

## Role of the STAT1-SH2 Domain and STAT2 in the Activation and Nuclear Translocation of STAT1\*

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**Interferon (IFN) induction of immediate-early response genes is mediated through the signal transducers and activators of transcription (STATs). Activation of STAT1 by IFN $\alpha$  or IFN $\gamma$  through its tyrosine phosphorylation involves members of the Jak tyrosine kinases. In addition, STAT2 is activated by IFN $\alpha$ , and, together with STAT1 and p48/ISGF3 $\gamma$ , forms the transcription factor complex ISGF3. Previous findings suggested that the STAT1-SH2 domain, which is required for the homo- or heterodimerization of STAT1, also participates in the recruitment of STAT1 to the IFN-receptors, because mutations in the SH2-domain abolished STAT1 activation by IFN $\gamma$ . Furthermore, STAT2 was reported to be required for the activation of STAT1 by IFN $\alpha$ . We were able to induce STAT1 tyrosine phosphorylation by IFN $\alpha/\beta$  in the absence of STAT2 or a functional STAT1-SH2 domain. In contrast, IFN $\gamma$  was unable to cause tyrosine phosphorylation of STAT1-(SH2:Arg  $\rightarrow$  Gln). Interestingly, although STAT1 was found in the nucleus in STAT2-deficient cells, the nuclear accumulation of the tyrosine phosphorylated SH2-mutant STAT1 was impaired. In summary, our results indicate that the SH2 domain of STAT1 is not required for its ligand-dependent activation by IFN $\alpha/\beta$ . Moreover, tyrosine phosphorylation is not sufficient to target STAT1 to the nucleus; rather, dimerization appears to play a critical role in the subcellular distribution of STAT1.**

Interferons as well as many other cytokines and growth factors mediate their biological effects through the induction of a set of immediate-early response genes (1–10). This process depends on the activation of a family of SH2 and SH3 domain containing signal transducers and activators of transcription (STATs) (11–16).<sup>1</sup> Activation of latent, cytoplasmic, or membrane-associated STAT proteins is accomplished through their

tyrosine phosphorylation (11, 15–17), which in most cases depends on the activity of the Janus protein-tyrosine kinases (Jaks) (18–25). IFN $\gamma$  initiation of STAT1 tyrosine phosphorylation requires the activity of Jak1 and Jak2 (18, 19), whereas IFN $\alpha/\beta$  mediates STAT1 and STAT2 activation through the kinases Jak1 and Tyk2 (18, 25). Activation of STAT1 by IFN $\alpha/\beta$  was also reported to depend on the presence of STAT2 (26, 27), implying a sequential activation of STAT proteins through the IFN $\alpha/\beta$  receptor. More recently, Li *et al.* demonstrated that STAT1 and STAT2 are prebound to the inactive IFNAR2c chain and that this association requires the N-terminal region of STAT2 (28).

After its tyrosine phosphorylation, STAT1 either homodimerizes or forms heterodimers when STAT2 is activated by IFN $\alpha/\beta$  to translocate to the nucleus where site-specific binding to enhancer elements leads to gene activation (29, 30). Nuclear import is often controlled by binding of a karyophilic protein that contains a single or bipartite nuclear localization signal to the nuclear pore, with subsequent import in a GTP hydrolysis-dependent manner. Although the dependence of STAT1 nuclear import in response to IFN $\gamma$  on the GTPase activity of Ran/TC4 has been demonstrated (31), no nuclear localization signal has been identified in any of the STAT proteins. Tyrosine phosphorylation of STAT1 is an absolute prerequisite for its nuclear translocation and its ability to bind DNA (32, 33). The SH2 domain of STAT1 has been implicated in the activation as well as in the dimerization process (29, 30, 34–36), because mutations in the SH2 domain of STAT1 also prevent it from becoming tyrosine phosphorylated (34, 37). This defect in the ability to activate an SH2 domain-mutated STAT1 (STAT1-SH2mut) made it thus far impossible to determine whether dimerization is required for nuclear translocation of STAT1 or whether the phosphorylated tyrosine residue serves as a binding site for a potential transport protein.

Here we demonstrate the activation of STAT1 by IFN $\alpha/\beta$ , but not IFN $\gamma$ , in the absence of a functional STAT1-SH2 domain; furthermore, we show STAT1 activation by IFN $\alpha/\beta$  in STAT2-deficient cells. Importantly, whereas the lack of STAT2 did not affect the nuclear localization of STAT1, the abrogation of STAT1-SH2 domain function resulted in the inability of STAT1 to translocate to the nucleus despite its tyrosine phosphorylation.

### MATERIALS AND METHODS

**Cells**—2ftgh, U3-SH2mut, and U6A cells were described previously (26, 38). Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin (Irvine Scientific).

**Interferons and Reagents**—IFN $\alpha$ , IFN $\beta$ , and IFN $\gamma$  were generous gifts from Hoffman LaRoche, Chiron, and Genentech, respectively. 50 mM sodium vanadate and 100 mM hydrogen peroxide (both from Fisher) were incubated in Dulbecco's modified Eagle's medium without fetal bovine serum for 15 min prior to addition to cells.

**Whole Cell Extracts**—Following treatment, cells were washed with PBS and lysed on the plates with lysis buffer (1 ml) containing 20 mM Hepes, pH 7.4, 1% Triton X-100, 100 mM NaCl, 50 mM NaF, 10 mM  $\beta$ -glycerophosphate, 1 mM sodium vanadate and 1 mM phenylmethylsulfonyl fluoride. Lysates were centrifuged at 13,000 rpm for 5 min, and protein concentration was determined by Lowry (Bio-Rad Protein Assay).

**Immunoprecipitation and Immunoblotting**—Lysates were precleared by incubation with protein G-Sepharose for 30 min; subsequently, lysates were incubated for 2 h with a polyclonal antibody against the C terminus of STAT1 and protein G-Sepharose for an additional hour. Immunoprecipitates were washed three times with ice-cold lysis buffer,

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<sup>1</sup> The abbreviations used are: STATs, signal transducers and activators of transcription; IFN, interferon; PBS, phosphate-buffered saline; EMSA, electrophoretic mobility shift assay; PIPES, 1,4-piperazinediethanesulfonic acid.

resuspended in SDS sample buffer, and resolved on a 7.5% SDS-polyacrylamide gel (Bio-Rad). After transfer onto polyvinylidene difluoride membrane, proteins were detected with anti-phosphoSTAT1 (1:500; New England Biolabs) or anti-STAT1 (1:1000; Transduction Labs) antibodies. Blots were developed with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Amersham Pharmacia Biotech).

**Electrophoretic Mobility Shift Assay**—EMSA were performed using whole cell extracts prepared as described above and an end-labeled oligonucleotide corresponding to the GRR sequence found in the promoter sequence of the FcγRI (5'-AATTAGCATGTTTCAAGGATTT-GAGATGTATTTCCAGAAAAG-3') as described previously (39).

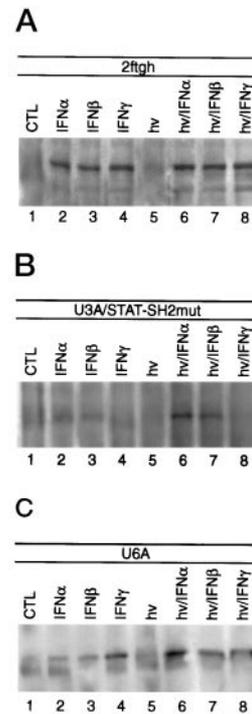
**Immunofluorescence**—Cells were seeded onto coverslips in 6-well plates and incubated overnight at 37 °C in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. After treatment, coverslips were washed once with PBS followed by one wash with PIPES buffer. Cells were fixed in methanol at room temperature for 6 min, and nuclei were permeabilized by incubating with 0.5% Nonidet P-40/PIPES buffer for 13 min at room temperature. Coverslips were washed three times with PBS, blocked with 10% goat serum for 35 min, and incubated with anti-STAT1 (Transduction Laboratory) for 50 min at room temperature. Cells were rinsed four times for 5 min in PBS before incubation with Cy3-conjugated secondary antibody for 40 min at room temperature. After washing, coverslips were mounted onto glass slides in 50% glycerol/PBS.

## RESULTS AND DISCUSSION

Significant progress has been made in our understanding of the mechanisms that lead to the activation of STAT proteins through numerous cytokine and growth factor receptors. However, very little is known about the process of nuclear translocation of STAT proteins after their tyrosine phosphorylation. Although tyrosine phosphorylation of STAT1 is required for its nuclear localization, it remains unclear whether phosphorylation of Tyr<sup>701</sup> is solely required for STAT dimerization or whether a cytosolic transport protein utilizes this phosphotyrosine residue as a docking site. Previous findings suggested that the SH2 domain of STAT1 was required to recruit latent STAT1 to activated receptors (29, 35, 36, 40–42). Consequently, the reverse approach of introducing an Arg → Gln mutation in the SH2 domain to render it incapable of binding phosphotyrosine and preventing dimerization was unsuccessful, because a STAT1 carrying such a mutation did not become tyrosine phosphorylated in response to IFN or epidermal growth factor (34, 37).

It has been previously shown that incubation of cells with a combination of 1 mM hydrogen peroxide and 0.1 mM orthovanadate (H/V) results in the ligand-independent activation of STAT proteins (43, 44). This treatment even proved effective in the activation of STAT1 in the absence of the Jak tyrosine kinases that are required for STAT activation (44), indicating that this procedure effectively bypasses the need for receptor-mediated signaling. We therefore decided to explore the possibility that this treatment might also activate a STAT1 carrying the Arg → Gln mutation in the SH2 domain. Indeed, exposure of either wild-type 2ftgh cells or STAT1-deficient U3A cells stably expressing STAT1-SH2mut to H/V resulted in the IFN-independent tyrosine phosphorylation of STAT1 or STAT1-SH2mut, respectively (data not shown). However, this treatment appeared to be extremely toxic to cells, because we found that it not only failed to target tyrosine phosphorylated wild-type STAT1 into the nucleus, but it also prevented the nuclear translocation of wild-type STAT1 when IFN was added after H/V, despite the fact that this costimulation resulted in a further increase in STAT1 tyrosine phosphorylation (data not shown).

We therefore decided to titrate the concentration of H/V to a level where it did not affect the normal nuclear translocation of wild-type STAT1 after stimulation of cells with IFN. It was found that a significantly lower concentration of H/V (referred



**FIG. 1. Tyrosine phosphorylation of STAT1 in the absence of STAT1-SH2 domain or STAT2.** 2ftgh (A), U3A reconstituted with STAT1-SH2mut (B), and U6A (C) were stimulated for 30 min with either IFN $\alpha$  (500 units/ml), IFN $\beta$  (500 units/ml), or IFN $\gamma$  (10 ng/ml) without (lanes 1–4) or with (lanes 5–8) prior priming of cells for 30 min with h/v (10  $\mu$ M/5  $\mu$ M). Whole cell extracts (15  $\mu$ g) were subjected to immunoprecipitation with antiserum directed against the C terminus of STAT1, and isolated proteins were resolved on a 7.5% SDS-polyacrylamide gel. After transfer to Immobilon polyvinylidene difluoride membrane, activated STAT1 was detected with a polyclonal anti-(Tyr<sup>701</sup>)PhosphoSTAT1 antibody. Loading of equal amounts of STAT1 was confirmed by reprobing with STAT1 antiserum (data not shown). CTL, control.

to as h/v, to indicate 10  $\mu$ M hydrogen peroxide and 5  $\mu$ M orthovanadate) did not interfere with the nuclear translocation of wild-type STAT1 after IFN stimulation (see Fig. 3A, lower panel) but was also unable to induce tyrosine phosphorylation of wild-type STAT1 or STAT1-SH2mut (Fig. 1, A and B, lanes 5). Surprisingly, however, although the exposure of cells to this low concentration of h/v did not cause tyrosine phosphorylation of STAT1 or STAT1-SH2mut by itself, it promoted the subsequent activation of STAT1-SH2mut by IFN $\alpha/\beta$ . In contrast, IFN $\gamma$  was still unable to induce tyrosine phosphorylation of STAT1-SH2mut (Fig. 1B, lane 8). Interestingly, we were also able to detect a weak but reproducible tyrosine phosphorylation of STAT1-SH2mut through stimulation with IFN $\alpha/\beta$  alone but not with IFN $\gamma$ . This is in contrast to previous reports suggesting that STAT1 could not become phosphorylated in the absence of a functional SH2 domain; however, these studies were predominantly focused on STAT1 activation by IFN $\gamma$  or epidermal growth factor. Furthermore, we believe that the use of phosphoSTAT1-specific antiserum results in increased sensitivity compared with phosphotyrosine blots, allowing for the detection of low levels of STAT1 phosphorylation. It thus appears that the priming of cells with a subthreshold concentration of h/v facilitates an increase in the activation of STAT1-SH2mut after IFN $\alpha/\beta$  while preserving the IFN-dependent nature of the stimulation. Earlier studies showed that in some cases the autophosphorylation sites of receptors are dispensable for STAT activation, leaving the possibility that the STAT-SH2 domain binds to a receptor-associated, tyrosine phosphorylated protein (24, 45). In contrast, our findings suggest for

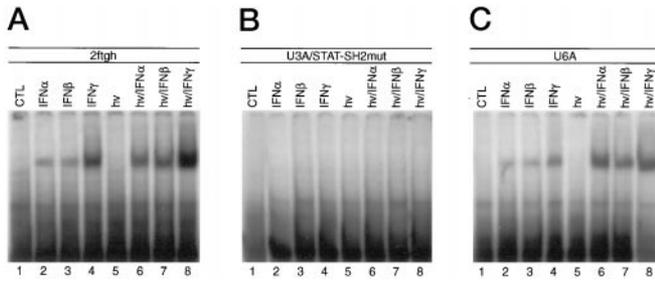


FIG. 2. Effect of h/v priming on EMSA of 2ftgh (A), U3A reconstituted with STAT1-SH2mut (B), and U6A (C) were stimulated for 30 min with either IFN $\alpha$  (500 units/ml), IFN $\beta$  (500 units/ml), or IFN $\gamma$  (10 ng/ml) without (lanes 1–4) or with (lanes 5–8) prior priming of cells for 30 min with h/v (10  $\mu$ M/5  $\mu$ M). Whole cell extracts were prepared, and EMSAs were performed using the end-labeled GRR as a probe. CTL, control.

the first time the presence of an alternate mechanism for recruitment of a STAT protein to an activated cytokine receptor that does not involve the SH2 domain of the STAT protein.

Because it had been reported that STAT2 is essential for activation of STAT1 by IFN $\alpha$  (27), we decided to test whether h/v priming could also overcome this requirement. Indeed, whereas IFN $\alpha/\beta$  alone activates STAT1 only weakly in STAT2-deficient U6A cells (Fig. 1C, lanes 2 and 3), the pretreatment with h/v before addition of IFN $\alpha/\beta$  restored the ligand-induced tyrosine phosphorylation to levels comparable with those seen in wild-type cells (Fig. 1C, lanes 6 and 7). As was the case with STAT1-SH2mut, h/v treatment alone did not lead to any detectable tyrosine phosphorylation in the U6A cells (Fig. 1C, lane 5).

To analyze the DNA binding capabilities of tyrosine phosphorylated STAT1-SH2mut or of STAT1 activated by IFN $\alpha$  in the absence of STAT2, we performed electrophoretic mobility shift assays using the GRR sequence of the high affinity Fc $\gamma$ RI promoter as a probe. As shown in Fig. 2C, tyrosine phosphorylated STAT1 in U6A cells lysates was able to bind to the GRR as expected and confirmed the results of the phosphoSTAT1 Western blots. In contrast, tyrosine phosphorylated STAT1-SH2mut was unable to form a complex capable of binding the GRR (Fig. 2B) or to assemble with activated STAT2 into the interferon-stimulated response element-binding ISGF3 complex (data not shown). These results confirm that the SH2 domain of STAT1 is required for formation of a dimerizing DNA binding complex but is expendable for achieving tyrosine phosphorylation in response to IFN $\alpha/\beta$ .

Our primary goal was to investigate whether the dimerization of STAT1 was required for its nuclear translocation or whether tyrosine phosphorylated STAT1 is transported into the nucleus as a monomer. We therefore resorted to immunohistochemistry to analyze the subcellular localization of tyrosine phosphorylated STAT1-SH2mut or of IFN $\alpha$ -activated STAT1 in the absence of STAT2. As shown in Fig. 3A (upper panel), IFN $\alpha$ -activated, tyrosine phosphorylated STAT1 translocated efficiently into the nucleus, with h/v priming apparently enhancing the level of translocation (Fig. 3A, lower panel). STAT2-deficient U6A cells displayed, in good correlation with the low level of tyrosine phosphorylation of STAT1, only marginal translocation of STAT1 to the nucleus in response to IFN $\alpha/\beta$  alone (Fig. 3C, upper panel). However, h/v priming was able to facilitate substantial nuclear presence of STAT1 in response to IFN $\alpha/\beta$  (Fig. 3C, lower panel).

In contrast, no STAT1-SH2mut could be detected in the nucleus after any of the treatments (Fig. 3B), despite proper phosphorylation of the protein on Tyr<sup>701</sup> in response to IFN $\alpha/\beta$  after h/v priming. These results demonstrate that the tyrosine phosphorylation per se, although required, is not sufficient to

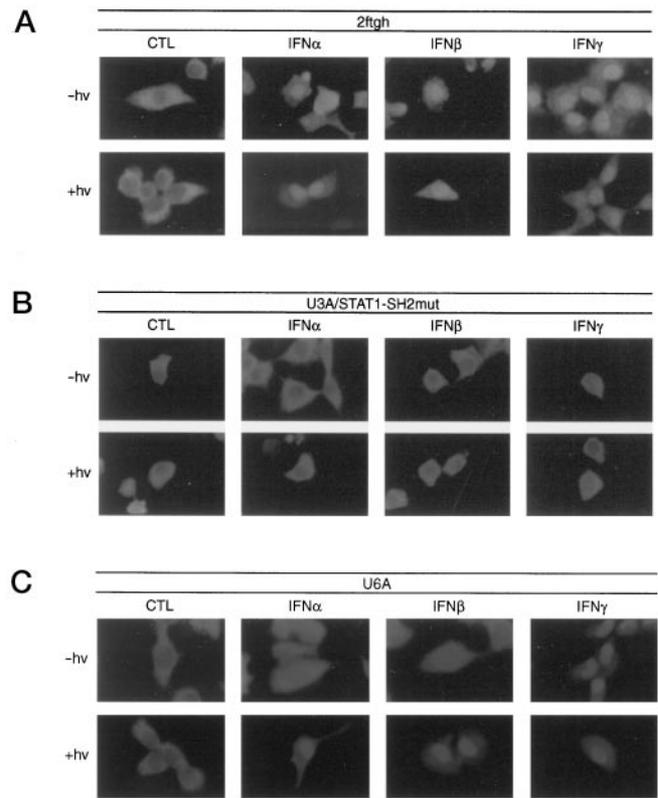


FIG. 3. Nuclear translocation of STAT1 in the absence of STAT1-SH2 domain or STAT2. 2ftgh (A), U3A reconstituted with STAT1-SH2mut (B), and U6A (C) were stimulated for 30 min with either IFN $\alpha$  (500 units/ml), IFN $\beta$  (500 units/ml), or IFN $\gamma$  (10 ng/ml) without (upper panels) or with (lower panels) prior priming of cells for 30 min with h/v (10  $\mu$ M/5  $\mu$ M). Subcellular localization of STAT1 was analyzed using a monoclonal antibody against STAT1. CTL, control.

target STAT1 to the nucleus. Rather, the dimerization of STAT proteins appears to be the essential mediator of nuclear translocation. It is theoretically possible that h/v priming is able to promote the tyrosine phosphorylation of STAT1-SH2mut in response to IFN $\alpha/\beta$  but is unable to facilitate any additional phosphorylation on serine/threonine residues that could be required for STAT1 translocation to the nucleus. However, thus far the only reported ligand-induced serine phosphorylation of STAT1 occurs on Ser<sup>727</sup> (46), a residue that is absent in the STAT1 $\beta$  splice variant. Nevertheless, STAT1 $\beta$  is still able to translocate to the nucleus in response to IFN stimulation.

In conclusion, our results show for the first time that STAT1 can be rapidly activated in a ligand-dependent manner even in the absence of a functional SH2 domain, demonstrating that the binding of the STAT1-SH2 domain to receptor phosphorylation sites is not the only mechanism that allows STAT1 to interact with an activated cytokine receptor. In contrast, an intact SH2 domain is required in order for STAT1 to be imported into the nucleus. These results lead to the conclusion that STATs need to form homo- or heterodimers in the cytoplasm to cross the nuclear membrane.

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#### REFERENCES

1. Driggers, P. H., Ennist, D. L., Gleason, S. L., Wai-Han, M., Marks, M. S., Levi, B.-Z., Flanagan, J. R., Appella, E., and Ozato, K. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 3743–3747
2. Friedman, R. L., and Stark, G. R. (1985) *Nature* **314**, 637–639

3. Larner, A. C., Jonak, G., Cheng, Y.-S. E., Korant, B., Knight, E., and Darnell, J. E. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 6733–6737
4. Luster, A. D., Unkeless, J. C., and Ravetch, J. V. (1985) *Nature* **315**, 672–676
5. Revel, M., and J., C. (1986) *Trends Biochem. Sci.* **11**, 166–170
6. Yan, C., Sehgal, P. B., and Tamm, I. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 2243–2247
7. Hallek, M., Lepisto, E. M., Slattey, K. E., Griffin, J. D., and Ernst, T. J. (1992) *Blood* **80**, 1736–1742
8. Staeheli, P., Danielson, P., Haller, O., and Sutcliffe, J. G. (1986) *Mol. Cell. Biol.* **6**, 4770–4774
9. Khan, K. D., Shuai, K., Lindwall, G., Maher, S. E., Darnell, J. E., Jr., and Bothwell, A. L. M. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 6806–6810
10. Finbloom, D. S., and Larner, A. C. (1995) *Arthritis Rheum.* **38**, 877–889
11. Fu, X.-Y. (1992) *Cell* **70**, 323–335
12. Fu, X.-Y., Schindler, C., Improta, T., Aebersold, R., and Darnell, J. E., Jr. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 7840–7843
13. Levy, D. E., Kessler, D. S., Pine, R., and Darnell, J. E., Jr. (1989) *Genes Dev.* **3**, 1362–1371
14. Schindler, C., Fu, X.-Y., Improta, T., Aebersold, R., and Darnell, J. E., Jr. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 7836–7839
15. Larner, A. C., David, M., Feldman, G. M., Igarashi, K., Hackett, R. H., Webb, D. A. S., Sweitzer, S. M., Petricoin, E. F., III, and Finbloom, D. S. (1993) *Science* **261**, 1730–1733
16. Larner, A. C., and Finbloom, D. S. (1995) *Biochim. Biophys. Acta* **1266**, 278–287
17. David, M., Romero, G., Zhang, Z. Y., Dixon, J. E., and Larner, A. C. (1993) *J. Biol. Chem.* **268**, 6593–6599
18. Muller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A. G., Barbieri, G., Witthuhn, B. A., Schindler, C., Pellegrini, S., Wilks, A. F., Ihle, J. N., Stark, G. R., and Kerr, I. M. (1993) *Nature* **366**, 129–135
19. Watling, D., Guschin, D., Muller, M., Silvennoinen, O., Witthuhn, B. A., Quelle, F. W., Rogers, N. C., Schindler, C., Ihle, J. N., Stark, G. R., and Kerr, I. M. (1993) *Nature* **366**, 166–170
20. Silvennoinen, O., Ihle, J. N., Schlessinger, J., and Levy, D. E. (1993) *Nature* **366**, 583–585
21. Johnston, J. A., Kawamura, M., Kirken, R. A., Chen, Y.-Q., Blake, T. B., Shibuya, K., Ortaldo, J. R., McVicar, D. W., and O'Shea, J. J. (1994) *Nature* **370**, 151–153
22. Ihle, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., Thierfelder, W. E., Kreider, B., and Silvennoinen, O. (1994) *Trends Biochem.* **19**, 222–227
23. Darnell, J. E., Kerr, I. M., and Stark, G. R. (1994) *Science* **264**, 1415–1421
24. David, M., Wong, L., Flavell, R., Thompson, S., Larner, A. C., and Johnson, G. (1996) *J. Biol. Chem.* **271**, 9185–9188
25. Velazquez, L., Fellous, M., Stark, G. R., and Pellegrini, S. (1992) *Cell* **70**, 313–322
26. Leung, S., Qureshi, S., Kerr, I., Darnell, J., and Stark, G. (1995) *Mol. Cell. Biol.* **15**, 1312–1317
27. Qureshi, S. A., Leung, S., Kerr, I. M., Stark, G. R., and Darnell, J. E., Jr. (1996) *Mol. Cell. Biol.* **16**, 288–293
28. Li, X., Leung, S., Kerr, I., and Stark, G. (1997) *Mol. Cell. Biol.* **17**, 2048–2056
29. Gupta, S., Yan, H., Wong, L. H., Ralph, S., Krolewski, J., and Schindler, C. (1996) *EMBO J.* **15**, 1075–1084
30. Shuai, K., Horvath, C. M., Huang, L. H., Qureshi, S. A., Cowburn, D., and Darnell, J. E., Jr. (1994) *Cell* **76**, 821–828
31. Sekimoto, T., Nakajima, K., Tachibana, T., Hirano, T., and Yoneda, Y. (1996) *J. Biol. Chem.* **271**, 31017–31020
32. Shuai, K., Stark, G. R., Kerr, I. M., and Darnell, J. E., Jr. (1993) *Science* **261**, 1744–1746
33. Shuai, K., Schindler, C., Prezioso, V., R., and Darnell, J. E., Jr. (1992) *Science* **258**, 1808–1812
34. Improta, T., Schindler, C., Horvath, C. M., Kerr, I. M., Stark, G. R., and Darnell, J. E., Jr. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4776–4780
35. Greenlund, A. C., Morales, M. O., Viviano, B. L., Yan, H., Krolewski, J., and Schreiber, R. D. (1995) *Immunity* **2**, 677–687
36. Heim, M. H., Kerr, I. M., Stark, G. R., and Darnell, J. E., Jr. (1995) *Science* **267**, 1347–1349
37. Shuai, K., Ziemiecki, A., Wilks, A. F., Harpur, A. G., Sadowski, H. B., Gilman, M. Z., and Darnell, J. E., Jr. (1993) *Nature* **366**, 580–583
38. Muller, M., Laxton, C., Briscoe, J., Schindler, C., Improta, T., Darnell, J. E., Jr., Stark, G. R., and Kerr, I. M. (1993) *EMBO J.* **12**, 4221–4228
39. Wilson, K. C., and Finbloom, D. S. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 11964–11968
40. Bach, E. A., Tanner, W., Masters, S., Ashkenazi, A., Aguet, M., Shaw, A. S., and Schreiber, R. D. (1996) *Mol. Cell. Biol.* **16**, 3214–3221
41. Greenlund, A. C., Farrer, M. A., Viviano, B. L., and Schreiber, R. D. (1994) *EMBO J.* **13**, 1591–1600
42. Yan, R., Krishnan, K., Greenlund, A. C., Gupta, S., Lim, J. T. E., Schreiber, R. D., Schindler, C. W., and Krolewski, J. J. (1996) *EMBO J.* **15**, 1064–1074
43. Igarashi, K., David, M., Larner, A. C., and Finbloom, D. S. (1993) *Mol. Cell. Biol.* **13**, 3984–3989
44. Haque, S., Wu, Q., Kammer, W., Friedrich, K., Smith, J., Kerr, I., Stark, G., and Williams, B. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 8563–8568
45. Ali, S., and Ali, S. (1998) *J. Biol. Chem.* **273**, 7709–7716
46. Wen, Z., Zhong, Z., and Darnell, J. E., Jr. (1995) *Cell* **82**, 241–250