resistant viruses and, for the moment, are the example par excellence of viruses which appear capable of inhibiting the IFN response at more than one level. The role of VA RNA in inhibiting the IFN and double-stranded RNA-dependent protein kinase is well documented [39-41]. In addition, however, Anderson and Fennie [42] showed that mutants defective in E1A showed a reduced ability to inhibit IFN action and an inhibitory effect of E1A on IFN-inducible gene expression was reported by Reich et al. [43]. E1A is an immediate early gene. When it is transcribed very early post-infection, it yields 12 S and 13 S mRNAs and the corresponding polypeptides. Three regions of these polypeptides, CR1, 2 and 3 are highly conserved in different strains of adenovirus. Expression of the products of the E1A 12 S or 13 S gene inhibits α and γ IFN-inducible gene expression in stably transfected cells. Experiments with mutant constructs indicate that inhibition is mediated through the CR1 domain. A similar inhibition is seen with cells stably expressing both 12 S and 13 S E1A products and in adenovirus infected cells. Interestingly, once again the induction of β IFN in response to double-stranded RNA is also inhibited in the cells expressing E1A indicating that, whether or not a common factor is involved, more than one signal transduction pathway is affected (A. Ackrill, G.R. Stark and I.M. Kerr, unpublished).

The mechanisms of inhibition by terminal protein and E1A are not the same. There is no homology between the proteins, and they affect different aspects of the response. In the case of terminal protein, the production or activation of the Eα subunit of the transcription factor E is inhibited whereas with E1A it is the production or activation of the Eγ subunit that is inhibited. It will be of considerable interest to determine the biochemical basis for these inhibitions. It will, of course, also be of interest and of clinical importance to determine how widespread and various are the mechanisms employed by different viruses to counter the production of and response to the IFNs.

REFERENCES

[1] Williams, B.R.G. (1991) Eur. J. Biochem. (in press).
[2] Levy, D. and Darnell, J.E. (1990) The New Biologist 2, 923-928.
[3] Pfeffer, L.M. and Tan, Y.H. (1991) Trends Biochem. Sci. (in press).
[4] Aguet, M., Dembic, Z. and Merlin, G. (1988) Cell 55, 273-280.
[5] Uze, G., Lutfalla, G. and Gresser, I. (1990) Cell 60, 225-234.
[6] Wiel, J., Epstein, C.J., Epstein, L.B., Sedmak, J.J., Sabran, J.L. and Grossberg, S.E. (1983) Nature 301, 437-439.
[7] Larner, A.C., Chaudhuri, A. and Darnell, J.E. (1986) J. Biol. Chem. 261, 453-459.
[8] Akai, H. and Larner, A.C. (1989) J. Biol. Chem. 264, 3252-3255.
[9] Schneck, J., Rager-Ziswan, B., Rosen, O.M. and Bloom, B.R. (1982) Proc. Natl. Acad. Sci. USA 79, 1879-1883.
[10] Faltyneck, C.R., Princler, G.L., Gusella, G.L., Varesio ,L. and Radzioch, D. (1989) J. Biol. Chem. 264, 14305-14311.
[11] Reich, N.C. and Pfeffer, L.M. (1990) Proc. Natl. Acad. Sci. USA 87, 8761-8765.
[12] Pestka, S., Langer, J.A., Zoon, K.C. and Samuel, C.E. (1987) Annu. Rev. Biochem. 56, 727-777.
[13] Benech, P., Mory, Y., Revel, M. and Chebath, J. (1985) EMBO J. 4, 2249-2256.
[14] Friedman, R.L., Manley, S.P., McMahon, M., Kerr, I.M. and Stark, G.R. (1984) Cell 38, 745-755.
[15] Luster, A.D., Unkeless, J.C., Ravetch, J.V. (1985) Nature 315, 672-676.
[16] Reich, N., Evans, B., Levy, D., Fahey, D., Knight, E. and Darnell, J.R. (1987) Proc. Natl. Acad. Sci. USA 84, 6394-6398.
[17] Hug, H., Costas, M., Staeheli, P., Aebi, M. and Weissmann, C. (1988) Mol. Cell. Biol. 8, 3065-3079.
[18] Porter, A.C.G., Chernajovsky, Y., Dale, T.C., Gilbert, C.S., Stark, G.R. and Kerr, I.M. (1988) EMBO J. 7, 85-92.
[19] Levy, D.E., Kessler, D.S., Pine, R., Reich, N. and Darnell, J.E. (1988) Genes and Develop. 2, 383-393.
[20] Cohen, B., Peretz, D., Varman, D., Benech, P. and Chebath, J. (1988) EMBO J. 7, 1411-1419.
[21] Rutherford, M.N., Hannigan, G.E. and Williams, B.R.G. (1988) EMBO J. 7, 751-759.
[22] Reid, L.E., Brasnett, A.H., Gilbert, C.S., Porter, A.C.G., Gewert, D.R., Stark, G.R. and Kerr, I.M. (1989) Proc. Natl. Acad. Sci. USA 86, 840-844.
[23] Lew, D.J., Decker, T., Strehlow, I. and Darnell, J.E. (1991) Mol. Cell. Biol. 11, 182-191.
[24] Ackrill, A.M., Reid, L.E., Gilbert, C.S., Gewert, D.R., Porter, A.C.G., Lewin, A.R., Stark, G.R. and Kerr, I.M. (1991) Nucleic Acids Res. 19, 591-598.
[25] Klar, D. and Hammerling, G.J. (1989) EMBO J. 8, 475-481.
[26] Yang, Z., Sugawara, M., Ponath, P.D., Wessendorf, L., Banerji, Y.L. and Strominger, J.L. (1990) Proc. Natl. Acad. Sci. USA 87, 9226-9230.
[27] Dale, T.C., Imam, A.M.A., Kerr, I.M. and Stark, G.R. (1989) Proc. Natl. Acad. Sci. USA 86, 1203-1207.
[28] Fu, X-Y., Kessler, D.S., Veals, S.A., Levy, D.E. and Darnell, J.E. (1990) Proc. Natl. Acad. Sci. USA 87, 8555-8559.

[29] Levy, D.E., Kessler, D.S., Pine, R. and Darnell, J.E. (1989) Genes and Develop. 3, 1362–1371.
[30] Kessler, D.S., Veals, S.A., Fu, X-Y. and Levy, D.E. (1990) Genes and Develop. 4, 1753–1765.
[31] Pine, R. Decker, T., Kessler, D.S., Levy, D.E. and Darnell, J.E. (1990) Mol. Cell. Biol. 10, 2448–2457.
[32] Miyamoto, M., Fujita, T., Kimura, Y., Maruyama, M., Harada, H., Sudo, Y., Miyata, T. and Taniguchi, T. (1988) Cell 54, 903–913.
[33] Imam, A.M.A., Ackrill, A.M., Dale, T.C., Kerr, I.M. and Stark, G.R. (1990) Nucleic Acids Res. 18, 6573–6580.
[34] Baeuerle, P.A. and Baltimore, D. (1988) Science 242, 540–546.
[35] Nolan, G.P., Ghosh, S., Liou, H.C., Tempst, P. and Baltimore, D. (1991) Cell 64, 961–969.
[36] Decker, T., Lew, D.J., Mirkovitch, J. and Darnell, J.E. (1991) EMBO J. 10, 927–932.
[37] Pellegrini, S., John, J., Shearer, M., Kerr, I.M. and Stark, G.R. (1989) Mol. Cell. Biol. 9, 4605–4612.
[38] Foster, G.R., Ackrill, A.M., Goldin, R.D., Kerr, I.M., Thomas, H.C. and Stark, G.R. (1991) Proc. Natl. Acad. Sci. USA 88, 2888–2892.
[39] Kitajewski, J., Schneider, R.J., Safer, B., Munemitsu, S.M., Samuel, C.E., Thimmappaya, B. and Shenk, T. (1986) Cell 45, 195–200.
[40] O'Malley, R.P., Mariano, T.M., Siekierka, J. and Matthews, M.B. (1986) Cell 44, 391–400.
[41] Katze, M.G., De Corato, D., Safer, B., Galabru, J. and Hovanessian, A.G. (1987) EMBO J. 6, 689–697.
[42] Anderson, K.P. and Fennie, E.H. (1987) J. Virol. 61, 787–795.
[43] Reich, N., Pine, R., Levy, D. and Darnell, J.E. (1988) J. Virol. 62, 114–119.