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Interferon-Regulatory Factor 1 Is an Immediate-Early Gene under Transcriptional Regulation by Prolactin in Nb2 T Cells

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The pituitary peptide hormone prolactin (Prl) is a potent inducer of Nb2 T lymphoma cell proliferation. To analyze the early genetic response to the mitogenic signals of Prl, a cDNA library was constructed from Nb2 T cells stimulated for 4 h with Prl and the protein synthesis inhibitor cycloheximide. Of 26 distinct clones isolated by differential screening, one clone, designated c25, exhibited extremely rapid but transient kinetics of induction by Prl and superinduction by Prl plus cycloheximide. Run-on transcription analysis indicated that c25 gene transcription was induced greater than 20-fold within 30 to 60 min of Prl stimulation. Surprisingly, DNA sequence analysis of c25 cDNA revealed that this Prl-inducible early-response gene is the rat homolog of the mouse transcription factor interferon-regulatory factor 1 (IRF-1), sharing 91% coding sequence similarity with mouse IRF-1. At the protein level, rat IRF-1 shares 97% and 92% homology with mouse IRF-1 and human IRF-1, respectively, suggesting that this molecule has been functionally conserved throughout evolution. Our studies show that the gene for IRF-1 is an immediate-early gene in Prl-stimulated T cells, which suggests that IRF-1 is a multifunctional molecule. In addition to its role in regulating growth-inhibitory interferon genes, IRF-1 may, therefore, also play a stimulatory role in cell proliferation. The gene for IRF-1 is one of the earliest genes known to be transcriptionally regulated by Prl.

The pituitary hormone prolactin (Prl) has been shown to be a differentiation-specific and potent growth-promoting factor for a wide variety of tissues (11, 14, 20, 31, 38, 41, 45, 46). Relatively little is known about the initial biochemical and genetic responses elicited by Prl binding to Prl receptors in these target tissues. As a model system for studying the mitogenic effects of Prl, a T-cell lymphoma line (Nb2) (15) which proliferates in response to picogram amounts of Prl was used. Prl administration to quiescent Nb2 cells results in rapid transcriptional induction of several growth-related genes (47), including c-*myc*, β-actin, ornithine decarboxylase, and heat shock protein 70. The rapid kinetics of transcriptional induction suggests that these genes represent G0-G1 genes that respond directly to the mitogenic signals of Prl. Further, induction of c-*myc* occurs without de novo protein synthesis (46), suggesting that modulation of preexisting factors is involved in its transcriptional induction by Prl. The rapid transcriptional response to Prl stimulation suggests that Nb2 T cells represent an excellent culture system with which to isolate novel, Prl-inducible early genes critical for T-cell activation and growth.

A number of early-response genes or competence genes have been isolated from diverse cell types, including growth factor-induced (5) or serum-induced (1, 3, 5, 22, 26, 27, 29, 43) fibroblasts, growth factor-induced pheochromocytoma PC12 cells (33, 34, 43, 44), and mitogen-induced T lymphocytes (25, 30, 39, 49; reviewed in reference 6). In most cases, the protein synthesis inhibitor cycloheximide (CHX) was used in the cloning strategy to isolate mRNA sequences whose expression does not require de novo protein synthesis, as well as to stabilize those rare gene transcripts that may be rapidly but transiently induced. Interestingly, overlapping sets of identical early genes have been isolated from these different cell types (3, 4, 21, 22, 28, 30, 33, 43), demonstrating that the same regulatory proteins appear to mediate a variety of cellular responses to different external signals. Some early-response genes encode nuclear DNA-binding proteins with zinc finger motifs (4, 22, 28, 33, 43), steroid hormone receptorlike molecules (19), transmembrane proteins (18), low-molecular-weight secreted molecules (5, 30, 39), and even an interferonlike molecule (44). How this battery of immediate-early genes functions in a program leading to cell proliferation and/or differentiation is not known.

In our studies, we set out to identify early-response genes that may play an important role in Prl-mediated growth control. A cDNA library was prepared from Nb2 T cells after 4 h of incubation with Prl and CHX. Interestingly, one of the early genes isolated was that for the transcription factor interferon-regulatory factor 1 (IRF-1). IRF-1 has been previously shown to be a potent transcriptional regulator of growth-inhibitory interferon (IFN) and IFN-inducible genes (12, 17, 23, 35). In the Nb2 T-cell system, rat IRF-1 (rIRF-1) demonstrated rapid, transient, and dramatic transcriptional induction by Prl. rIRF-1 was also found to be expressed in a number of different cell types, as well as a variety of tissues, suggesting that expression of the gene for IRF-1 can be regulated by other stimuli, in addition to a specific response to Prl in Nb2 T cells. A dual role of rIRF-1 as an early regulatory gene in the T-cell activation pathway and as a regulator of growth-inhibitory genes is therefore suggested. Prl is a potent stimulator of IRF-1 gene transcription in T cells.

MATERIALS AND METHODS

Cell culture. Nb2 T cells were maintained in Fischer medium for leukemic mice (GIBCO Laboratories, Grand Island, N.Y.) as previously described (47), except that newborn calf serum (Hazelton, Lenexa, Kans.) was used instead of fetal bovine serum. Quiescent cells were prepared as previously described (47). To reinitiate growth, 10 ng of Prl (NIH-P-S13 from the National Institute of Diabetes and

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Digestive and Kidney Diseases) per ml was added. Protein synthesis inhibitors were used at either 10 μg of CHX per ml (10 mg/ml stock in water) or 30 μM anisomycin (8,000× stock in ethanol).

Lambda ZAP cDNA library and differential screening. A 10-μg sample of poly(A)$^+$ RNA from Nb2 T cells that had been treated for 4 h with Prl and CHX were used to generate cDNAs by using a combination of oligo(dT) and random-priming procedures as already described (41). Double-stranded cDNAs were size selected for lengths greater than 500 base pairs (bp) and cloned into the lambda ZAP bacteriophage cloning system (Stratagene, San Diego, Calif.) (41). An unamplified library of 10^7 recombinant clones was obtained. Phage plates (15 cm^2) were prepared with medium plaque density, and two identical filters (nitrocellulose or nylon) were made from each plate. One filter was hybridized with ^{32}P-labeled first-strand cDNA probes derived from quiescent cells, and the second filter was hybridized with cDNAs from Prl-plus-CHX-stimulated cells. Routinely, 2 × 10^6 cpm/ml was used for each filter. Filters were hybridized at 42°C for 48 h and extensively washed at 50°C as already described (41).

Isolation and characterization of cDNA clones. Differentially reacting phage clones were excised from the lambda ZAP phage vector by using a one-step excision protocol with a helper phage as already described (41). The excised, single-stranded phagemid was then recovered through bacterial transformation as double-stranded pBluescript(SK−) plasmids. From 1 to 5 mg of recombinant plasmid DNA was obtained from 100-ml bacterial cultures by using a modified alkaline lysis procedure (2). *Eco*RI or *Eco*RI-*Hin*dIII double-digest cDNA inserts were prepared by agarose gel electrophoresis, followed by glass-milk isolation (Bio101, La Jolla, Calif.). For analysis of induction by Prl plus CHX, control and Prl-plus-CHX-stimulated poly(A)$^+$ RNAs were directly labeled by using T4 kinase (37) and hybridized to duplicate slot blots containing 5 μg of DNA from various cDNA clones.

RNA blot analysis. To determine the relative contributions of Prl and CHX to increasing steady-state mRNA levels, RNA slot blots were made containing 5 μg of total RNA from control and Prl-, Prl-plus-CHX-, and CHX-treated cells and hybridized with individual cDNA inserts. To estimate the sizes of the corresponding mRNAs, 10 μg of total RNA was analyzed by hybridization with random-primed cDNAs as already described (48). To determine cell type-specific expression, NIH 3T3 fibroblasts were made quiescent in 1% fetal bovine serum for 24 h and stimulated with 20% fetal bovine serum plus CHX for 4 h. Mouse mammary epithelial cells, Comma-D (7), were cultured as already described (48) and stimulated with 1 μg each of insulin, hydrocortisone, and Prl per ml plus CHX for 4 h. To determine clone 25 (c25) mRNA expression in various tissues, 50 μg of total RNA from a panel of rat tissues was hybridized with a c25 cDNA probe. The hybridized signals were quantitated by densitometric scanning of multiple exposures of the autoradiograms with a Quick Scan integrator (Helena Laboratories, Beaumont, Tex.).

Nuclear run-on transcription assay. To determine relative transcriptional activities of genes in quiescent versus Prl-stimulated cells, 90 × 10^6 nuclei were analyzed by an in vitro run-on transcription assay as already described (47). DNA dot filters contained 5 μg each of c25 (1.7 kilobases [kb]), c34 (2.6 kb), and pGemblue (2.9 kb; Promega Biotec, Madison, Wis.). In each hybridization reaction, 27 × 10^6 cpm was used, and three to four individual reactions were used for each time point. Hybridized counts were eluted and quantitated by liquid scintillation counting. After correction for the plasmid DNA background (average, 4 ppm), the transcription rate was determined as parts per million input counts hybridized per kilobase of DNA (ppm/kb).

TABLE 1. Prl-plus-CHX-responsive cDNA clones from an Nb2 T-cell library[a]

Clone[b]	Insert size (kb)	Response[c]
1	1.2	+
2	2.6	+
4	1.6	+
5	1.7	+
15	1.1	++
22	4.7	+
24	1.3	+
25	1.7	+++
26	1.6	40% inhibition
29	1.0	+
30	1.5	+
35	2.8	+
4b	2.1	+++

[a] This table represents a partial list of Prl-plus-CHX-responsive cDNA clones.

[b] c4b was identified by rescreening the library with c25. c34 (2.6-kb insert [not listed]), which does not respond to Prl plus CHX, was used as a control cDNA.

[c] Treatment with Prl plus CHX for 4 h. +, Low; ++, moderate; +++, high.

DNA sequencing. For DNA sequencing of c25, several overlapping cDNA clones were used. A full-length cDNA clone, c4b, was isolated from the lambda ZAP cDNA library by using a 0.9-kb insert representing the most 5′ portion of c25 as the probe. Either single-stranded phagemids or double-stranded plasmids were sequenced by the chain termination method by using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) together with M13 reverse primer (Stratagene), T7 primer (Stratagene), or custom-made specific primers. Exonuclease III-Mung bean nuclease deletion and sequencing protocols (Stratagene) were used to generate nested deletions for sequencing. All sequences were determined in both directions through all overlapping sites or at least three times in one direction.

RESULTS

Cloning and isolation of Prl-plus-CHX-responsive genes. To identify genes that may play a role in Prl-mediated growth control, the strategy was to isolate genes that are expressed early after Prl stimulation of quiescent Nb2 T cells without protein synthesis. Incubation with Prl for 4 h was chosen because previous studies have determined that two growth-related genes, c-*myc* (13, 47) and Nb29, a heat shock protein 70 homolog (8, 47), were induced 5- to 10-fold at the transcriptional level at 4 h after Prl stimulation (47). A lambda ZAP Nb2 T-cell cDNA library was differentially screened with first-strand cDNAs prepared from quiescent versus Prl-plus-CHX-stimulated cells. Of 143,000 plaques screened, 96 primary positive plaques were obtained which, after multiple rounds of screening, generated 26 Prl-plus-CHX-responsive clones. cDNA insert sizes estimated on the basis of *Eco*RI and *Eco*RI-*Hin*dIII restriction digestion of pBluescript(SK−) plasmids ranged from 1 to 5 kb (Table 1). Cross-hybridization analyses showed that these clones did not hybridize to each other, to a panel of known oncogenes (c-*fos*, c-*myc*, c-*ras*, c-*src*, c-*mos*, and c-*abl*), or to T-cell

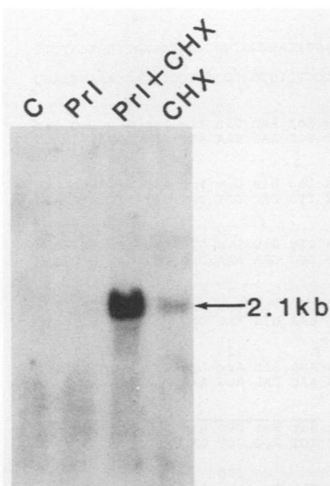

FIG. 1. c25 gene expression in Nb2 T cells. Total RNAs (10 μg per lane) isolated from control cells (C) versus cells treated for 4 h with Prl, Prl plus CHX, or CHX were hybridized with a c25 cDNA probe. Autoradiographic signals were quantitated by densitometric scanning as described in Materials and Methods.

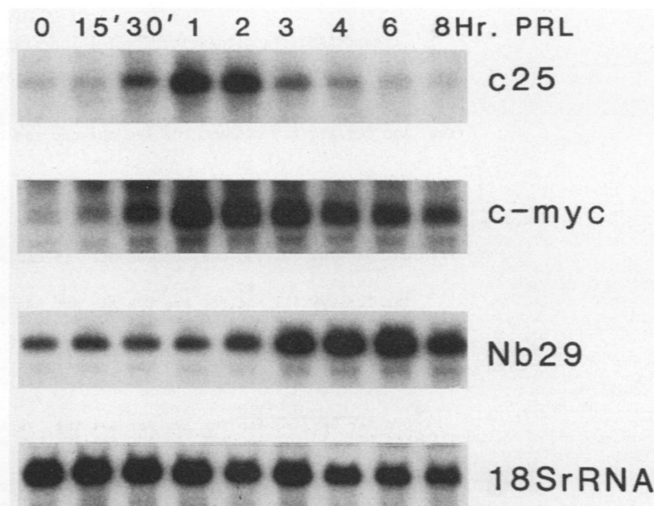

FIG. 2. Early time course of Prl-inducible c25 gene expression in Nb2 T cells. Total RNA (10 μg per lane) was isolated at various times after Prl stimulation of quiescent Nb2 T cells and hybridized with various DNA probes. RNA sizes: c25, 2.1 kb; c-*myc*, 2.4 kb; Nb29, 2.4 kb; 18S rRNA, 1.9 kb.

activation genes (interleukin 2 and its receptor), indicating that they represent unique and distinct cDNAs (data not shown).

To determine the abundance and inducibility of the 26 mRNAs represented by the Prl-plus-CHX-responsive cDNAs, poly(A)$^+$ RNA from either quiescent or Prl-plus-CHX-treated cells was directly labeled with T4 kinase (37) to ensure full representation of all mRNA sequences and used as hybridization probes (data not shown). Two identical slot blots containing all 26 unknown cDNAs were hybridized with the two RNA probes. Following densitometric scanning, these clones were grouped into those that were highly inducible, moderately inducible, or inhibited by Prl plus CHX (Table 1). Selected cDNAs were further analyzed by RNA slot blots for inducibility with Prl or CHX (data not shown). Most of the unknown cDNAs fell into the moderate-inducibility category, with Prl plus CHX treatment resulting in a two- to threefold change in steady-state mRNA levels. One clone, c26, was inhibited by CHX alone. Another clone, c25, was inducible more than 15-fold by Prl plus CHX and was further analyzed for its pattern and kinetics of induction in response to either Prl or CHX alone.

c25 gene expression: early response to Prl. By using c25 cDNA as a probe, a single mRNA species at 2.1 kb was observed in Nb2 T cells, which was stabilized by CHX alone and superinduced by Prl plus CHX (Fig. 1). Since c25 mRNA was superinducible with Prl plus CHX, lack of a c25 signal in the RNA treated with Prl for 4 h suggests that c25 was transiently induced by Prl in less than 4 h. An early time course study (Fig. 2) confirmed that, indeed, c25 mRNA levels were rapidly induced within 30 min by Prl and that maximum stimulation of about 15-fold above basal levels occurred at 1 h. This rapid and dramatic increase was transient, as c25 mRNA had returned to nearly basal levels by 4 h of Prl incubation. In comparison, c-*myc* mRNA was induced by Prl with similar rapid kinetics, while Nb29 was maximally induced at 4 to 6 h. However, unlike c25 mRNA, c-*myc* and Nb29 mRNA levels were not transiently induced but remained elevated in the continued presence of Prl. The induction of early growth response genes by Prl in Nb2 T cells was specific, as the level of 18S rRNA remained relatively unchanged with Prl incubation.

c25 gene expression: transcriptional regulation by Prl. The unusually rapid, transient, and dramatic increase in c25 mRNA levels suggested that this gene is regulated by Prl at the transcriptional level. To analyze the relative transcription rate of c25, nuclear run-on transcription assays were performed (Fig. 3). A rapid 14-fold induction by Prl of the c25 transcription rate was observed at 15 min, which increased to greater than 22-fold by 30 min but was completely gone by 4 h. This very rapid but transient increase in c25 transcriptional activity is specific, as the transcription rate of a control gene, c34, was relatively unchanged in response to

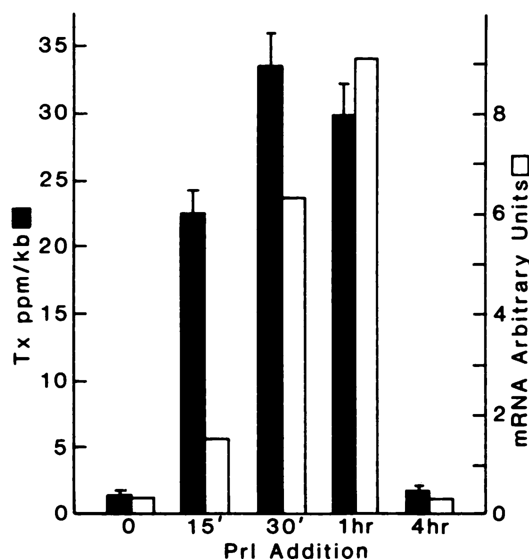

FIG. 3. The c25 gene is transcriptionally regulated by Prl in Nb2 T cells. The transcriptional activities of nuclei isolated from quiescent cells versus cells treated with Prl at various times were analyzed by nuclear run-on transcription (Tx) assay as described in Materials and Methods. Relative rates of c25 transcription in response to Prl are shown. Hybridization signals for c25 mRNA in the same experiment were quantitated by densitometric scanning.

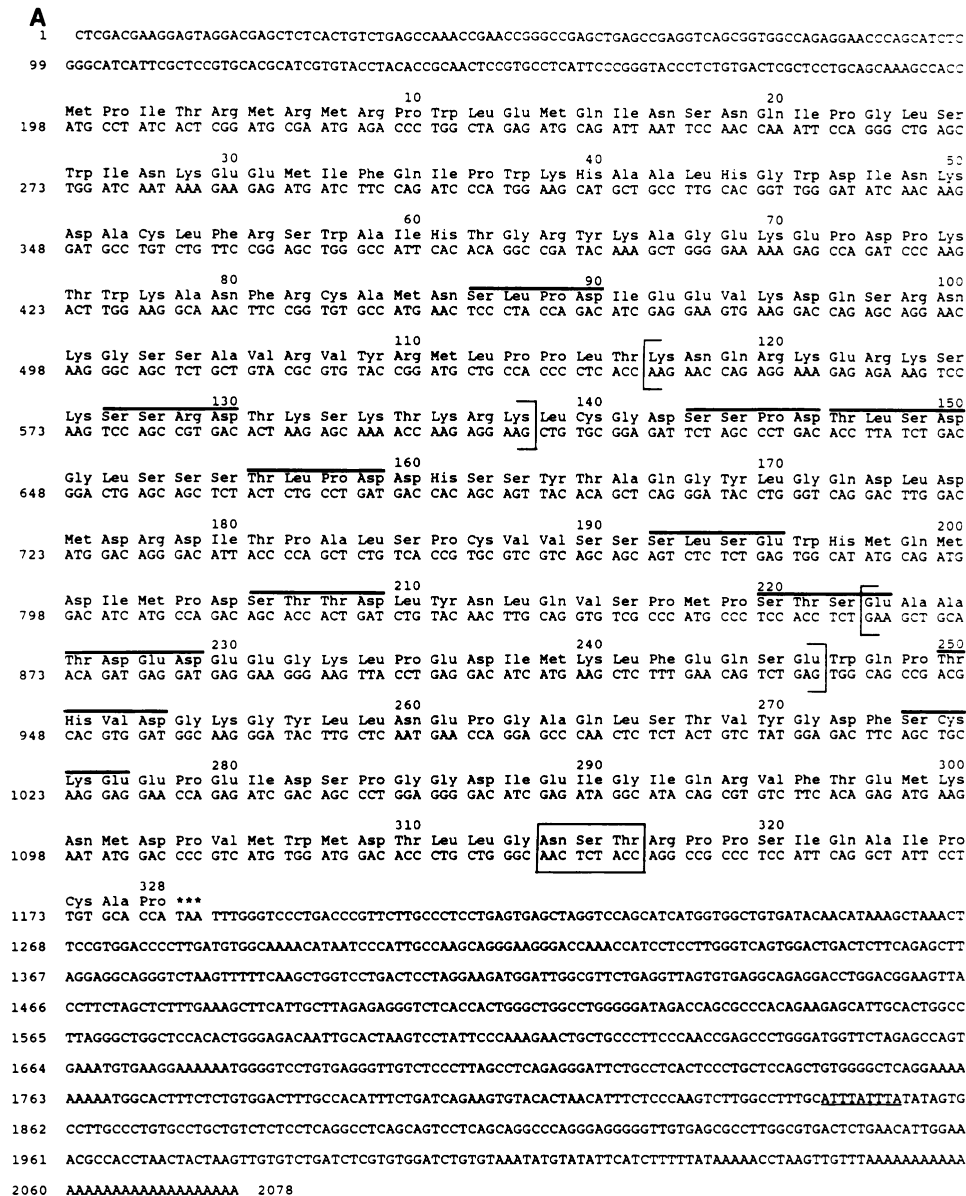

FIG. 4. Structural analysis of c25 cDNA. (A) Nucleotide sequence and deduced amino acid sequence of c25-c4b. Lys-Arg basic and Glu-Asp acidic domains are shown in brackets. Potential CK-II phosphorylation sites, Ser/Thr-X-X-Asp/Glu, are overlined. A potential N-linked glycosylation site, Asn-X-Thr, is boxed. The AUUUA motif sequences are underlined. (B) Nucleotide sequence comparison between rIRF-1 and mIRF-1 cDNAs. Dashes represent identical sequences, dots represent deletions, and the sequences in the corresponding regions are shown in lowercase letters.

Prl (data not shown). In the same experiment, a rapid, transient, and dramatic increase in steady-state c25 mRNA was observed, with 18-fold stimulation above basal levels at 1 h. The parallel induction by Prl of both gene transcription and mRNA accumulation demonstrated for the first time that c25 is one of the earliest Prl-inducible genes shown to be regulated primarily at the transcriptional level.

Sequence analysis: c25 is the rat homolog of mouse IRF-1 and human IRF-1. Since c25 contains only a 1.7-kb insert, a 5'-end 0.9-kb fragment of c25 cDNA was used to rescreen the lambda ZAP cDNA library. Several overlapping clones containing more 5' sequences were obtained. c4b was found to contain an additional 400 bp of 5' sequences. DNA sequence analyses of these and Exonuclease III-Mung bean nuclease deletion clones generated from c25 with either primers flanking the DNA inserts or custom-made oligomer primers specific for c4b sequences (data not shown) provided a complete 2.1-kb cDNA sequence with a single open reading frame of 328 amino acids (Fig. 4A). Surprisingly, this open reading frame was found to share 91% sequence similarity with the mouse interferon-regulatory factor 1 (mIRF-1)-coding-region cDNA (35) (Fig. 4B), suggesting that c25-c4b is likely to be the rat homolog of mIRF-1.

The 5' sequence of c4b extended 15 bp beyond the reported mIRF-1 cDNA clone, but appeared to end 15 nucleotides 3' of the major capping site in the genomic mIRF-1 gene (Fig. 4B) (35). Interestingly, the high degree of similarity between the rIRF-1 and mIRF-1 sequences was

not observed in the 15-bp extended 5′ noncoding rat cDNA sequence (Fig. 4B). A simple repeat, AACCG, occurs 10 times in the mouse 5′ noncoding region but only 6 times in the rat sequence as a result of a 20-bp deletion in the rat cDNA. The overall sequence identity between the rat and mouse 3′ noncoding regions is about 85%, and several deletions and insertions are found in both rat and mouse sequences. One exception is ATTTATTTA and the surrounding 25-bp sequences in the 3′ ends of both cDNAs, which are completely identical (Fig. 4). The ATTTA sequence motif has been implicated in mediating rapid mRNA turnover of a variety of lymphokine genes and oncogenes via posttranscriptional mechanisms (40).

Comparisons of the rIRF-1, mIRF-1, and human IRF-1 (hIRF-1) proteins showed that the rIRF-1 protein is also highly enriched in Lys and Arg residues, which constitute 55% of the amino acids between residues 117 and 138, generating a very basic domain in the protein (Fig. 5). This domain is followed by a region enriched in Ser-Ser residues (Fig. 5B). Between amino acids 223 and 246 is an acidic domain in which Glu and Asp make up 42% of the residues. The last 27 amino acids constitute the most hydrophobic domain (59%) of the protein and contain a single potential N-linked glycosylation recognition site, 314-Asp-Ser-Thr, which is not conserved in either mIRF-1 or hIRF-1. A single amino acid deletion following the potential glycosylation site makes rIRF-1 one amino acid shorter than mIRF-1. In addition to the conserved charged domains, another strikingly conserved feature is the cluster of potential phosphorylation sites found in the middle of all three IRF-1 proteins. For example, the potential casein kinase II (CK-II) recognition sequence Ser/Thr-X-X-Asp/Glu is present 11 times in rIRF-1 and hIRF-1, and 9 times in mIRF-1 (Fig. 4A and 5B). In particular, one such site in hIRF-1, 192-Thr-X-X-Asp, has been conserved in position but not in sequence relative to rIRF-1 and mIRF-1, i.e., 192-Ser-X-X-Glu. Interestingly, the first half of the IRF-1 molecule, believed to bind to specific DNA sequences through the basic Lys/Arg domain, is con-

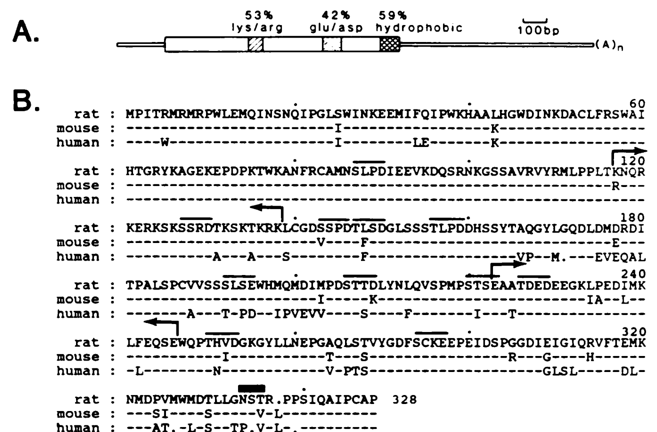

FIG. 5. Comparison of rIRF-1, mIRF-1, and hIRF-1. (A) Conserved domains in IRF-1. 5′ and 3′ noncoding regions are illustrated as open lines. Coding sequences are depicted as open boxes in which the conserved basic (Lys-Arg) and acidic (Glu-Asp) domains are highlighted. (B) Amino acid sequence comparisons of rIRF-1, mIRF-1, and hIRF-1. rIRF-1, mIRF-1, and hIRF-1 are 328, 329, and 325 amino acids long, respectively. Potential CK-II sites are overlined. Basic (K-R) and acidic (D-E) domains are bracketed by arrows. The potential N-linked glycosylation site (NST) in rIRF-1 is marked by a solid bar. The numbers conform to the rat protein sequence. Single-letter amino acid codes are shown.

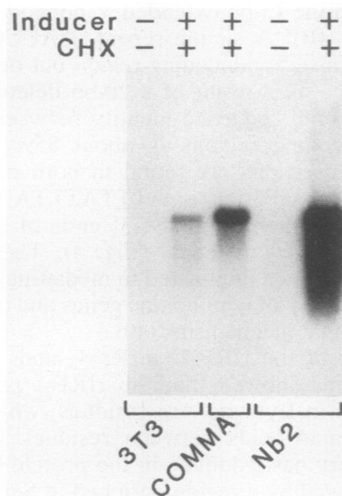

FIG. 6. c25–rIRF-1 expression in different cell types. The various cell cultures were "deinduced" in the appropriate medium for 24 h before stimulation with either serum or hormones plus CHX for 4 h as described in Materials and Methods. Inducer: 20% fetal bovine serum for 3T3 cells; insulin, hydrocortisone, and Prl at 1 μg/ml for Comma-D cells; and Prl at 10 ng/ml for Nb2 T cells. A 1.5-μg sample of poly(A)$^+$ RNA from 3T3 cells and 5 μg each of poly(A)$^+$ RNAs from Comma-D and Nb2 T cells were hybridized with a c25 cDNA probe. No hybridization signals were obtained with control Comma-D cell RNA (data not shown).

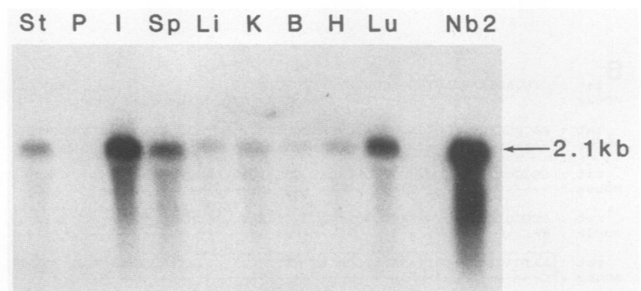

FIG. 7. c25–rIRF-1 expression in normal rat tissues. Total RNAs (50 μg per lane) from various rat tissues were isolated from 24-day old rats and examined for c25–rIRF-1 expression by hybridization with a c4b cDNA probe. The Nb2 T-cell control lane contained 5 μg of total RNA. St, Stomach; P, pancreas; I, small intestine; Sp, spleen; Li, liver; K, kidney; B, brain; H, heart; Lu, lung.

served in all three IRF-1 proteins. The second half of the molecule, which is believed to mediate *trans*-activating functions (17, 35), is more divergent among the three proteins, suggesting potential differences in function.

c25–rIRF-1 expression in various cell types. Since rIRF-1 is dramatically induced by Prl in Nb2 T cells, it was next determined whether IRF-1 is expressed in another cell type that is also responsive to Prl stimulation. The mammary epithelial cell line Comma-D (7) responds to Prl by expression of differentiated functions (Fig. 6). As a control, mouse 3T3 fibroblasts were used. Since c25–rIRF-1 mRNA might also be unstable in these cell types, an inducer-plus-CHX protocol was used. Under superinduction conditions, a 2.1-kb signal was observed in poly(A)$^+$ RNAs from all three types of cells, indicating that c25–rIRF-1 can be expressed in T cells, fibroblasts, and epithelial cells. It is not known whether serum alone can induce c25–rIRF-1 expression in fibroblasts. Interestingly, c25–rIRF-1 mRNA levels were elevated in actively proliferating mammary epithelial cells, while fully differentiated mammary cells did not express c25–rIRF-1 mRNA, even in the continued presence of Prl (data not shown). It appears that c25–rIRF-1 mRNA accumulates in rapidly growing mammary epithelial cells but becomes unstable as the cells are induced to differentiate with lactogenic hormones. These results reinforce the notion that c25–rIRF-1 is a growth-related gene and also suggest that c25–rIRF-1 can respond to signals other than Prl in other cell types.

c25–rIRF-1 expression in normal rat tissues. rIRF-1 expression was also examined in various rat tissues exposed only to normal circulating levels of Prl (Fig. 7). Except for the pancreas, rIRF-1 expression was detected at different abundancies in all of the tissues examined. In particular, significant rIRF-1 mRNA levels were found in the small intestine, as well as in stomach, spleen, and lung tissues. Thus, it appears that c25–rIRF-1 expression is not limited to one particular tissue or cell type (Fig. 6) and that IRF-1 has a general role in cell growth and differentiation processes, in addition to exhibiting a specific response to Prl in Nb2 T cells.

DISCUSSION

Prl is a multifunctional neuroendocrine hormone which plays a role in cell proliferation and differentiation in a variety of tissues and cell types. Despite extensive analysis, the molecular basis and regulatory mechanisms underlying Prl action remain unclear. This study reports the cloning of a panel of Prl-inducible early genes and the detailed characterization of one immediate-early gene from a T-cell line as a first step toward understanding the complex genetic response of cells initiated by interactions between Prl and its receptors.

rIRF-1 is a Prl-inducible, transiently expressed, immediate-early gene. One of the immediate-early genes isolated from Prl-plus-CHX-induced Nb2 T cells is transcription factor rIRF-1. Expression of the gene for rIRF-1 is regulated by rapid and dramatic 20-fold transcriptional up regulation by Prl (Fig. 3). The transcriptional induction by Prl is transient, with maximum stimulation between 30 and 60 min and a complete return to basal levels by 4 h of Prl treatment. It is not known which mechanism is involved in the transcriptional shutoff of the gene for rIRF-1 and whether this process would require ongoing protein synthesis. Transcriptional induction slightly precedes the increase in steady-state rIRF-1 mRNA and is sufficient to account for the large 18-fold transient rise in rIRF-1 mRNA levels (Fig. 3). The intrinsic lability of rIRF-1 mRNA contributes to the transient nature of rIRF-1 expression. rIRF-1 mRNA levels can be increased by CHX alone (Fig. 1), suggesting that steady-state rIRF-1 mRNA is under negative regulation by a labile protein factor. In fact, inclusion of CHX in the 4-h Prl induction regimen proved to be critical for IRF-1 detection, because CHX stabilized the unstable rIRF-1 transcripts. Hence, IRF-1 superinduction by Prl plus CHX reflects in part a stabilization of rIRF-1 mRNA levels by a posttranscriptional mechanism, possibly involving the AUUUA sequence motif located in the 3′ noncoding region of rIRF-1 mRNA (Fig. 4A).

It is not known whether rIRF-1 protein activity is also transiently regulated by Prl. rIRF-1, mIRF-1, and hIRF-1 are highly conserved in the first half of the molecule, including the basic domain which is implicated in DNA binding (Fig. 5) (17). A number of potential CK-II consensus sites lie within and border this conserved basic domain (Fig. 4 and 5). Phosphorylation of the IRF-1 protein at these sites may alter

its efficiency of binding to DNA (18, 35). Recent studies suggest that the c-*myc*-encoded protein is phosphorylated by a CK-II-like enzyme in vivo (32) and that such phosphorylation of the c-*myc*-encoded protein might play a role in signal transduction to the nucleus. Interestingly, this basic domain also contains the sequence Arg-Lys-X-Arg-Lys-X-Lys, which is reminiscent of the highly conserved nuclear localization signal Arg-Lys-X-(Arg-Arg/Lys-Lys)-X-X-(Arg/Lys) found in steroid hormone receptors and the simian virus 40 large T antigen (16). Thus, phosphorylation at the CK-II sites around this basic domain could potentially influence important regulatory regions in IRF-1 involved in DNA binding and/or nuclear translocation. Rapid modulation through protein phosphorylation may be another way of transiently modifying IRF-1 protein activity.

Novel role of rIRF-1 in cell proliferation? The function of rIRF-1 in Prl-mediated cell proliferation is unclear. The transient nature of rIRF-1 expression suggests that the rIRF-1 gene product is important for initiation of the G0-to-G1 transition within the cell cycle, and its continued presence may not be needed for progression through G1. Interestingly, the gene for IRF-1 was originally isolated on the basis of its properties as a transcription factor regulating the expression of the genes for IFN in Newcastle disease virus-induced mouse L929 fibroblasts (35). Since IFNs represent highly potent polypeptides that inhibit cell growth (10, 42), the paradox arises that in Prl-stimulated Nb2 T cells poised for cell growth, its transcription factor, rIRF-1, should be isolated as an immediate-early gene. In this regard, it will be interesting to see whether Nb2 T cells are induced by Prl to synthesize IFN through rIRF-1 induction. Temporal restriction in the length of rIRF-1 expression might be needed to prevent overproduction of these potent growth-inhibitory molecules. If this is true, then the mitogenic signal from Prl would be inherently self-limiting through eventual induction of antiproliferative IFN and IFN-inducible genes by the transcription factor rIRF-1. Alternatively, rIRF-1 may simply regulate other growth response genes not related to the growth-inhibitory pathways.

rIRF-1 expression is not limited to one cell type, as its expression was observed in proliferating T cells, mammary epithelial cells, and fibroblasts (Fig. 6). rIRF-1 is also widely expressed in various normal rat tissues (Fig. 7). In particular, rIRF-1 mRNA was detected at significant levels in small intestine tissue, which contains an abundance of rapidly proliferating crypt cells (24). IRF-1 expression appears to be correlated with the proliferating status of some tissues, as rapidly growing mammary epithelial cells, as well as concanavalin A-stimulated splenocytes also exhibit increased levels of IRF-1 mRNA (L. A. Schwarz and L.-Y. Yu-Lee, unpublished data). At least two other stimuli, Newcastle disease virus and IFN-β, also induce transient IRF-1 expression in mouse fibroblasts (17, 35). These observations show that rIRF-1 may be inducible by multiple stimuli in the other tissues and cell types, in addition to a specific response to Prl in Nb2 T cells.

Transcriptional and posttranscriptional mechanisms involved in Prl action. Another tissue addressed by our studies is the mechanism by which Prl regulates gene expression in its target tissues. A handful of Prl-regulated, differentiation-specific marker genes from mammary gland tissue appear to be regulated by Prl primarily at the posttranscriptional level (11, 36), although a specific effect of Prl on gene transcription has been observed (9; H. S. Goodman and J. M. Rosen, J. Cell Biol. **109**:102a, 1989). Moreover, maximal induction of these differentiation-specific genes by Prl occurs only after several days (11). This study is the first to demonstrate that a gene that encodes a potent transcription factor, IRF-1, is regulated by Prl at the transcriptional level (Fig. 3). Furthermore, rIRF-1 gene transcriptional induction by Prl is maximal within 1 h of hormone stimulation, demonstrating that it is also one of the earliest-responding genes regulated by Prl. We suspect that IRF-1 is a nuclear mediator of the Prl response in Nb2 T cells.

rIRF-1, a multifunctional molecule. These studies suggest that rIRF-1 is a highly inducible immediate-early gene whose expression is part of an early mitogenic response to Prl in Nb2 T cells. These studies also indicate that IRF-1 plays a pleiotropic role in the regulation of cell proliferation and/or differentiation, that is, that it may be growth promoting in one cell type and growth inhibiting in another via expression of IFN genes. In this manner, IRF-1 may be a master switch gene, capable of responding to multiple external signals, in addition to a specific response to Prl in T cells. Thus, we suspect that IRF-1 and the recently identified transcriptional repressor IRF-2, which share identical DNA-binding specificities (17), are a new class of transcription factors which may be involved in the expression of a network of other early genes whose transcriptional activity may be amplified or limited by IRF-1 and IRF-2. These studies further show that rIRF-1 represents an excellent molecular marker with which to examine the role of Prl in T-cell growth regulation and activation and should provide new insights into the immunoregulatory properties manifested by this versatile neuroendocrine hormone.

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