Deletion of *IRF-1*, Mapping to Chromosome 5q31.1, in Human Leukemia and Preleukemic Myelodysplasia

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One of the most frequent cytogenetic abnormalities in human leukemia and myelodysplasia is an interstitial deletion within chromosome 5q. A tumor suppressor gene has been hypothesized to lie in 5q31, the smallest commonly deleted region. *IRF-1*, a gene whose product manifests anti-oncogenic activity, was mapped to 5q31.1. *IRF-1* lies between *IL-5* and *CDC25C* and is centromeric to *IL-3* and *GM-CSF*. Among these genes, only *IRF-1* was consistently deleted at one or both alleles in 13 cases of leukemia or myelodysplasia with aberrations of 5q31. Inactivating rearrangements of one *IRF-1* allele, accompanied by deletion of the second allele, were also identified in one case of acute leukemia. Thus, *IRF-1* may be a critically deleted gene in human leukemia and myelodysplasia.

An interstitial deletion within the long arm of chromosome 5 [del(5q) or "5q−"] or loss of a whole chromosome 5 are among the most frequent cytogenetic abnormalities in human leukemia and the preleukemic myelodysplastic syndromes (MDS). Del(5q), initially described as the hallmark of a unique type of MDS with refractory anemia (the "5q− syndrome") (*1*), is now known to occur in 30% of patients with MDS, in 50% of patients with acute myelogenous leukemia (AML) arising secondary to MDS or prior chemotherapy, in 15% of de novo AMLs, and in 2% of de novo acute lymphocytic leukemias (ALL) (*2–4*). Although the proximal and distal breakpoints of the del(5q) vary from patient to patient, the smallest commonly deleted segment is band 5q31 (*5, 6*); rare de novo AMLs with translocations of 5q31 have also been described (*3*). Thus, a tumor suppressor gene has been hypothesized to lie in the 5q31 region.

We have now mapped the gene encoding interferon regulatory factor-1 (IRF-1) to chromosome 5q31.1 (*7*) (Fig. 1A). This protein, which functions as a transcriptional activator of *IFNα*, *IFNβ*, and other IFN-inducible genes (*8*), has recently been shown to possess growth inhibitory and anti-oncogenic activities (*9–11*). The *IRF-1* gene is itself IFN-inducible and may thus be one of the critical target genes mediating IFN action (*8*). To map *IRF-1* relative to other genes in this region, including the cytokine genes *IL-3, IL-4, IL-5,* GM-CSF (*5, 12*), and the mitotic inducer *CDC25C* (*13*), we created a physical map by pulsed-field gel electrophoresis (PFGE) (*14*). The *IRF-1* gene was determined to be approximately 200 kb telomeric to *IL-5* and 100 kb centromeric to *CDC25C*; the *IL-3* and *GM-CSF* genes were located at least 200 kb, but not more than 1600 kb, telomeric to this region (Fig. 1B).

With a full-length *IRF-1* cDNA as probe (*15*), we performed Southern blot and quantitative slot-blot analyses on DNA samples from 11 patients with the types of leukemia and MDS commonly associated with del(5q), from two patients with de novo AML who had translocations involving 5q31, and from four control individuals (Table 1). To provide an internal standard against which to quantitate *IRF-1* deletions, we hybridized each filter with a cDNA probe for the complement component 9 gene (C9), which maps to 5p13 (*16*), and determined the *IRF-1*:C9 hybridization ratio for each sample. Relative to the controls, there was a substantial reduction in the *IRF-1*:C9 ratio in each case of MDS and acute leukemia with del(5q) (Table 1). The magnitude of the reduction corresponded closely with the percentage of leukemic blasts in each sample and with the cytogenetic frequency of cells with del(5q). Unexpectedly, a substantial reduction in the *IRF-1*:C9 ratio was also seen in sample 10, a patient with de novo ALL who had no microscopically detectable del(5q) at disease presentation. A reduced ratio was also seen in each case of de novo AML with a translocation involving 5q31 (samples 12 and 13), indicating that DNA encompassing the *IRF-1* locus is lost from translocations that appeared to be balanced at the microscopic level of resolution (*3*).

To determine the extent of the del(5q) in each sample, we also performed Southern blot and quantitative slot-blot analyses with probes for *IL-4, IL-5, CDC25C,* and GM-CSF. Comparison of the extent of the deletion in each sample indicated that *IRF-1* was the only gene to be consistently deleted (*17*) (Table 1). On Southern analysis, structural rearrangements of *IRF-1* were also observed in sample 10 (Fig. 2); in contrast, no rearrangements were observed in the *IL-4, IL-5, CDC25C,* and GM-CSF genes in any sample. The deletion of several *IRF-1* restriction fragments and the appearance of novel rearranged bands in the Bgl II, Hind III, and Bam HI digests (Fig. 2B) were consistent with the presence of a deletion involving the 5′ proximal region of the *IRF-1* gene in the majority of cells in sample 10; the predominant breakpoint lies ~0.4 kb upstream of the Bgl II site in intron 1 (Fig. 2A). To localize this breakpoint, we used a modification of the polymerase chain reaction (PCR) to amplify

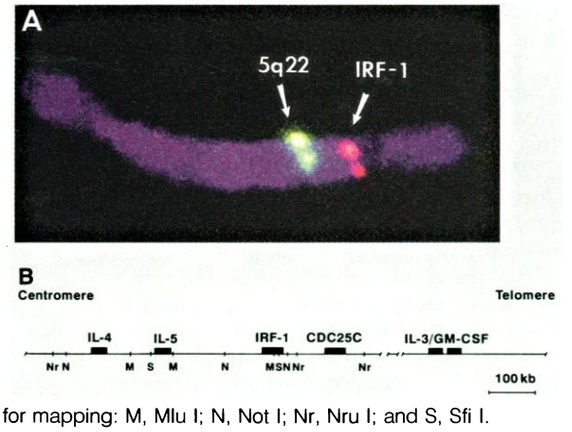

Fig. 1. (**A**) Computer-assisted fluorescence microscopic analysis of chromosome 5 from a normal metaphase hybridized with an *IRF-1* probe (red) and a unique genomic probe complementary to sequences at 5q22 (green) (*7*). The *IRF-1* gene was mapped to 5q31.1 by analyzing 15 hybridized metaphase samples and is reported as a fractional location relative to the short arm telomere of chromosome 5. (**B**) Physical map of the 5q31 chromosome region constructed by PFGE. Boxes represent coding exons of *IL-4, IL-5, IRF-1, CDC25C, IL-3,* and *GM-CSF*. Restriction enzymes used for mapping: M, Mlu I; N, Not I; Nr, Nru I; and S, Sfi I.

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Table 1. Cytogenetic, molecular, and FISH analyses of 5q31 deletions and translocations in leukemia and myelodysplasia.

Sample number	Disease*	Karyotype†	Leukemic blasts (%)‡	Hybridization ratios relative to *C9* control§					FISH analysis of *IRF-1*‖
				IL-4	*IL-5*	*IRF-1*	*CDC25C*	*GM-CSF*	
Myelodysplasias									
1	RA	46,XX,del(5)(q13q33)[10]/46,XX[2]		ND	0.63	0.36	0.76	1.2	2(66.7) 1(23.9) 0(9.4)
2	MDS	46,XY,del(5)(q13q33)[3]/46,XY[22]		ND	ND	0.62	ND	ND	2(71.9) 1(23.6) 0(4.5)
3	RA	45,XX,del(5)(q13q35),−6,+i(6p), del(7)(q22q22),−10,−12, +der(12)t(12;?)(p13;?)[4]/46,XX[14]		0.66	0.66	0.68	0.91	1.3	2(46.0) 1(48.3) 0(5.7)
4	MDS	52,X,−Y,+1,del(5)(q13q33),+8,+9, +11,i(14q),+18,+19,+22[15]		ND	ND	0.56	ND	ND	ND
Secondary leukemias									
5	RAEB → AML	40-46,XX,del(5)(q13q33), −11,+13,+16,−18,−22[20]	59	0.32	0.60	0.58	0.42	0.65	2(8.2) 1(84.5) 0(7.3)
6	RAEB → AML	46,XX,del(5)(q15q33)[25]	60	0.55	0.62	0.58	0.60	0.78	2(14.5) 1(78.1) 0(7.4)
De novo acute leukemias									
7	AML	44,XX,del(5)(q11.2q33),del(7)(q11.2), −8,−10,+der(10)t(10;11)(q22;q13), −11,+der(11),t(11;?)(q13;?),−13, +der(13)t(13;?)(q3?2;?),−16[17]/ 44,idem,del(6)(q?15;q?23)[3]	80	0.43	0.47	0.35	0.55	0.95	2(2.7) 1(86.3) 0(11.0)
8	AML	45,X,−X,der(4)t(4;?)(p16;?), del(5)(q13q33),del(8)(q21.3q24.2), del(9)(q12q32),del(17)(p11.2),dmins	80	0.46	0.54	0.35	0.91	0.82	2(1.2) 1(84.7) 0(14.1)
9	AML	46,XY,del(3)(q2?5q2?7),del(5)(q2?2q31), del(7)(q11.2),−12,+der(12)t(12;?) (p12;?)/45,idem,−7	90	0.34	0.62	0.45	0.62	0.47	ND
10	ALL: presentation	46,XX,t(4;11)(q21;q23),i(7q)[17]/46,XX[3]	75	0.93	0.94	0.55	0.91	1.08	2(55.9) 1(25.7) 0(18.4)
10	ALL: relapse	46,XX,t(4;11)(q21;q23),del(5)(q15q35), i(7q)[3]/46,XX[18]	ND	ND	ND	ND	ND	ND	ND
11	AML: presentation	46,XY,t(8;21)(q22;q22)	95	0.94	1.10	1.00	1.08	ND	ND
11	AML: relapse	46,XY,t(2;6)(p23;q25),del(5)(q31q35), t(8;21)(q22;q22)[13]/46,idem, t(13;18)(q14;q23)[3]	40	1.10	1.20	0.81	0.66	1.12	2(67.8) 1(25.7) 0(6.5)
12	AML	46,XY,t(5;13)(q31;q14),−7,del(15) (q21q26),+21[18]/46,XY[2]	95	0.53	0.54	0.46	0.52	1.08	2(2.5) 1(87.7) 0(9.8)
13	AML	46,XX,t(5;6)(q31;q21)	70	0.94	0.94	0.72	1.07	0.91	2(2.5) 1(77.3) 0(20.2)
		Selected controls							
Hematopoietic neoplasms with 5q abnormalities other than 5q31									
14	MDS/CMMoL	46,XY,t(5;12)(q33;p13),del(7)(q22q32)[20]		ND	ND	1.02	ND	ND	2(84.6) 1(15.0) 0(0.4)
15	Lymphoma	46,XX,t(2;5)(p23;q35)		1.02	1.05	1.06	1.08	1.30	2(88.2) 1(9.0) 0(2.8)
Normal tissues									
16	Blood (cryopreserved)	46,XY		1.30	1.14	1.03	1.04	1.04	2(89.7) 1(8.1) 0(2.2)
17	Bone marrow (cryopreserved)	46,XX		ND	ND	0.92	ND	ND	2(88.0) 1(8.0) 0(4.0)

*Abbreviations: MDS, myelodysplasia; RA, refractory anemia; RAEB → AML, refractory anemia with excess blasts in transformation to acute myeloid leukemia (AML); ALL, acute lymphoid leukemia; CMMoL, chronic myelomonocytic leukemia; ND, not determined. †Representative metaphase chromosome spreads were karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN, 1991). The number of cells identified with each clonal abnormality is given in brackets; idem, same as previous clone; dmins, double minutes. ‡Determined by morphological criteria. §Ratio of the hybridization signal intensity of each gene mapping to 5q31 relative to the signal obtained with the *C9* control probe. Signal intensity was determined by laser scanning densitometry; each hybridization assay was performed in triplicate. ‖In each sample, 1000 cells were scored (*7, 21*) for the number of alleles, with the frequency (%) indicated in parentheses.

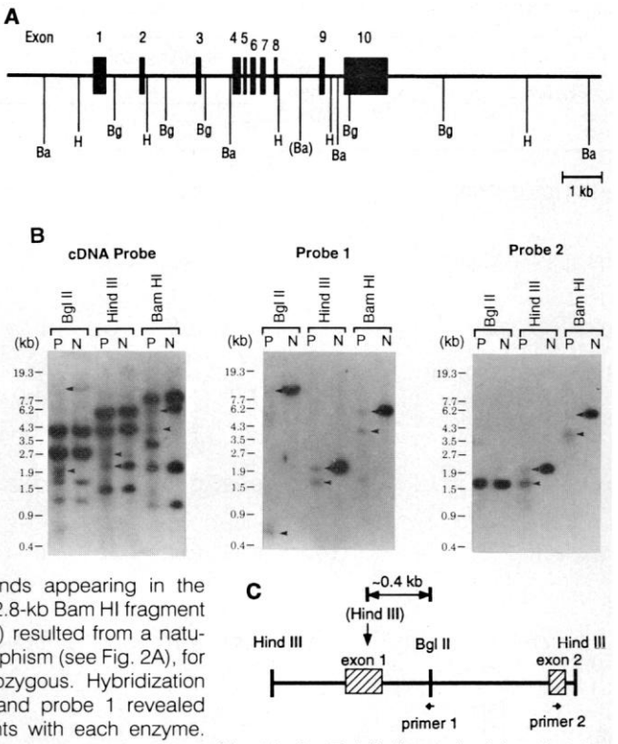

Fig. 2. Characterization of an *IRF-1* structural rearrangement in sample 10. (**A**) Exon map of the human *IRF-1* gene; positions of the exons are indicated by filled boxes. Restriction enzyme sites: H, Hind III; Ba, Bam HI; Bg, Bgl II. (Ba) indicates a polymorphic Bam HI site. (**B**) Southern blot analysis of genomic DNA from normal nonleukemic DNA (N) and patient sample 10 (P). The same filter was hybridized with a full-length *IRF-1* cDNA probe (*15*), probe 1 (the 1.0-kb Hind III–Bgl II fragment containing *IRF-1* exon 1), and probe 2 (the 1.0-kb Bgl II–Hind III fragment containing *IRF-1* exon 2). Arrowheads indicate deletions and novel bands appearing in the leukemic DNA. The additional 2.8-kb Bam HI fragment in the patient sample (left blot) resulted from a naturally occurring Bam HI polymorphism (see Fig. 2A), for which the patient was heterozygous. Hybridization with the *IRF-1* cDNA probe and probe 1 revealed *IRF-1* structural rearrangements with each enzyme. With probe 2, no deletions or rearrangements were evident in the Bgl II digest, implying that the breakpoint must lie upstream of the Bgl II restriction site in intron 1. (**C**) Map of the 1.9-kb Hind III–Hind III region encompassing *IRF-1* exons 1 and 2 and intron 1. The novel Hind III site in the leukemic sample and resultant 0.4-kb Hind III fragment are indicated above the map. The primers and orientations used for inverse PCR are also indicated (*18*).

and sequence the *IRF-1* gene in the region encompassing exon 1 and intron 1 in both normal and leukemia DNA from sample 10 (*18*). In addition to a 1.1-kb Hind III fragment seen in normal DNA, the leukemic DNA generated a 0.4-kb fragment (Fig. 2C). Sequencing of the two fragments revealed that the leukemic DNA diverged from the normal *IRF-1* sequence ten nucleotides beyond the primer 1 sequence (*18*). These results indicate that one allele of the *IRF-1* gene has likely been inactivated in the majority of leukemic cells in sample 10 by a deletion of the promoter region and exon 1.

To date, no mutations have been detected in the coding exons of the residual *IRF-1* allele in any of the leukemia or MDS samples, as monitored by ribonuclease protection analysis (*19*). Whether inactivating mutations can occur in other regions of the *IRF-1* gene remains to be determined. In addition, we have not detected abnormalities in the *IRF-2* gene, which encodes a protein that represses IRF-1 (*8*), in any patient sample (*20*).

To gain more insight into the nature of the *IRF-1* deletions in the leukemia and MDS samples, we performed fluorescence in situ chromosomal hybridization (FISH) studies (*7, 21*). With the 19-kb *IRF-1* genomic probe (*9*), we detected the loss of one *IRF-1* allele in a substantial proportion (24% to 88%) of cells in each MDS and leukemia sample with del(5q) or translocation 5q31; these observations were consistent with the slot-blot analysis (Table 1). The loss of one *IRF-1* allele was confirmed in the case of de novo ALL at presentation (sample 10). In addition, several leukemia and MDS samples (samples 1, 7, 8, 10, 12, and 13) also appeared to have a substantial proportion of cells (≥10%) with no *IRF-1* hybridization domain, suggesting that both *IRF-1* alleles may have been deleted in a subpopulation of cells in these samples. This possibility was further supported by the results of dual-color FISH studies with the *IRF-1* and 5q22 genomic probes on seven samples (including sample 10) in which residual cells were available (*7, 21*). Collectively, our results with sample 10 are particularly informative; in addition to the structural rearrangements that inactivated one *IRF-1* allele (Fig. 2), the residual *IRF-1* allele was deleted in a subpopulation of blasts. Such cells may have emerged at relapse when the del(5q) became detectable at the microscopic level of resolution (Table 1).

Our results suggest an unusual instability of the 5q region. Deletion of one *IRF-1* allele may be accompanied by the rearrangement or deletion of the residual allele. Thus, deletions or rearrangements, rather than point mutations, may be more frequent at this genetic locus in human leukemia and MDS. In light of the recent observation that subtle changes in the IRF-1:IRF-2 ratio perturb cell growth control (*10*), we suggest that loss of a single *IRF-1* allele may have biologic significance, analogous to the loss of the *APC, NF-1, p53,* and *WT-1* tumor suppressor genes (*22*). A clone of cells that had lost only a single *IRF-1* allele would be expected to have a slow capacity for expansion and would be a target population for further genetic mutations. Interestingly, these more indolent biologic characteristics are observed clinically in the majority of patients with the preleukemic 5q− syndrome (*3*). Loss of an additional *IRF-1* allele and the acquisition of mutations at other genetic loci may be critical steps for full leukemic transformation. Whether deletion of the *IRF-1* gene is solely responsible for the biological characteristics of the del(5q) and the 5q− syndrome remains to be determined.

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7. The *IRF-1* gene was mapped to normal metaphase chromosomes by fluorescence in situ chromosomal hybridization [D. Pinkel, T. Straume, J. W. Gray, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2934 (1986); D. Pinkel *et al.*, *ibid.* **85**, 9138 (1988); W. L. Kuo *et al.*, *Am. J. Hum. Genetics* **49**, 112 (1991)]. A 19-kb *IRF-1* genomic probe (*9*) (Fig. 2A) and a 19-kb single copy genomic DNA sequence from 5q22 used as a reference and control (Cyn 5.120; kindly provided by R. White, University of Utah, Salt Lake City, UT) were differentially labeled to allow dual-color visualization of hybridized domains. The 5q22 probe was nick translated with dinitrophenol (DNP)-11-dUTP (Novagen, Madison, WI) and was visualized by treatment first with a rat antibody to DNP (Boehringer Mannheim, Indianapolis, IN) and then with a goat antibody to rat immunoglobulin G conjugated to fluorescein isothiocyanate (CalTag, Burlingame, CA). The *IRF-1* probe was chemically modified with digoxigenin-11-dUTP (Boehringer Mannheim) and was visualized with a rhodamine-labeled antibody to digoxigenin (Novagen). The *IRF-1* probe hybridized only to sequences on chromosome 5q and was precisely mapped by computer-assisted fluorescence microscopy.
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17. Striking heterogeneity was observed in the deletion of *IL-4, IL-5, CDC25C,* and *GM-CSF* among different samples. Although Samples 3 and 8 were initially shown to have distal breakpoints at 5q35 and 5q33, respectively, deletion analysis indicated that the distal breakpoint in each case was between *IRF-1* and *CDC25C* (Table 1). Analysis of patients with the smallest deletions (Sample 10, *IRF-1* only; Sample 11, *IRF-1* and *CDC25C*; Sample 13, *IRF-1* only) revealed that *IRF-1* was the only consistently deleted gene.
18. Genomic DNA (1 μg) from the leukemic sample (Table 1, Sample 10) and normal DNA were digested in a 100-μl reaction volume with Hind III (10 units) at 37°C for 3 hours. Digested DNA was diluted to 2 μg/ml in ligase buffer [50 mM tris-HCl (pH 7.6), 10 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol] and incubated with 2.8 units of T4 DNA ligase at 14°C for 20 hours. After ligation, the DNA was resuspended in distilled H_2O (30 μl), and nicks were introduced by heating at 95°C for 10 min. The regions encompassing *IRF-1* exon 1 and intron 1 were amplified by PCR (DNA thermal cycler, Perkin-Elmer Cetus) with the primers indicated in Fig. 2C. Primer 1 was 5′-CGCGCACGCAGATCTGCGAAACCTG-3′ (the Bgl II site is underlined), and primer 2 was 5′-TTCCAACCAAATCCCGGGGCTCATC-3′. The reaction was carried out (95°C for 30 sec, 60°C for 1 min, 70°C for 2 min, 40 cycles) in 50-μl volumes with 1 μM $MgCl_2$, 0.01% gelatin, and 1.25 units Taq I DNA polymerase (Perkin-Elmer Cetus). The PCR products were checked by agarose gel electrophoresis, and the appropriate bands (a 1.1-kb Hind III fragment from normal DNA and a 0.4-kb Hind III fragment from leukemic DNA) were cloned into the Bluescript plasmid. The sequence from primer 2 to the 3′ Hind III site (Fig. 2C) in six independently isolated clones was identical in normal and leukemic DNA; the leukemic DNA sequence diverged from the normal sequence beginning ten nucleotides 5′ to primer 1 and was thereafter replaced with an unknown sequence. The PCR fragment obtained from normal DNA hybridized to the expected *IRF-1* restriction fragments, whereas the 0.4-kb fragment derived from leukemic DNA hybridized to novel bands detected in Southern blots (Fig. 2B).
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21. Probes detecting *IRF-1* or 5q22 sequences were differentially labeled to allow single (*IRF-1*) or dual (*IRF-1* and 5q22) color visualization of hybridized domains in interphase cells from MDS and leukemic samples (*7*). Single- and dual-color hybridizations were performed by a modification of the procedures described in Kuo *et al.* (*7*). (The authors will supply a detailed procedure upon request.) Fluorescence microscopy with appropriate filters was accomplished as described in Pinkel *et al.* (*7*). In single-color studies, all cells in the microscopic field were scored as having two, one, or no *IRF-1* domains (Table 1). In dual-color studies, *IRF-1* domains were scored in cells showing at least one 5q22 hybridization domain. Blast populations definitively lacking one *IRF-1* hybridization domain but retaining one 5q22 hybridization domain were identified in 4 of 16 residual cells that could be analyzed in Sample 10.
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Anti-Oncogenic and Oncogenic Potentials of Interferon Regulatory Factors–1 and –2

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Interferon regulatory factor–1 (IRF-1), a transcriptional activator, and IRF-2, its antagonistic repressor, have been identified as regulators of type I interferon and interferon-inducible genes. The *IRF-1* gene is itself interferon-inducible and hence may be one of the target genes critical for interferon action. When the *IRF-2* gene was overexpressed in NIH 3T3 cells, the cells became transformed and displayed enhanced tumorigenicity in nude mice. This transformed phenotype was reversed by concomitant overexpression of the *IRF-1* gene. Thus, restrained cell growth depends on a balance between these two mutually antagonistic transcription factors.

Interferons (IFNs) are a family of cytokines that exhibit antiproliferative activity on many normal and transformed cells and can block growth factor–stimulated cell cycle transitions (*1*). IFNs are induced by growth factors, which suggests that they participate in a feedback mechanism that regulates cell growth (*1*). In previous studies, we identified two DNA binding factors that regulate *IFN* gene expression, IRF-1 and IRF-2 (*2–5*). These factors are structurally related, particularly in the amino-terminal region, which confers DNA binding specificity, and they independently bind to a promoter element shared by the *IFN-α* and *IFN-β* genes as well as many IFN-inducible genes. This promoter element has the consensus sequence motif G(A)AAA(G or C)(T or C)GAAA(G or C)(T or C) (*5, 6*). IRF-1 and IRF-2 are distantly related in structure to two other DNA binding factors involved in IFN signaling, ICSBP and ISGF3γ (*3*).

Gene transfection studies have demonstrated that IRF-1 functions as an activator for IFN and IFN-inducible genes, whereas IRF-2 represses the action of IRF-1 (*5, 7–10*). Expression of the *IRF-1* gene itself is IFN-inducible. The *IRF-2* gene is also induced in IFN-stimulated cells, but this induction occurs only after induction of *IRF-1* (*5*). In IFN-treated or virus-infected cells, the IRF-2 protein is more stable than the IRF-1 protein (half-lives of 8 hours and 30 min, respectively) (*11*). Thus, in growing cells IRF-2 is more abundant than IRF-1, but after stimulation by IFN or viruses the amount of IRF-1 increases relative to IRF-2 (*11*). These observations suggest that a transient decrease in the IRF-2:IRF-1 ratio may be a critical event in the regulation of cell growth by IFNs. Consistent with this notion are the findings that IRF-1 manifests antiproliferative properties both in vivo and in vitro (*4, 12*).

We first quantified expression of *IRF-1* and *IRF-2* mRNAs in mouse NIH 3T3 cells throughout the cell cycle. Cells were growth-arrested by serum starvation for 24 hours (G_1 arrest) and were then induced to transit the cell cycle by serum restoration (*13*). A [^{3}H]thymidine uptake assay and

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