# Communication

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# Growth Hormone Induces a DNA Binding Factor Related to the Interferon-stimulated 91-kDa Transcription Factor*

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**Signaling mechanisms leading to regulation of gene transcription by growth hormone (GH) and other molecules that signal via the cytokine receptor family have been elusive. Based upon recent findings that GH and interferons activate JAK family tyrosine kinases, we have identified a novel signaling pathway leading from the GH receptor to the nucleus. We report that in 3T3-F442A fibroblasts, GH stimulates tyrosyl phosphorylation of a protein recognized by antibody to p91, a component of DNA-binding complexes that are activated by tyrosyl phosphorylation in response to interferons α and γ. In addition, a GH-inducible DNA binding factor (GHIF) is identified that binds to the c-*sis*-inducible element of the c-*fos* promoter. GHIF contains a protein antigenically related to p91 and is tyrosyl-phosphorylated. These findings indicate that in signaling between their receptors and the nucleus, GH and interferons utilize related or identical components, including JAK family tyrosine kinases and proteins in the p91 family. When combined with recent findings that many members of the cytokine receptor family activate JAK kinases, including some cytokines that activate p91-related proteins, these findings suggest that signaling pathways involving JAK kinases and p91 family members may be broadly distributed.**

Signaling by GH has recently been shown to involve activation of the tyrosine kinase JAK2 (1). JAK2, a member of the Janus family of protein tyrosine kinases (2–4), also serves as a signaling molecule for interferon (IFN)[1] γ, erythropoietin, and various other cytokine receptors (5–7). Tyk2, another kinase in the JAK family, mediates responses to IFN α/β (8). In investigating events subsequent to activation of JAK2, we found that GH stimulated tyrosyl phosphorylation of a protein of approximately 90 kDa in murine 3T3-F442A fibroblasts (9). Interestingly, a 91-kDa tyrosyl-phosphorylated protein (p91, also known as Stat 91) (10) has been identified as a component of transcription factor complexes induced by IFNα and IFNγ. In response to IFNα, p91 forms a complex with two other tyrosyl-phosphorylated proteins (p84 and p113) and a DNA binding subunit (p48) to form the IFN-stimulated gene factor 3 complex (ISGF3), which initiates transcription of IFNα-stimulated genes (11–13). With IFNγ treatment, p91 appears to be tyrosyl-phosphorylated, to translocate to the nucleus, and to bind directly to IFNγ responsive DNA sequences (10, 14–16). p91 has recently been implicated in stimulation of gene expression by a variety of cytokines (17) and by epidermal growth factor (EGF) (18–21). In this paper, we examine whether a GH-induced signaling pathway involves p91.

## EXPERIMENTAL PROCEDURES

*Materials*—3T3-F442A cells were provided by Dr. H. Green (Harvard University) and Dr. M. Sonenberg (Sloan-Kettering), and were maintained as previously described (22). Recombinant human GH was provided by Genentech and Eli Lilly. Murine IFNγ was provided by Genentech. Anti-p91 serum (αp91) was prepared against the 39 COOH-terminal amino acids unique to p91 (17) and thus does not cross-react with p84, an alternately spliced form of p91 (23). Antiserum αp113 was raised against the 113-kDa protein of ISGF3. A mouse monoclonal antibody against phosphotyrosine (αTyr(P) 4G10) was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Oligonucleotides used to probe for the c-*sis*-inducible element (SIE) contained the sequences of a high affinity binding site for c-*sis*-inducible factor (m67) or a non-binding mutant of the SIE (m57), as described (24).

*Immunoblotting*—Confluent 3T3-F442A fibroblasts were deprived of serum overnight and treated as indicated. Following treatment, cells were washed three times with 3 ml of PBSV buffer (10 mM sodium phosphate, 150 mM sodium chloride, 1 mM sodium orthovanadate, pH 7.4), then scraped in 0.5 ml of lysis buffer (10 mM Tris, 150 mM sodium chloride, 2 mM EGTA, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and 10 μg/ml each of leupeptin and aprotinin, pH 7.4). Insoluble material was cleared by centrifugation. αp91 (1:500 dilution) or αp113 (1:100 dilution) was added to the supernatant and incubated on ice for 2 h. Immune complexes were collected on 50 μl of protein A-agarose beads during a 1-h incubation at 8 °C. The beads were washed three times with 1.5 ml of wash buffer (10 mM Tris, 150 mM sodium chloride, 2 mM EGTA, 0.1% Triton X-100, pH 7.4), and the immunoprecipitated proteins were stripped from the beads by heating for 5 min at 100 °C in 100 μl of quench (4 parts lysis buffer to 1 part 250 mM Tris, pH 6.8, 10% SDS, 10% β-mercaptoethanol, 40% glycerol). The proteins were resolved on a 3–10% SDS-polyacrylamide gel and electrophoretically transferred to nitrocellulose membranes. Western blotting with αTyr(P) (0.2 μg/ml) was done as previously described (9). In some experiments, blots were reprobed with αp91 (1:2500 dilution).

*Electrophoretic Mobility Shift Assay*—Confluent 3T3-F442A fibroblasts (22) were incubated at 37 °C in medium containing 0.5% calf serum for 40 h prior to treatment with GH, murine IFNγ or vehicle at 37 °C for 30 min unless indicated otherwise. Nuclear extracts were prepared essentially as described (25) using hypotonic buffer (20 mM Hepes, pH 7.9, 1 mM EDTA, 0.2% Nonidet P-40, 1 mM EGTA, 20 mM NaF,

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[1] The abbreviations used are: IFN, interferon; ISGF3, IFN-stimulated gene factor 3; GHIF, GH-inducible DNA binding factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; SIE, c-*sis*-inducible element.

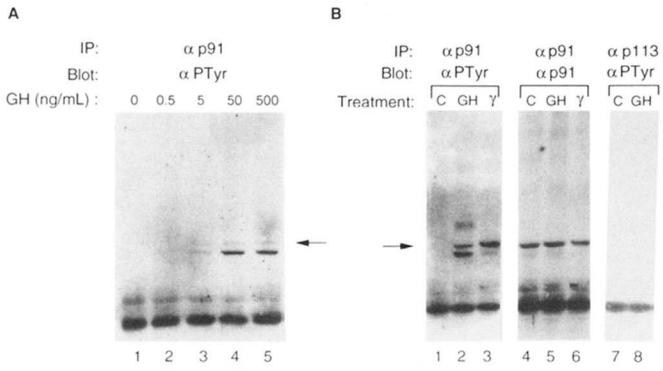


FIG. 1. **GH promotes tyrosyl phosphorylation of proteins antigenically related to p91.** *A*, dose response. 3T3-F442A fibroblasts were incubated with the indicated concentrations of GH for 15 min as described (8). Whole cell lysates were prepared and immunoprecipitated (*IP*) with αp91. The resulting immune complexes were analyzed by Western blotting using αTyr(P). *B*, the p91 proteins phosphorylated in response to GH and IFNγ co-migrate. 3T3-F442A fibroblasts were incubated with GH (500 ng/ml) (*lanes 2*, *5*, and *8*), murine IFNγ (20 ng/ml) (*lanes 3* and *6*), or vehicle (*lanes 1*, *4*, and *7*) for 15 min at 37 °C. Solubilized proteins were immunoprecipitated with αp91 (*lanes 1–6*) or αp113 (*lanes 7* and *8*), then subjected to sequential Western blot analysis first with αTyr(P) (*lanes 1–3*, *7* and *8*) and then with αp91 (*lanes 4–6*). The *arrows* indicate the migration of p91.

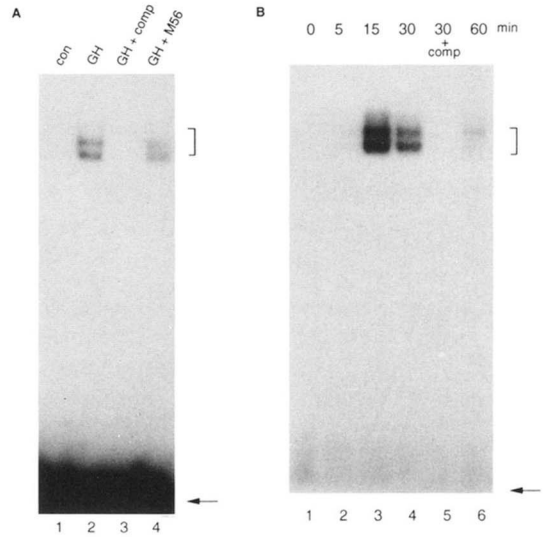


FIG. 2. **GH induces GHIF DNA binding activity in 3T3-F442A fibroblasts.** *A*, induction of GHIF binding activity. Electrophoretic mobility shift assays were performed using a high affinity SIE probe (23) and nuclear extracts from 3T3-F442A fibroblasts treated without (*lane 1*) or with 500 ng/ml GH (*lanes 2–4*). The GH-induced binding (*lane 2*) is competed by 100-fold excess of unlabeled SIE competitor oligonucleotide (*comp*, *lane 3*). A 100-fold excess of unlabeled mutated SIE DNA sequence (m56) competes poorly for binding to the GHIF complex (*lane 4*). *B*, GHIF binding activity is transiently activated. Binding was analyzed after treatment with 500 ng/ml GH for 0 (*lane 1*), 5 (*lane 2*), 15 (*lane 3*), 30 (*lane 4*), or 60 min (*lane 6*). *Lane 5* shows competition by 100X unlabeled SIE oligonucleotide. The *bracket* marks the position of the retarded protein-SIE complex, and the *arrow* indicates migration of free SIE probe.

1 mM $Na_3VO_4$, 1 mM $Na_4P_2O_7$, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml each of leupeptin, aprotinin, and pepstatin) to lyse the cells and high salt buffer (hypotonic buffer with the addition of 420 mM NaCl and 20% glycerol) for nuclear extraction. For the mobility shift assays, 10 μl of incubation buffer (10 mM Hepes, pH 7.9, 50 mM KCl, 1 mM EDTA) containing 8 μg of nuclear extract was mixed with 10 μl of $^{32}$P-labeled SIE probe (40,000 cpm) in the presence of 10 mM Hepes, pH 7.9, 10% glycerol, 1 mM dithiothreitol, 1 μg of poly(dI·dC), and 5 μg of bovine serum albumin. 100-fold excess of unlabeled competitor oligonucleotide was added as indicated. Binding reactions proceeded for 30 min at 30 °C. When indicated, nuclear extracts were preincubated for 20 min at room temperature with αp91, rabbit nonimmune serum, αTyr(P), or an irrelevant monoclonal antibody, anti-p53 (4 μg, Santa Cruz Biotechnical). Binding was analyzed on 5% polyacrylamide gels (39:1, acrylamide:bisacrylamide ) containing 2.5% glycerol in 0.25 × TBE (22.5 mM Tris borate, 0.5 mM EDTA, pH 8.0). Gels were fixed, dried, and subjected to autoradiography.

## RESULTS

To determine whether a protein related to p91 is tyrosyl-phosphorylated in response to GH, antibodies made against p91 of the ISGF3 complex (αp91) were used to immunoprecipitate protein from 3T3-F442A fibroblasts treated with GH. Tyrosyl-phosphorylated proteins in the immunoprecipitates were identified by immunoblotting with a monoclonal antibody against phosphotyrosine (αTyr(P)). Fig. 1*A* demonstrates that in GH-treated cells αp91 precipitates two tyrosyl-phosphorylated proteins with apparent $M_r$ of 94,000 and 85,000 that are absent in immunoprecipitates from control cells. The amount of both tyrosyl-phosphorylated proteins increases with GH concentrations from 0.5 to 500 ng/ml, within the physiological range for GH secretory episodes in rodents (26, 27). These two proteins co-migrate with tyrosyl-phosphorylated proteins immunoprecipitated from IFNγ-treated cells (Fig. 1*B*). The larger of these proteins co-migrates with p91, as determined by sequentially probing the immunoblot first with αTyr(P) (Fig. 1*B*, *lanes 1–3*) and then with αp91 (Fig. 1*B*, *lanes 4–6*). The identity of the more rapidly migrating tyrosyl-phosphorylated protein, which is seen prominently in the αp91 precipitates from GH-stimulated cells (Fig. 1*B*, *lane 2*) and more weakly in those from IFNγ-stimulated cells (*lane 3*), is unknown. This 85-kDa phosphoprotein (p85) is not recognized in Western blots by an antibody that recognizes both p91 and p84, an alternately spliced form of p91 (not shown). The relative intensities of p91 and p85 varied from experiment to experiment, but the ratio of p91 to p85 was always higher in IFNγ-treated cells than in GH-treated cells. A third tyrosyl-phosphorylated protein detected in *lane 2* was seen in only one of seven experiments. It is unlikely to be p113, since αp113 did not precipitate any tyrosyl-phosphorylated proteins from GH-treated cells (Fig. 1*B*, *lane 8*), although the αp113 antibody did precipitate a tyrosyl-phosphorylated 113-kDa protein from IFNα-treated 2fTGH cells (data not shown).

Because p91-containing complexes induced by IFNs α or γ, when tyrosyl-phosphorylated, bind to DNA of IFN-regulated genes (11, 12, 28–30), we investigated whether p91-related proteins might play a comparable role when induced by GH. Since GH stimulates the transcription of the proto-oncogene c-*fos* in 3T3-F442A cells (22), the c-*fos* promoter was used to probe for GH-inducible DNA-binding complexes that might contain proteins related to p91. In these cells, GH was found to induce the binding of a nuclear factor to the SIE of the c-*fos* promoter, as analyzed by electrophoretic mobility shift assays using a high affinity SIE probe (24) (Fig. 2). The SIE lies 346 base pairs upstream of the c-*fos* transcription start site and was identified as a binding site for c-*sis*-inducible factor (31). The SIE contains sequences very similar to the GAS (γ-interferon-activated site) and GRR (IFNγ response region) recognized by p91 after IFNγ stimulation (9, 32).

This newly identified DNA-binding factor, termed GH-inducible factor (GHIF), produces multiple bands in the mobility shift assay (Fig. 2*A*, *lane 2*), suggesting that GHIF is a complex of multiple components and/or is present in multiple phosphorylation states. Binding to the SIE was completely competed by unlabeled probe (Fig. 2, *panel A*, *lane 3*; *panel B*, *lane 5*) but poorly by an oligonucleotide containing a mutated SIE sequence (m56), which failed to bind c-*sis*-inducible factor or to mediate c-*fos* induction by PDGF (24) (Fig. 2*A*, *lane 4*). GHIF binding activity peaked in 15 min and subsided within 60 min

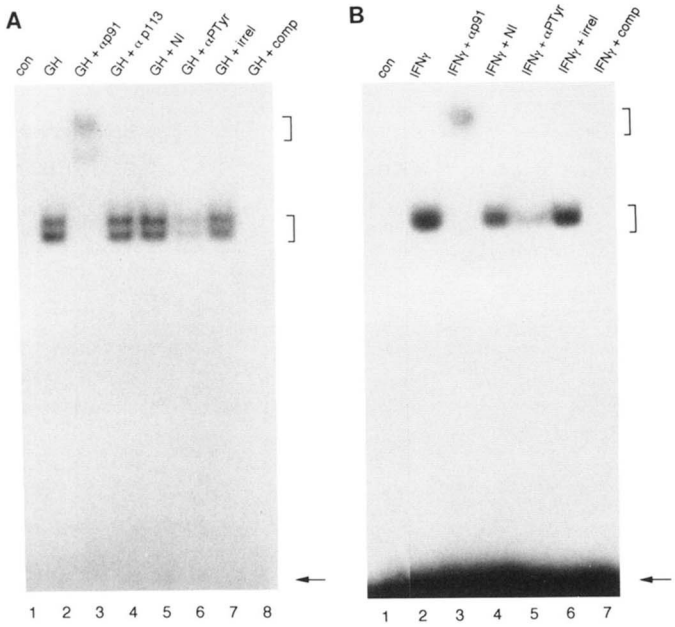


FIG. 3. **GHIF contains p91-related proteins and phosphotyrosine.** *A*, nuclear extracts from 3T3-F442A cells treated with GH (500 ng/ml) (*lanes 2–8*) or vehicle (*lane 1*) were preincubated for 20 min at room temperature with αp91 (*lane 3*), αp113 (*lane 4*), or rabbit nonimmune serum (*NI*, *lane 5*) (each 1:40 dilution in the final binding reaction) or with αTyr(P) (1 μg, *lane 6*) or an irrelevant (*irrel*, *lane 7*) monoclonal antibody, anti-p53 (4 μg, Santa Cruz Biotechnical), prior to use in the mobility shift assay. When extracts from GH-treated cells were preincubated with αp91 prior to the addition of the SIE probe, a supershift of the GH-induced bands is visible (*lane 3*, *upper bracket*), whereas αp113 (*lane 4*) or nonimmune rabbit serum (*NI*, *lane 5*) had no effect. GH-induced binding (*lower bracket*) is specifically competed by addition of 100-fold excess of unlabeled SIE (*lane 8*). *B*, IFNγ induces DNA-binding activity containing p91-related proteins. 3T3-F442A fibroblasts were treated with IFNγ (10 ng/ml, *lanes 2–6*) or vehicle (*lane 1*) for 30 min, and nuclear extracts were used in mobility shift assays as described above. The presence of p91 in the induced protein-SIE complex (*lane 2*, *lower bracket*) is indicated by the supershift when αp91 was added (*lane 3*, *upper bracket*). Non-immune serum (*lane 4*) had no effect. The presence of phosphotyrosine is indicated by a decrease in intensity of the shifted band when αTyr(P) was added (*lane 5*); the irrelevant monoclonal antibody had no effect (*lane 6*). 100-fold unlabeled SIE successfully competes for binding (*lane 7*). The *arrow* indicates migration of free probe.

(Fig. 2*B*) and was detectable at GH concentrations as low as 0.5 ng/ml (data not shown).

To investigate whether GHIF contains components related to the DNA binding factors induced by IFNs, the influence of αp91 on the binding of GHIF was tested. Addition of αp91 to nuclear extract just prior to its interaction with the GHIF-binding sequence results in a supershift of the GHIF·SIE complex in the mobility shift assay (Fig. 3*A*, *lane 3*, *upper bracket*), consistent with the presence of a protein in GHIF antigenically related to p91. In contrast to the supershift induced by αp91, neither αp113 (Fig. 3*A*, *lane 4*) nor non-immune serum (*lane 5*) altered the GHIF·SIE complex. Furthermore, a supershift does not occur when αp91 is used with an unrelated probe for the c-*fos* serum response element (data not shown).

Phosphorylation of p91 on $Tyr^{701}$ has recently been shown to be required for its nuclear translocation, DNA binding, and gene activation (10). The importance of tyrosyl phosphorylation of GHIF is suggested by the observation that preincubation of nuclear extracts from GH-treated cells with αTyr(P) reduces GH-induced binding (Fig. 3*A*, *lane 6*), while an irrelevant antibody (*lane 7*) has no effect. Furthermore, treatment of extracts with a recombinant tyrosine phosphatase from *Yersinia enterocolitica* (33) also reduces GHIF binding activity (not shown).

To determine whether the DNA binding factor induced by IFNγ is similar to GHIF, 3T3-F442A fibroblasts were treated with IFNγ. Like nuclear extracts from GH-treated cells, extracts from IFNγ-treated cells contain proteins that bind to the SIE sequence (Fig. 3*B*, *lane 2*). However, only the lower band appears prominently in the mobility shift assay when cells are stimulated with IFNγ. As with GHIF, the binding of the IFNγ-induced complex was reduced by αTyr(P) (Fig. 3*B*, *lane 5*) and the complex was supershifted by αp91 (Fig. 3*B*, *lane 3*), indicating that the DNA-binding complex is tyrosyl-phosphorylated and contains protein antigenically related to p91.

## DISCUSSION

The present findings indicate that GH, which signals via the tyrosine kinase JAK2 (1), induces binding of the GHIF complex containing p91-related proteins to the SIE of the c-*fos* promoter. This suggests that there are common features in the signaling cascades for GH, IFNγ, and IFNα, and most likely for other members of the cytokine receptor superfamily that signal via JAK kinases (*i.e.* receptors for erythropoietin, interleukin-3, prolactin, granulocyte macrophage colony stimulating factor, granulocyte colony stimulating factor, the gp130 subunit of receptors for interleukin-6, ciliary neurotrophic factor, leukemia inhibitory factor, and oncostatin M (5–7, 42).[2] The shared features include activation of JAK family kinases, tyrosyl phosphorylation of p91-related proteins and participation of p91-related proteins in DNA-binding complexes, presumably culminating in regulation of gene transcription. While activation of JAK family members appears to be required for phosphorylation of p91 (8, 9), it remains to be determined whether JAK kinases phosphorylate p91 directly, whether additional p91 family proteins exist, what other proteins complex with p91, and whether and how such a cascade might intersect with other signaling pathways. The SH2 domains present in p91 have been shown to be essential for SIE-mediated gene activation (21). Thus, interaction of p91 with itself or other tyrosyl-phosphorylated proteins is highly probable.

While the implications suggested by the shared features in GH and IFN signaling are intriguing, it is also important to consider differences between responses to GH and IFNγ. The difference in the pattern of bands in the shifted complexes from GH- and IFNγ-treated cells suggests that GHIF and the IFNγ-stimulated factor are not identical. One possible explanation for this difference is that GH- and IFNγ-stimulated DNA-binding complexes contain multiple proteins, only some of which are shared (*e.g.* p91). This is consistent with observations (20) that the SIE binding activity induced by EGF is detected as three bands in electrophoretic mobility shift assays using nuclear extracts from A431 cells, but αp91 recognizes only two of the bands. Presumably, the third band contains a different DNA binding component in the SIE-binding complex. In GHIF complexes, a third band is only occasionally observed (data not shown), suggesting that this component of the complex is somewhat labile in 3T3-F442A cells. The 85-kDa phosphoprotein that co-precipitates with p91 in GH-treated 3T3-F442A cells may contribute to DNA-binding complexes, but its role remains to be determined. Overall, differences in p91-related proteins associated with the SIE, or the presence of additional proteins in the complex not related to p91, could be the basis for specificity in signaling involving GH, IFNs, cytokines, or EGF in their respective target cells. The involvement of p84 and p113 in IFNα but not in IFNγ signaling (14, 23) further suggests that specificity in signaling by these ligands derives at least in part from such differences in the transcription factor complexes.

[2] G. S. Campbell, L. S. Argetsinger, J. N. Ihle, P. A. Kelly, J. A. Rillema, and C. Carter-Su, submitted for publication.

Differences in the protein composition of the DNA binding factors induced by these various ligands also raise the possibility that some of these factors may interact with proteins that bind to other elements in the c-*fos* promoter. Since the serum response element contributes to induction of c-*fos* by GH (34) and lies close to the SIE, it is possible that GHIF interacts with proteins associated with the serum response element (35) to regulate c-*fos* transcription.

The identification of GHIF binding activity represents the first identification of a distinct DNA-binding complex rapidly induced by GH. Proteins that bind to the SIE are also inducible by c-*sis*/platelet-derived growth factor (PDGF), EGF, and IFNγ (24, 25, 36, 37). Furthermore, p91-related protein is present in SIE-binding complexes induced by PDGF (19).[3] or by EGF and other growth factors in A431 cells and fibroblasts overexpressing specific growth factor receptors (19–21), as well as in nuclei from liver of EGF-treated mice (18). It has yet to be determined precisely how the SIE-binding complexes induced by cytokines and growth factors compare to GHIF. The ability of the SIE to mediate induction of gene transcription in response to PDGF has been demonstrated in NIH 3T3 fibroblasts (24), and in response to EGF in COS cells overexpressing p91 (21). Although the role of the SIE alone in mediating induction of c-*fos* by GH has not yet been demonstrated, it is of note that the induction of GHIF binding to the SIE occurs at physiological GH concentrations in 3T3-F442A fibroblasts, while other growth factors and cytokines are often studied at superphysiological concentrations. Furthermore, in contrast to studies with cells overexpressing growth factor or cytokine receptors, the 3T3-F442A cells respond to GH in the context of the endogenous, physiological levels of GH receptors, JAK2, and p91. This is consistent with a potential role for the SIE in physiological regulation of c-*fos* expression by GH, a major regulator of normal growth. This role is of particular interest in light of recent observations that mutation of the SIE obliterates normal expression of endogenous Fos in transgenic mice and Fos inducibility in fibroblasts derived from them.[4] A physiological role for Fos in GH-dependent 3T3-F442A cells is consistent with the observations that Fos protein participates in the transcriptional regulation of differentiation-dependent genes in these cells (38, 39), and that GH is required for their differentiation to adipocytes (40, 41). Thus, understanding the regulation of c-*fos* by GH, as well as the participation of p91 in such regulation, can provide insight into the physiological mechanism of action of GH and possibly that of other cytokines and factors that signal via pathways involving p91.

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[3] L. Johnson and B. H. Cochran, personal communication.
[4] T. Curran, personal communication.

REFERENCES

1. Argetsinger, L., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N. & Carter-Su, C. (1993) *Cell* **74,** 237–244
2. Wilks, A. F., Harpur, A. G., Kurban, R. R., Ralph, S. J., Zurcher, G. & Ziemiecki, A. (1991) *Mol. Cell Biol.* **11,** 2057–2065
3. Firmbach-Kraft, I., Byers, M., Shows, T., Dalla-Favera, R. & Krolewski, J. J. (1990) *Oncogene* **5,** 1329–1336
4. Harpur, A. G., Andres, A. C., Ziemiecki, A., Aston, R. R. & Wilks, A. F. (1992) *Oncogene* **7,** 1347–1353
5. Watling, D., Guschin, D., Muller, M., Silvennoinen, O., Witthuhn, B. A., Quelle, F. W., Rogers, N. C., Schindler, C., Stark, G. R., Ihle, J. N. & Kerr, I. M. (1993) *Nature* **366,** 166–170
6. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O. & Ihle, J. N. (1993) *Cell* **74,** 227–236
7. Silvennoinen, O., Witthuhn, B., Quelle, F. W., Cleveland, J. L., Yi, T. & Ihle, J. N. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 8429–8433
8. Velasquez, L., Fellous, M., Stark, G. R. & Pellegrini, S. (1992) *Cell* **70,** 313–322
9. Campbell, G. S., Christian, L. J. & Carter-Su, C. (1993) *J. Biol. Chem.* **268,** 7427–7434
10. Shuai, K., Stark, G. R., Kerr, I. M. & Darnell, J. E., Jr. (1993) *Science* **261,** 1744–1746
11. Schindler, C., Shuai, K., Prezioso, V. R. & Darnell, J. E., Jr. (1992) *Science* **257,** 809–813
12. Fu, X. Y., Schindler, C., Improta, T., Aebersold, R. & Darnell, J. E., Jr. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 7840–7843
13. Kessler, D. S., Veals, S. A., Fu, X. Y. & Levy, D. E. (1990) *Genes & Dev.* **4,** 1753–1765
14. Shuai, K., Schindler, C., Prezioso, V. R. & Darnell, J. E., Jr. (1992) *Science* **258,** 1808–1812
15. Pearse, R. N., Feinman, R., Shuai, K., Darnell, J. E., Jr. & Ravetch, J. V. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 4314–4318
16. Wilson, K. C. & Finbloom, D. S. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 11964–11968
17. Larner, A. C., David, M., Feldman, G. M., Igarishi, K., Hackett, R. H., Webb, D. S., Sweitzer, S. M., Petricoin, E. F., III & Finbloom, D. S. (1993) *Science* **261,** 1730–1733
18. Ruff-Jamison, S., Chen, K. & Cohen, S. (1993) *Science* **261,** 1733–1736
19. Silvennoinen, O., Schindler, C., Schlessinger, J. & Levy, D. E. (1993) *Science* **261,** 1736–1739
20. Sadowski, H. B., Shuai, K., Darnell, J. E., Jr. & Gilman, M. Z. (1993) *Science* **261,** 1739–1744
21. Fu, X.-Y. & Zhang, J.-J. (1993) *Cell* **74,** 1135–1145
22. Gurland, G., Ashcom, G., Cochran, B. H. & Schwartz, J. (1990) *Endocrinology* **127,** 3187–3195
23. Schindler, C., Fu, X. Y., Improta, T., Aebersold, R. & Darnell, J. E., Jr. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 7836–7839
24. Wagner, B. J., Hayes, T. E., Hoban, C. J. & Cochran, B. H. (1990) *EMBO J.* **9,** 4477–4484
25. Sadowski, H. B. & Gilman, M. Z. (1993) *Nature* **362,** 79–83
26. Tannenbaum, G. S. & Martin, J. B. (1976) *Endocrinology* **98,** 562–571
27. Eden, S. (1979) *Endocrinology* **105,** 555–563
28. Gutch, M. J., Daly, C. & Reich, N. C.(1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 11411–11415
29. David, M., Romero, G., Zhang, Z. Y., Dixon, J. E. & Larner, A. C. (1993) *J. Biol. Chem.* **268,** 6593–6599
30. Igarashi, K., David, M., Finbloom, D. S. & Larner, A. C. (1993) *Mol. Cell Biol.* **13,** 1634–1640
31. Hayes, T. E., Kitchen, A. M. & Cochran, B. H. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 1272–1276
32. Pearse, R. N., Feinman, R., Shuai, K., Darnell, J. E., Jr., Ravetch, J. V. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 4314–4318
33. Guan, K. & Dixon, J. E. (1990) *Science* **257,** 813–815
34. Meyer, D. J., Stephenson, E. W., Johnson, L., Cochran, B. H. & Schwartz, J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 6721–6725
35. Treisman, R. (1992) *Trends Biochem. Sci.* **17,** 423–426.
36. Herrera, R. E., Shaw, P. E. & Nordheim, A. (1989) *Nature* **340,** 68–70
37. Hannigan, G. E. & Williams, B. R. (1992) *J. Interferon Res.* **12,** 355–361
38. Distel, R., Ro, H. S., Rosen, B. S., Groves, D. & Spiegelman, B. M. (1987) *Cell* **49,** 835–844
39. Rauscher, F. J., III, Sambucetti, L. C., Curran, T., Distel, R. J. & Spiegelman, B. M. (1988) *Cell* **52,** 471–480
40. Morikawa, M., Nixon, T. & Green, H. (1982) *Cell* **29,** 783–789
41. Nixon, T. & Green, H. (1984) *Endocrinology* **14,** 527–532
42. Stahl, N., Boulton, T. G., Farruggella, T., Ip, N. Y., Davis, S., Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J. N. & Yancopoulos, G. D. (1994) *Science*, in press