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REVIEW

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Interferon regulatory factors: growth control and histone gene regulation – it's not just interferon anymore

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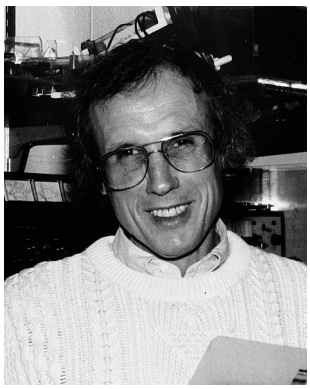
Abstract Interferon-regulatory factors (IRFs) are a related family of proteins originally identified by their ability to bind a DNA sequence found in the β-interferon gene and many interferon-stimulated genes. Two well-studied members of this family, IRF-1 and IRF-2, have antagonistic roles in interferon-β gene regulation: IRF-1 activates this gene, and IRF-2 represses the activation by IRF-1. IRF-1 and IRF-2 have more recently been linked to growth control by displaying tumor suppressor and oncogenic activities, respectively. A possible explanation for the oncogenic activity of IRF-2 is the discovery that IRF-2 can activate a histone gene that is functionally coupled to cell cycle progression. This first report of native IRF-2 playing the role of activator of a gene essential for growth may lead to the discovery of a more general involvement of interferon regulatory factors in mediating growth control.

Key words Interferon · Histone · Transcription · Cell cycle · Proliferation

Abbreviations *CAT* Chloramphenicol acetyl transferase · *CCE* Cell cycle element · *EMSA* Electrophoretic mobility shift assay · *HiNF* Histone nuclear factor · *ICSBP* IFN consensus sequence binding protein · *IFN* Interferon · *IL* Interleukin · *IRF* IFN-regulatory factor · *ISGF* IFN-stimulated gene factor · *ISRE* IFN stimulated response element · *OAS* Oligoadenylate synthetase · *PKR* Double-stranded RNA-activated protein kinase · *PRD* Positive regulatory domain · *STAT* Signal transducer and activator of transcription



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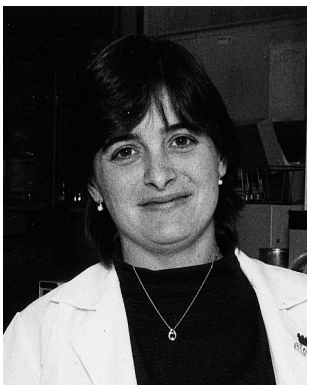

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Introduction

Interferons (IFNs) are a family of cytokines involved in the antiviral response and play an important role in cell growth and differentiation (reviewed in [1]). In particular, IFNs function as negative growth factors in many normal and transformed cell types [1]. Viral infections can induce the type I IFN (IFN-α and IFN-β) genes whose products can in turn induce a group of IFN inducible genes (Table 1), some of which are presumably responsible for the antiproliferative and antiviral effects of IFNs. IFNs are also induced to some extent by growth factors, which suggests that they are participants in a feedback loop mechanism of growth control [17–21].

The IFN-β gene has been widely studied as a model gene to dissect the IFN response and to understand transcriptional induction and repression (see review by Mania-

Table 1 Interferon responsive gene products[a]

Gene product	Function	Reference
HLA class I, II	MHC antigens	2, 3
2′-5′ OAS	Antiproliferative	4
RNase L	Antiproliferative	4
PKR	Antiproliferative	4
Guanylate binding protein	Guanylate binding	5
ISG54	Unknown	6
ISG15	Unknown	7
56 kDa protein	Unknown	8
6–16	Unknown	9
9–27	Unknown	9
IP-10	Cytokine	10
Complement factor D	Humoral immunity	11
β_2-Microglobulin	Associated with MHC class I	12
Mx (mouse)	Antiviral	13
Inducible nitric oxide synthetase	Antiviral	14
IRF-1	Tumor suppressor	15
IRF-2	Oncogene	16

[a] All gene products are human unless otherwise noted

tis et al. [22]). IFN-β mRNA levels increase over 2000-fold within hours of virus infection and then rapidly decrease. Following this transient increase in IFN-β mRNA the IFN-β protein is secreted and binds a specific cell surface receptor, thus initiating a signal transduction cascade that leads to the activation of a large number of genes.

The induction of the IFN-β gene has been shown to occur at the transcriptional level, and the positive and negative regulatory elements required for this on/off switch are located within 200-bp immediately upstream of the start site of transcription [23–30]. The mechanism of viral induction is thought to involve the inactivation or displacement of cellular repressor proteins and the production of virus inducible cellular factors that bind to the positive regulatory elements. Several protein factors have been identified that interact with these *cis*-acting sequences, including ATF-2, NF-κB, HMGI(Y), positive regulatory domain (PRD) binding factors, and the IFN-regulatory factor (IRF) family of proteins [15, 16, 22, 31–36].

The interferon regulatory factor family of proteins: primary regulators of the IFN response or functional redundancies?

IRF-1 and IRF-2

IRF-1 was the first member of the IRF family to be isolated and characterized [15]. IRF-1 binds to PRD I and PRD III of the IFN-β gene. Point mutations that reduce IRF-1 binding significantly reduce the level of induction by virus, suggesting a role for this binding activity in IFN induction [15, 37]. Recombinant IRF-1 has been shown to stimulate transcription of promoters containing multimerized PRD I binding sites as well as the endogenous IFN-α and IFN-β genes in transient transfection assays [16, 38]. IRF-1 is nearly identical (except for three amino acids) to an independently cloned protein designated IFN-stimulated gene factor (ISGF) 2, which is reported to bind the IFN-β promoter but is not a primary activator of endogenous IFN-β [39].

IRF-1 is maintained at nearly undetectable levels in unstimulated cells but is itself induced by IFN and by viral infection, which suggests a role for IRF-1 in the viral induction of IFN-β [15, 39, 40]. However, no new protein synthesis is required for viral induction of IFN transcription, as evidenced by the insensitivity of this process to cycloheximide [1]. The induction of IRF-1 by virus is dependent on new protein synthesis and therefore sensitive to cycloheximide, implying that IRF-1 is not absolutely required for the viral induction of IFN-β [39]. Nevertheless, IRF-1 does appear to play a role in IFN-β transcription as the presence of IRF-1 augments the level of viral induction of IFN [39].

Shortly after IRF-1 was discovered, a highly related protein was isolated by cross-hybridization with IRF-1 cDNA. This protein, designated IRF-2, is homologous to IRF-1 at its amino-terminus and binds PRD I with the same affinity as IRF-1 [16]. IRF-2 was later shown to be identical to ISGF1 and PRD I BF_c, two constitutive factors that bind the IFN-β promoter [41, 42]. As with IRF-1, IRF-2 is inducible by virus and IFN, albeit with a slower rate of induction than IRF-1, but has a function distinct from that of IRF-1 [16]. In transient transfection experiments IRF-2 alone has no effect on the IFN-β promoter, but it can antagonize the IRF-1 mediated activation of this promoter [16].

In unstimulated cells IRF-2 protein levels are approximately tenfold higher than IRF-1, due in part to the much longer half life of IRF-2 protein (8 h versus 30 min for IRF-1) [43]. In response to IFN there is a transient increase in IRF-1 levels, suggesting that one level of control of the IFN promoter is the competition between IRF-1 and IRF-2 [43]. Another level of control may involve the IFN-inducible and cycloheximide-insensitive processing of IRF-2 to a truncated form. This truncated protein, characterized by several groups and designated PRD I BF_i, TH3, or In 4, no longer antagonizes IRF-1 activation of IFN-β, and in some reports high levels of this factor parallel IFN-β induction [42, 44–46].

Interrelationships of IRFs and cellular signaling mechanisms

Other members of the IRF family (Table 2), based on their homology to IRF-1, include ISGF3γ, the DNA binding protein of the signal transducer and activator of transcription (STAT) 1, 2 complex, which is a primary regulator of IFN-γ stimulated genes, and IFN consensus sequence binding protein (ICSBP), an IFN inducible protein that binds an element in MHC class I genes [48, 49]. These proteins share homology with the IRFs in their amino-termini and bind similar recognition sequences, although ISGF3γ binds only IFN-stimulated genes and

Table 2 Interferon regulatory factor family members

Protein	Function	Reference
IRF-1 (ISGF2)	Activator	15
IRF-2 (ISGF1, PRDI-BF_c)	Repressor/activator	16, 47
ISGF3γ	DNA binding of STAT	48
ICSBP	Repressor	49
Pip/LSIRF/ICSAT	Repressor/activator	51–53
cIRF-3 (chicken)	Unknown	54
Truncated IRF-2 (TH3, PRDI-BF_i, In4)	Activator?	42, 44–46

not the IFN-β promoter itself. ISGF3γ is also implicated in IFN-α mediated induction of IFN-responsive genes, although recently an ISGF3-independent signaling mechanism for IFN-α induction of IRF-1 has been described [50]. ICSBP is predominantly expressed in cells of the lymphoid/macrophage lineage and, as IRF-2, can antagonize transcriptional activation by IRF-1 [49]. An avian IRF family member has recently been cloned which is rapidly and transiently induced by double-stranded RNA. Although the protein product cIRF-3 can bind an IFN-stimulated response element, no functional studies have yet been carried out to examine its role in the IFN response. The newest members of the mammalian IRF family are Pip/LSIRF and ICSAT, which are lymphoid-specific proteins from mouse and human, respectively [51–53]. These proteins, as ICSBP and IRF-2, can abrogate the stimulatory effect of IRF-1 or IFN [51–53]. Interestingly, Pip/LSIRF has also been shown to be a transcriptional activator in the presence of a second protein, PU.1 [51].

Functional diversity based on selective heterogeneity

The IRF family members are grouped together because of the high homology of their amino-termini. This region has been shown to contain the DNA binding domain of all these proteins. The C-terminal regions are very diverse among the IRF family members. The carboxyl half of IRF-1 contains an activation domain [38]. There is some debate as to the functional domains of IRF-2. The IFN-inducible, truncated IRF-2 proteins mentioned above have in most cases been described as having lost the ability to repress IRF-1 activation [42, 44–46]. However, one report suggests that a truncated form of IRF-2 is actually a stronger repressor [55]. These conflicting results may be explained by the observations of Yamamoto et al. [47]. They report that the C-terminus of IRF-2 possesses a repression domain which when fused to a generic DNA binding domain can repress IRF-1 or other activators. The deletion of this repression domain converts IRF-2 to an activator of transcription [47]. Therefore the inducible processing of IRF-2 may lead to deletion of the repression domain and possibly expose a latent activation domain. Under some conditions only the DNA binding domain of IRF-2 remains after processing, and this domain has been reported as a potent repressor of IRF-1 [55, 56]. Consequently IRF-2 may exert its repressive effect in two ways: (a) by silencing nearby activator proteins and (b) by competing with IRF-1 for its cognate site. The reported latent activation domain of IRF-2 also suggests that there may be some biological situations in which IRF-2 acts as a transcriptional activator.

Role of IRFs in proliferation: a feedback loop of growth control?

As mentioned above, IFNs are known regulators of cell growth and differentiation, showing antiproliferative activity in many cell types [1]. The IFNs exert their activities by inducing a number of proteins (Table 1), some of which appear to be regulators of growth control. These include a double-stranded RNA-activated protein kinase (PKR), RNaseL, IRF-1, and IRF-2 [4, 15, 16].

IRF-1 has been shown to be regulated by a number of cytokines in addition to IFN-α, IFN-β, and IFN-γ, [e.g., prolactin, tumor necrosis factor, leukemia inhibitory factor, interleukin (IL) 1 and 6] which would imply a general role for IRF-1 in growth control [15, 40, 57, 58]. Consistent with this hypothesis is that IRF-1 itself displays antiproliferative properties both in vivo and in vitro [58, 59]. IRF-2 in turn is regulated in part by IRF-1, suggesting a feedback loop of gene regulation [60, 61].

IRF knockout mice reveal limited roles for IRF-1 and IRF-2

To examine the relative contributions of IRF family members to the IFN response and growth control, mice devoid of IRF-1 or IRF-2 were generated by targeted gene disruption [62, 63]. IRF-$1^{-/-}$ mice showed normal development and reproductive behavior and had no obvious changes in their internal organs. However, some very discrete phenotypic changes were observed. A severe reduction in $CD8^+$ cells was observed in these IRF-1 deficient mice, suggesting that IRF-1 is necessary for proper T-cell development [62]. Embryonic fibroblasts from IRF-$1^{-/-}$ mice display a normal level of type I IFN induction except under certain induction conditions [e.g., poly(I):poly(C) mediated induction], suggesting that there are IRF-1 dependent and independent mechanisms for IFN induction [62, 63]. In vivo these mice show no changes in the inducibility of type I IFNs, and no decrease in the antiviral activity of the serum is seen in response to most viruses (with the exception of encephalomyocarditis virus, which causes accelerated mortality in IRF-$1^{-/-}$ mice) [62–64]. These results corroborate earlier findings that IRF-1 activity is not required for the induction of type I IFNs.

The phenotype of IRF-$2^{-/-}$ mice supported a role for IRF-2 in growth control. These mice displayed physical susceptibilities, including a relatively high frequency of premature death and death after parturition [62]. Lymphocytic choriomeningitis virus infection leads to death in IRF-$2^{-/-}$ mice, and they also display abnormal B lym-

phopoiesis and hematopoiesis. The role of IRF-2 as a repressive factor in type I IFN gene regulation was confirmed as the level of IFN-α and IFN-β mRNAs was shown to be enhanced in IRF-2 deficient cells which were induced by a specific virus [62]. However, induction of IRF-2$^{-/-}$ fibroblasts by dsRNA resulted in no change in IFN levels, again suggesting multiple pathways for IFN gene regulation and/or a functional redundancy between IRF-2 and other factors, possibly ICSBP or Pip/LSIRF [62]. Furthermore, neither IRF-1 or IRF-2 is essential for the regulation of several type I IFN stimulated genes [e.g., 2′-5′ oligoadenylate synthetase (OAS), H-2K^{b}, and PKR], reaffirming the hypothesis that the IRFs are not primary regulators of these genes [62].

IRFs and programmed cell death

The embryonic fibroblasts from IRF-1$^{-/-}$ mice were useful in demonstrating a role for IRF-1 in programmed cell death. Expression of the c-Ha-*ras* oncogene combined with a block to cell proliferation caused wild-type cells but not IRF-1$^{-/-}$ cells to undergo apoptosis [65]. This characteristic is reminiscent of the tumor suppressor p53 which is required for *ras*-induced apoptosis [66]. p53 has also been shown to be involved in DNA damage induced apoptosis in thymocytes but not mature T-lymphocytes [67]. However, mature T-cells do require IRF-1 for DNA damage induced apoptosis, suggesting at least two apoptotic pathways in T-lymphocytes [68]. The IRF-1 mediated pathway may be manifested through induction of the mammalian cell death gene, *Ice* (interleukin-1β converting enzyme) [69, 70]. *Ice* mRNA levels are lower in IRF-1$^{-/-}$ embryonic fibroblasts than in wild type, and overexpression of IRF-1 leads to induction of *Ice* [68]. Furthermore, an IRF element has been found in the promoters of both the mouse and human *Ice* genes [68]. IRF-1 and p53 also appear to be involved in the DNA damage-induced activation of the cell-cycle inhibitor gene *p21/WAF1/CIP1* [71].

The role of IRFs in cancer: IRF-1 is a tumor suppressor and IRF-2 has oncogenic potential

IRF-1 mRNA and protein levels oscillate during the NIH 3T3 growth cycle following arrest by serum starvation and subsequent restoration of serum [72]. The highest levels of IRF-1 protein and mRNA are seen during growth arrest, and a smaller peak is seen prior to the onset of DNA synthesis. In contrast, IRF-2 mRNA levels remain constant during the NIH 3T3 cell cycle [72]. During the cell cycle of prolactin-induced lymphocytes IRF-1 mRNA shows two peaks of activity, in early G_1 and later at the G_1/S boundary [73].

To examine the effect of disturbing the IRF-1/IRF-2 ratio IRF-2 was overexpressed in NIH 3T3 cells. These cells grew to a high cell density, displayed anchorage-independent growth and caused tumor formation when injected into nude mice [72]. Therefore an oncogenic potential was ascribed to IRF-2. Deletion analysis of IRF-2 demonstrated that the amino-terminal 160 amino acids, which contain the DNA binding domain, are sufficient for this oncogenic activity [74]. When IRF-1 is introduced into the IRF-2 expressing NIH 3T3 cells, the tumorigenicity is suppressed, with the extent of suppression dependent on the level of IRF-1 expression [72]. Accordingly, IRF-1 has been designated a tumor suppressor. This activity is not limited to IRF-2 induced transformation as the introduction of IRF-1 can also suppress cellular transformation induced by c-Ha-*ras*, c-*myc*, or *fosB* [65, 75]. Furthermore, the deletion of the IRF-1 locus on human chromosome 5 has been implicated in many types of human leukemias, although one report suggests that the IRF-1 locus is not within the 5q region consistently lost in these diseases [76, 77]. It may be that IRF-1 is merely one of numerous tumor suppressors that are located within a small region of chromosome 5.

Multiple mechanisms of IRF-2 mediated tumorigenicity

One explanation for the oncogenic activity of IRF-2 is that overexpression of IRF-2 induces transformation by antagonizing the antiproliferative properties of IRF-1. IRF-1 is involved in the activation of many IFN-stimulated genes in vitro, some of which are implicated in the inhibition of cell proliferation (e.g., 2′-5′ OAS, PKR and lysyl oxidase; Table 3). The observation that the overexpression of the N-terminal half of IRF-2 is sufficient to achieve a transformed phenotype supports the notion that IRF-2 represses the activity of IRF-1 by simply competing for the same DNA recognition site on these proliferation-specific genes [74]. This explanation may be valid for lysyl oxidase, a putative tumor suppressor, which is downregulated in IRF-1$^{-/-}$ embryonic fibroblasts and NIH 3T3 cells overexpressing IRF-2 [80]. However, the data from IRF-1$^{-/-}$ fibroblasts suggesting that IRF-1 is not the principal activator of 2′-5′ OAS and PKR do not support a simple competition model to explain IRF-2 tumorigenicity [62, 64, 92].

Another interpretation of the oncogenic properties of IRF-2 which is not mutually exclusive with that presented above is that IRF-2 is involved in the activation of genes critical for cell proliferation. This theory is supported by the slightly abnormal growth phenotype of the IRF-2$^{-/-}$ mice described above and by the recent observation that IRF-2 can activate a histone gene which is functionally coupled to cell cycle progression [93].

IRFs and cell cycle regulated histone gene expression: the missing link?

Multiple histone genes undergo numerous levels and mechanisms of cell cycle regulation

The histones are a family of proteins that are absolutely required for the packaging and maintenance of intact chromosomes. The production of histone proteins is tightly coupled to the cell cycle, with maximal accumu-

lations appearing coincidentally with DNA replication during S phase (for reviews see [94–97]). The replication-dependent core histones H2A, H2B, H3, H4, and the linker histone H1 are all members of multigene families in eukaryotic cells. The expression of most histone genes is regulated during the cell cycle at both the transcriptional and post transcriptional levels, with histone mRNA levels increasing approximately tenfold during S phase. One-third to one-half of this increase is due to enhanced transcription and the remainder to processing of immature mRNAs in the nucleus and increased stability of histone mRNAs in the cytoplasm [94].

The phenomenon of coordinated up-regulation of the multiple histone genes during S phase prompted a search for a responsible common element among the histone genes. The promoters of replication-dependent histone genes show a modular organization of regulatory elements similar to other RNA polymerase II transcribed genes [98]. These elements include a TATA box and binding motifs for other general transcription factors such as ATF and SP1 [98–101]. However, the organization of upstream regulatory elements varies widely among the various classes of histone genes and even within a particular class of histones. A histone family specific hexamer is present in most histone gene promoters examined thus far and has been considered a likely candidate for a cell cycle control element [98]. It is now known that this region is responsible only for maximal transcriptional levels and does not appear to be involved in cell cycle fluctuations [94]. There are also elements that are common to a particular class of histone genes. The H1-, H2B-, and H3-specific elements are implicated in the cell cycle regulation of these genes [102–104].

The fact that each histone gene class has its own regulatory elements indicates that the transcriptional control of the histone genes is a much more complex situation than was previously imagined. The higher level of complexity may be due to the need for variable histone subtype specific expression in different cell types. Further insights into the details of each subtype specific regulation may elucidate the underlying cause for the coordinated synthesis of all the histone genes. It is suspected that although each histone subclass has its own DNA element and corresponding binding proteins, there is a common mechanism of regulation of these factors.

Organization of a cell cycle regulated human histone gene: a paradigm for proliferation specific and S phase enhanced transcription

Several regions in the 5′ flanking region of the human H4 histone gene FO108 are involved in protein-DNA interactions that affect the overall transcription of this gene (Fig. 1) [105]. These regions were identified through both in vivo and in vitro techniques. In vivo genomic footprinting and fingerprinting techniques identified two protected regions: Site I, the distal promoter element, between positions –130 and –87 bp; and Site II, the proximal element, between –70 and –20 bp, which includes the TATA box between –32 and –28 (Fig. 1a) [89]. These are the only protein-DNA binding sites determined in vivo for a histone H4 gene and therefore are the only interactions with demonstrated biological relevance. Both Site I and Site II are protected from DNase I throughout the HeLa cell cycle [89]. However, in vivo footprinting of terminally differentiated HL-60 cells in which histone synthesis is completely shut down showed that Site II alone was no longer occupied [106]. These results suggest that Site II is important for the cell growth related regulation of histone H4 gene transcription.

Identification of a histone H4 cell cycle promoter element

Promoter elements of the H4 gene FO108 have been further dissected through expression studies. Deletion and site-directed mutants of histone promoter–chloramphenicol acetyl transferase (CAT) fusion genes were constructed and established as stable cell lines [107]. Transcription levels were analyzed at various stages during the cell cycle of synchronized HeLa cell cultures. These studies established that the promoter region of the FO108 gene (approximately 1 kb of 5′ flanking region), when fused to the CAT gene, was capable of initiating CAT gene expression and conferring a two- to threefold elevation in transcription of the CAT gene during S phase, comparable to endogenous H4 genes (Fig. 1b) [107]. A series of 5′ promoter deletions showed that regions far upstream (–1018 to –216) were not involved in the cell cycle regulation of this gene but had a threefold effect on the overall level of transcription [107]. Deletion of nucleotides –215 through –71, which includes Site I, had a drastic effect on the levels of transcription but no effect on the cell cycle regulation of this gene (Fig. 1b) [89, 107]. The most proximal promoter site, Site II, appears to be sufficient for the enhanced transcription of this gene during S phase (Fig. 1b). Further deletions of nucleotides –70 through –41 result in a loss of regulated transcription of FO108, suggesting that the distal portion of Site II is critical for the cell cycle regulation of this gene [107].

At this stage point mutations were introduced to establish more precisely which nucleotides in Site II are required for the regulated transcription of this gene. Nucleotides which had been shown in vivo and in vitro to be protein contact sites were mutated in clusters. Mutations within an 11-bp element resulted in the abrogation of cell cycle regulation of human histone H4 gene FO108, implying that this region is a cell cycle element (CCE) [107]. This element had been previously defined as a protein-DNA interaction site, termed the M-box, and the protein factor involved was designated histone nuclear factor (HiNF) M [108, 109].

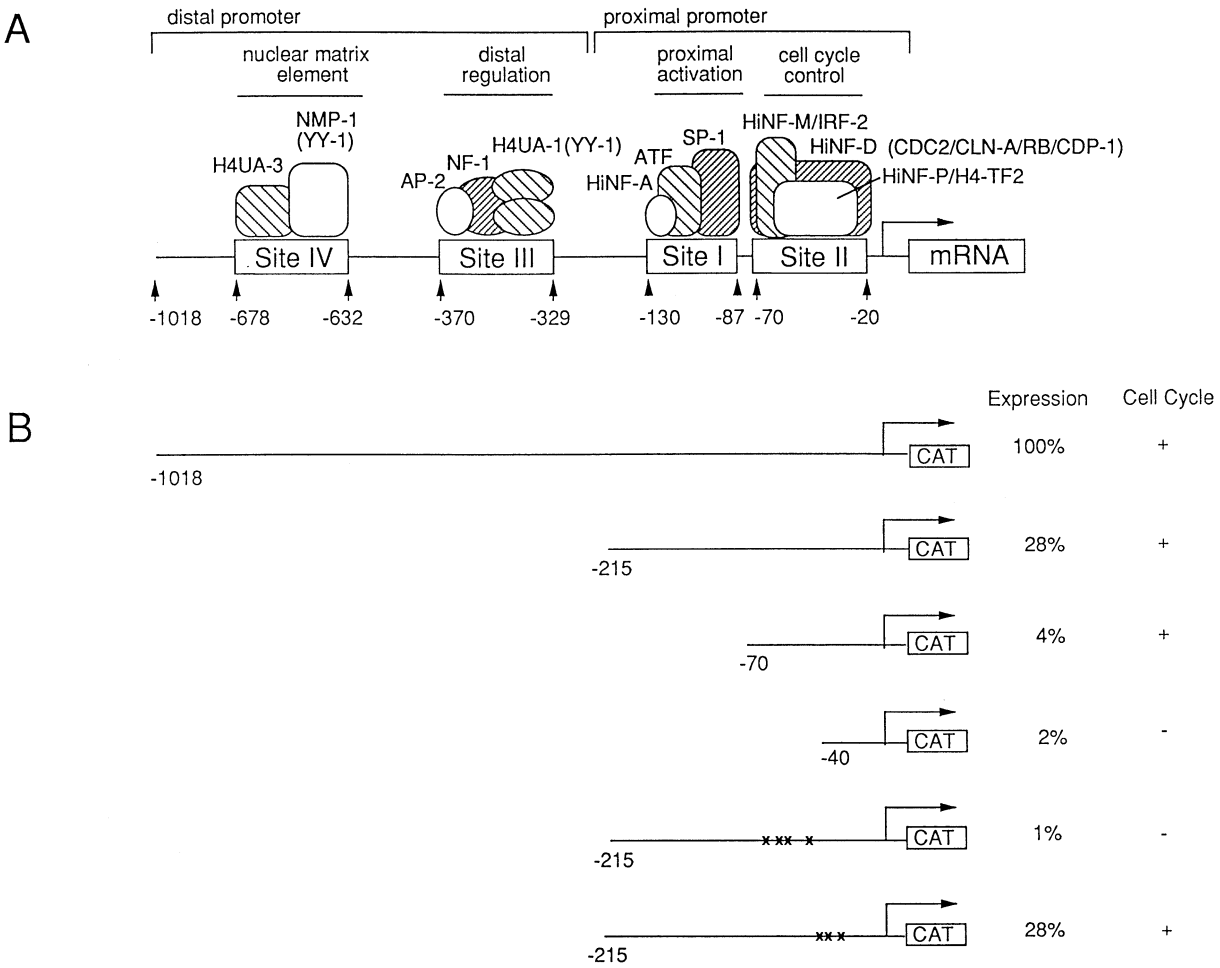

Fig. 1 **A** Schematic diagram of the FO108 H4 histone gene promoter showing locations of regulatory sequences and sites of protein/DNA interactions (for review see [97, 105]). **B** Summary of deletion/mutation analysis studies to identify the cell cycle element of histone H4 described in [107]

Histone nuclear factor M is critical for the cell cycle regulation of histone H4

Three distinct protein-DNA interactions at Site II have been identified in vitro using nuclear extracts (Fig. 1a). HiNF-D is a mosaic factor of several proteins and shows sites of nuclease protection and nucleotide interactions spanning the entire Site II region [108–110]. Some of the proteins involved in factor D binding have recently been identified and consist of cdc2, cyclin A, a retinoblastoma protein family member, and CDP/*cut*, which is the DNA binding moiety [111, 112]. The two other protein-DNA interactions at Site II, HiNF-M and HiNF-P (which may be identical to H4TF-2) make contacts within the HiNF-D binding region but appear to be distinct entities [108, 109, 113]. HiNF-M therefore acts within an element that supports interactions with cell cycle regulatory factors and phosphorylation signaling pathways.

The importance of HiNF-M has been confirmed by binding studies with mutated Site II fragments. Fragments containing point mutations which abolish cell cycle regulation could no longer interact with HinF-M or HiNF-P but were still bound by HiNF-D, although the binding was reduced [107]. Point mutations which abolished HiNF-P binding alone had no effect on cell cycle regulation. This suggests that cell cycle regulation of histone H4 is related to HiNF-M binding at Site II. Recent observations from our laboratory indicate that binding site mutations that affect any of the Site II factors alone have very little effect on the cell cycle regulation of histone H4, suggesting a complex level of control involving multiple factors. Unraveling of this complicated situation may require studies involving the withdrawal of the various Site II factors.

IRF-2 is the cell cycle element binding factor and a key regulator of histone H4

HiNF-M, the CCE binding factor, was purified from HeLa cell nuclear extracts and found to be a protein with an apparent molecular weight of 48 kDa. Microsequencing of four internal peptides of HiNF-M showed identity with IRF-2 [93]. Upon examination of the HiNF-M binding site, the CCE, it was discovered to be highly homologous to the IFN-stimulated response element (ISRE) and the IRF consensus element determined by oligonucle-

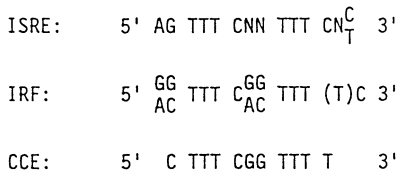

Fig. 2 The histone H4 CCE has high homology to IFN regulatory elements. The ISRE as reported in [114], the IRF element as reported in [115], and the CCE of H4 histone gene FO108 [107]

otide binding site selection (Fig. 2) [107, 114, 115]. HiNF-M has been shown to bind the ISRE, and recombinant IRF-1 and IRF-2 can bind the CCE in an electrophoretic mobility shift assay (EMSA) [93]. Specific antibodies to IRF-2, and not IRF-1, are immunoreactive with the HiNF-M complex [93]. This result in itself does not preclude a role for IRF-1 in histone gene regulation as IRF-1 is not readily detectable by EMSA in uninduced cells [15].

To assess the functional role of the IRFs on histone gene regulation transient transfections were performed in several cell types by coexpressing IRF-1 or IRF-2 with an H4 histone promotor CAT construct. Surprisingly, both IRF-1 and IRF-2 stimulated transcription of this promoter but not a promoter containing a mutation in the HiNF-M/IRF binding site [93]. Unlike the IFN-β and IFN-stimulated gene promoters, IRF-2 did not repress the IRF-1 stimulatory effect. Some of the studies were carried out in IRF-$1^{-/-}$, IRF-$2^{-/-}$, or IRF-$1^{-/-}$/IRF-$2^{-/-}$ (double-knockout) embryonic fibroblasts. One important finding with these cells was that endogenous IRF-1 is incapable of compensating for the lack of IRF-2 in IRF-$2^{-/-}$ cells, suggesting that IRF-2 is the key IRF involved in histone gene regulation [93].

Further evidence for the importance of IRF-2 specifically in H4 gene regulation was shown in a cell cycle analysis of the FDC-P1 myeloid stem cell line. Following isoleucine deprivation and subsequent cytokine stimulation two distinct, differentially regulated HiNF-M complexes were observed in an EMSA, and both complexes were immunoreactive with IRF-2 but not IRF-1, antibodies [93, 116]. The level of the more slowly migrating form of HiNF-M/IRF-2 peaked at the G_1/S boundary. Since previous results indicate that IRF-2 mRNA levels do not change during the cell cycle [72], this result suggests that a posttranslational modification of IRF-2 or a variation in partner proteins of IRF-2 is occurring during the cell cycle. Therefore IRF-2 may play an intrinsic role in the cell cycle control of histone H4 gene regulation. This effect may not be limited to a single human histone gene as potential IRF elements can be found in several mammalian histone genes (Table 3). This result may represent a key link between IRF-2 and a gene required for growth, thereby suggesting a mechanism for the oncogenic activity of IRF-2, and may help elucidate a mechanism for coordinate control of genes at the G_1/S transition. However, a disregulation of histone H4 has not yet been examined in cells with abnormal levels of IRF-2; therefore it will be interesting to monitor the levels of H4 histone in IRF-$2^{-/-}$ and IRF-2 transformed fibroblasts.

Table 3 IRF binding sites in cell growth related genes[a]

Gene	Sequence	Reference
IRF-2	ATTTTCATTTTC	60, 61
2′-5′ OAS	GGTTTCGTTTC	78
PKR (mouse)	TGTTTCGTTTTC	79
Lysyl oxidase (mouse)	ATTTTCACTTTG	80
Ice	ACTTTCAGTTTC	81
IL-4	GGTTTCATTTC	82
IL-5	CATTTCCATTTC	83
IL-7 receptor	CTCTTCCATTTC	84
p53	GCTTTGCGTTTG	85
E-cadherin (mouse)	GGTTTCCGTTTTG	86
H4/a	CTTTTCAGTCTC	87
H4.A (distal)	TATTTCGGTTTG	88
H4.A (proximal)	TCTTTCAGGTTCT	88
H4/h	GCTTTCAGTCTTC	87
H4-FO108	GCTTTCGGTTTTC	89
H4-AST (mouse)	GCTTTCAGTTTTC	90
H1.2 (HB1)	TAATTCTGTTTC	91
H1.2 (HB2)	ACTTTCTGTTTT	91

[a] All genes are human unless otherwise noted

Conclusions and perspectives: pleiotropic regulatory roles of IRFs

The studies discussed above suggest that IFN regulatory factors are not solely involved in the IFN response and in fact may not be essential for this activity. Overexpression of IRF-1 can result in an induction of endogenous type I IFN mRNA levels while the expression of ISGF2 (which is nearly identical to IRF-1) does not affect IFN-β message [15, 39]. Furthermore, IFN-β mRNA levels increase with viral induction in the presence of cycloheximide, which prevents induction of IRF-1 protein [1, 39]. IRF-1 therefore does not appear to be vital for IFN-β induction and may share redundant functions with another activation factor(s).

The phenotype of IRF-$1^{-/-}$ mice also suggests that there are multiple mechanisms of type I IFN induction: IRF-1 dependent and IRF-1 independent pathways. Some IFN-stimulated genes are unaffected in IRF-$1^{-/-}$ or IRF-$2^{-/-}$ mice (2′-5′ OAS, H-2K^b, and PKR) while others are severely impaired (inducible nitric oxide synthase, guanylate binding protein, and lysyl oxidase) [62, 64, 80, 92, 117, 118]. The mechanism by which the IFN response is induced [i.e., poly(I):poly(C) or virus] can affect the level of response in IRF-$1^{-/-}$ or IRF-$2^{-/-}$ mice, and different viruses can also lead to different effects [62, 64]. IRFs are important for the antiviral action of IFNs against some viruses, but the IFN response is obviously quite diverse, utilizing multiple pathways through many target genes. These target genes may be activated using redundant mechanisms involving the various IRF family members and/or other unknown activators and re-

pressors that may be expressed when IRF-1 and IRF-2 are unavailable.

The role of IRFs in growth control is becoming clearer. Overexpression of IRF-2 in NIH 3T3 cells leads to an oncogenic phenotype, and coexpression of IRF-1 suppresses the tumorigenicity of IRF-2 [72]. IRF-1 has been mapped to human chromosome 5q31.1, a region that is frequently deleted in certain leukemias and myelodysplastic syndromes [76]. Other evidence for the importance of IRFs in cell growth is the result from IRF knockout mice that lymphopoiesis and hematopoiesis are impaired in IRF-$1^{-/-}$ and IRF-$2^{-/-}$ mice, respectively [62].

Recent evidence suggests a role for IRF-1 in programmed cell death as IRF-1 appears to be necessary for Ha-*ras* oncogene-induced apoptosis [65]. IRF-1 has also been implicated in DNA damage-induced apoptosis in mature T lymphocytes [68]. This phenomenon may be related to the reported induction of the mammalian cell death gene, *Ice*, by IRF-1 [68].

The regulation of genes directly involved with growth may be the mechanism by which IRFs affect growth control. It is known that IRF-1 activates PKR which possesses tumor suppressor activity (although IFN-β induction of this gene is unaffected in IRF-$1^{-/-}$ cells, suggesting redundant forms of regulation of PKR) [62, 119]. Another putative antiproliferative gene is lysyl oxidase, which may be the best candidate thus far as a target of IRF-1 [80]. IRF-2 may antagonize these activities and/or be involved in the activation of genes required for growth. There are potential IRF binding sites in many other genes involved in growth control (Table 3), some of which have antiproliferative activity, and some of which are necessary for growth. One set of the latter category are the histone genes.

Histone proteins are required for the ordered assembly of DNA into chromatin and are therefore essential for cell growth. Maintenance of intact chromosomes is such a critical function of the cell, that there are likely numerous mechanisms of maintaining proper histone production, and this control may vary in different cell types. Maintaining multiple copies of the various histone genes which are regulated by different means is one mechanism which the cell has utilized to ensure chromatin integrity. Coordinate control of the various histone classes may be regulated through a common mechanism. For example, HiNF-D binds the promoters of histones H4, H3, and H1 [111, 120]. Subtype regulation appears to involve multiple, specific factors that have limited scope. This mechanism ensures that the lack of any one particular factor would not be fatal to the cell. Many histone H4 genes have potential IRF-2 binding sites (Table 3), and IRF-2 is likely important for their regulation. However, since IRF-$2^{-/-}$ mice are viable, there must be alternative mechanisms of histone H4 production. It may be that these mice have some minor defects that are related to the lack of adequate histone H4.

These diverse types of regulation the cell employs are often achieved through posttranslational modifications such as phosphorylation and protein/protein interactions. IRF-1 is a phosphoprotein, but the role of this phosphorylation is not known [39, 43]. Members of the IRF family have been shown to interact with other proteins, including TFIIB and other IRFs [121–123]. In some cases these interactions can change the nature of a protein from an activator to a repressor. The IRF-2-like repressing protein Pip can activate certain promoters in the presence of PU.1 [51]. This phenomenon is also well documented for other transcription factors such as YY1, RAP-1, and Dorsal [124–126]. The role of phosphorylation or protein/protein interactions in IRF-2 activity has not been examined, but the close proximity within Site II of CDP/*cut*, a retinoblastoma protein related protein, cyclin A, and cdc2 (in the HiNF-D complex) provides potential mechanisms for cell cycle regulated protein/protein interactions or modification of IRF-2 by the cdc2 kinase activity [111]. This arrangement is not unique to histone H4 as the IRF-2/CDP motif is seen in other genes such as *gp91^{phox}*, a differentiation-specific gene of myelomonocytic cells [112, 127]. Interestingly, it has very recently been shown that IRF-2 transactivates *gp91^{phox}* promoter activity, again reminiscent of the histone H4 promoter [128].

In conclusion, the IRFs are multifunctional proteins involved in the IFN response, apoptosis, growth control, and possibly differentiation. The role of IRFs in these processes may not always be a primary one, but they are likely important for subtle responses by the cell in various situations. They are another example of the many redundant mechanisms which the cell has to maintain viability under diverse conditions, and further definition of the function of IRFs will help elucidate these mechanisms.

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