STAT1 Is Inactivated by a Caspase*

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Apoptosis involves the activation of a cascade of interleukin-1β converting enzyme-like proteases (caspases), a group of cysteine proteases related to the prototype interleukin-1β-converting enzyme (caspase-1). These proteases cleave specific intracellular targets such as poly(ADP-ribose) polymerase, DNA-dependent protein kinase, and nuclear lamins. We show here that apoptosis can be induced by double-stranded RNA. The induction of apoptosis by double-stranded RNA and other agents leads to the cleavage by a caspase of the signal transducer and activator of transcription factor, STAT1 which is pivotal in the signal transduction pathways of the interferons and many other cytokines and growth factors. The product of this cleavage is no longer able to mediate interferon-activated signal transduction and the cleavage event may play a role in regulating the apoptosis response itself.

Apoptosis is the process of programmed cell death, and is a common occurrence in embryogenesis and in the destruction of infected, damaged, or senescent cells (1, 2). The biochemical processes involved in apoptosis can be divided into two discrete phases. A variable period of time elapses following exposure to apoptosis-inducing signals before cells become committed to undergo apoptosis. This phase is followed by the execution phase (more correctly a suicide phase, since apoptosis is cell autonomous) which, unlike the commitment phase, is irreversible. The execution phase is the best understood, and is characterized by changes in the cytoplasmic membrane, breakdown of the nuclear envelope, condensation of chromatin structure, and eventual destruction of the chromatin (laddering). Execution is associated with the activation of a family of cysteine-proteases (caspases) which are related to the enzyme required to process interleukin-1β (IL-1β)1 from its precursor form (IL-1β-converting enzyme, caspase-1). It is not clear whether any single caspase can cause cell death, since these enzymes appear to be arranged in a complex network. Furthermore, to date, although several substrates have been identified for caspases, no single substrate cleavage has been shown to be essential for apoptosis, and the consensus of opinion is that cell death occurs by a thousand cuts" (3).

In addition to activation through occupation of surface receptors such as Fas or tumor necrosis factor, apoptosis can also be induced by intracellular signals such as DNA damage, inhibition of protein synthesis, and viral infection. We are interested in the specific means by which viral infection can cause cells to undergo apoptosis (4). Apoptosis of virally infected cells is important since it allows macrophages to destroy a cell before the completion of the viral life cycle and the release of infectious particles (5). The significance of this is reflected by the fact that many viruses encode specific inhibitors of caspases (6–11). One of the best viral inducers of apoptosis is influenza (12–14). It has been proposed that double-stranded RNA (dsRNA) is generated during influenza viral replication, and that this leads to the transcriptional activation of Fas antigen that is a key factor in inducing apoptosis (15, 16).

In this paper we show that dsRNA is capable of inducing apoptosis without the requirement for de novo protein synthesis, indicating that susceptible cells contain a pre-existing pathway for the execution of apoptosis if exposed to dsRNA. This is highly reminiscent of the transcriptional induction of β-interferon (β-IFN) in response to viral infection or dsRNA treatment (17). While investigating the effects of this dsRNA-induced apoptosis on the induction of β-IFN we discovered that the signal transducer and activator of transcription molecule, STAT1, becomes cleaved after aspartic acid 694 by a caspase. STAT1 is a transcription factor that becomes phosphorylated on tyrosine 701 by members of the JAK kinase family in response to IFNs and many other cytokines and growth factors (18–20); this phosphorylation is required for dimerization and subsequent DNA binding and is thus essential for effective signal transduction. The removal of the C terminus of STAT1 by proteolysis consequently renders STAT1 unable to participate in signal transduction. This cleavage is also caused by inducers of apoptosis other than dsRNA, and may play a role in the down-regulation of cellular responses during cell death.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—HeLa (subclone E 211) and U3A (22) cells were grown in Dulbecco’s modification of Eagle’s medium supplemented with Glutamax (Life Technologies) + 10% fetal bovine serum (Life Technologies). For dsRNA treatments, HeLa cells were grown until they were 80–90% confluent, primed by treatment with 500 units/ml human α-IFN (Wellferon, a mixture of naturally produced α-IFN subtypes; Glaxo-Welcome) for 18 h, and then induced using 100 μg/ml dsRNA (poly(I)-poly(C); Pharmacia) for the lengths of times shown in the figures. Where indicated, cycloheximide was added to the medium at 50 μg/ml, at this concentration, protein synthesis is inhibited to >99.9% as determined by incorporation of labeled amino acids into total protein (23). The caspase inhibitor ZVAD (benzyloxy carbonyl-Val-Ala-Asp-fluoromethylketone; Enzyme System Products) was dissolved in Me2SO and added to cells at 100 μM at the same time as dsRNA. TPCK (Sigma) was dissolved in methanol and added to cells at 100 μM at the same time as dsRNA. Etoposide was dissolved in Me2SO and added to cells for 18 h at 50 μM. IFN treatment of U3A cells used 500 units of human α-IFN/ml or 500 units of human γ-IFN/ml for 3 h where indicated.

**Western Blotting Analysis**—Cells were lysed in sample buffer (24)

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1The abbreviations used are: IL, interleukin; STAT, signal transducer and activator of transcription; PARF, poly(ADP-ribose) polymerase; IFN, interferon; TPCK, N'-p-tosyl-L-phenylalanine chloromethyl ketone; dsRNA, double-stranded RNA; PKR, protein kinase R; ZVAD, benzoylcarbonyl-Val-Ala-Asp-fluoromethylketone; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]1-propanesulfonic acid. 

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Clonals, respectively. For re-probing, filters were stripped as described above and enhanced chemiluminescence (Amersham) using horseradish peroxidase conjugated to secondary antibody (MAbSV5-P-k) (25) recognizing amino acids 95–108 of the P and V proteins of simian virus 5 was obtained from Dr. R. Randall (University of St. Andrews). All protein-antibody interactions were detected with enhanced chemiluminescence (Amersham) using horseradish peroxidase conjugated sheep anti-mouse (Amersham) and donkey anti-rabbit (Amersham) IgG as secondary antibodies for monoclonal and polyclonal antibodies, respectively. For re-probing, filters were stripped as described by the manufacturers.

Plasmid Construction—A DNA sequence for human STAT1a was obtained by polymerase chain reaction and verified by partial sequencing and functional complementation in U3A cells (see Fig. 4). This cDNA was inserted between the NcoI and XhoI sites of the EF1α promoter vector pEF-S2 (a kind gift of Dr. R. H. Treisman, Imperial Cancer Research Fund) to generate pEFSTAT1a. The STAT1α cDNA was then epitope-tagged at the N terminus using oligonucleotides encoding the amino acids 95–108 (GKIPNPPLGLDST) of the P and V proteins of simian virus 5 to generate pEFSTAT1αSV5. Single amino acid changes to alanine or glutamic acid at aspartic acid 694 or to a stop codon at glycine 695 were introduced into pEFSTAT1αSV5 using recombinant polymerase chain reaction to generate pEFSTAT1αSV5(D694A), pEFSTAT1αSV5(D694E), and pEFSTAT1αSV5(G695STOP), respectively. The amino acid changes were confirmed by sequencing. The wild type STAT1α sequence and STAT1α genes containing the D694A and G695STOP changes were transferred from these plasmids into the vector pT7βlink2 (26) as NcoI to XhoI fragments to generate pT7βSTAT1α, pT7βSTAT1α(D694A), and pT7βSTAT1α(G695STOP), respectively. The reporter plasmid for α-IFN contained 4 copies of the 9–27 ISRE core (27) (AGGAATAAGAAACTG) arranged in tandem upstream of the BamHI site of ptk3(−39)luc (21), while the reporter plasmid for γ-IFN contained 2 copies of the IRF-1 GAS site (28) (TTTCCCCGAAA) arranged in tandem upstream of the BamHI site of ptk3(−39)luc. (21)

Transfections—HeLa cells were transfected with 2 μg of DNA and 8 μl of LipofectAMINE (Life Technologies Inc.) according to the manufacturers instructions. U3A cells were transfected using calcium phosphate precipitation with 10 μg of effector, 10 μg of reporter, and 2 μg of transfection control plasmid (pSV2CAT (29)). For reporter gene assays, lysates were prepared and assayed for luciferase and chloramphenicol acetyltransferase activity as described previously (21). Luciferase activity was corrected to the chloramphenicol acetyltransferase activity to normalize for variations in the transfection efficiency. Transfection experiments were repeated at least three times.

Apoptosis Assay—Laddered cytoplasmic DNA was prepared from Triton X-100 lysates and labeled with α-[32P]dATP using the terminal transferase activity of T4 DNA polymerase (30). Reaction products were fractionated by electrophoresis on 1.8% agarose gels in 0.8% M trisborate, 0.002 M EDTA, and after drying the gel, visualized by autoradiography.

In Vitro Cleavage Assay—Bacterial lysates were prepared from Escherichia coli B21L3De3pLysS carrying a pet plasmid encoding recombinant caspase-3 (kindly provided by Dr. N. McCarthy, ICRF) and the control plasmid pet15b, as described previously (31). One microgram of lysate was incubated with 1 μl of 35S-labeled STAT1α or mutant forms of STAT1α (prepared by in vitro translation of linearized plT7STAT1α, plT7STAT1α(D694A), or plT7STAT1α(G695STOP) in a standard TnT T7 quick coupled transcription/translation kit reaction; Promega Corp.) in a 10-μl reaction containing 25 mM Hepes, pH 7.5, 5 mM dithiothreitol, 5 mM EDTA, 0.1% CHAPS at 37 °C for 1 h. The reactions were stopped by the addition of sample buffer (24) then analyzed by fractionation on a 7.5% polyacrylamide/SDS gels followed by autoradiography.

RESULTS

We have previously shown that β-IFN expression can be induced in HeLa cells in response to treatment with dsRNA (21, 23). Induction occurs at the level of transcriptional initiation and does not require new protein synthesis, although the extent of induction can be much enhanced by a pretreatment with type I IFN (priming). We noted that during the induction process, cells exhibited an altered morphology consistent with the activation of apoptosis. To determine whether apoptosis could be induced by dsRNA, we treated HeLa cells with poly(I)-poly(C), which was added directly to the growth medium, and prepared DNA from induced cells. Fig. 1 shows that a nucleosomal ladder characteristic of cells undergoing apoptosis is produced in response to dsRNA. When cycloheximide was included in the induction medium the degree of laddering was significantly enhanced, although consistent with results in other systems cycloheximide alone could cause some laddering (32–36). Although it is not possible to determine from these results whether dsRNA and cycloheximide induce apoptosis through a common mechanism, the fact that the extent of apoptosis seen with the combination of dsRNA and cycloheximide is greater than that seen with either inducer alone demonstrates that cycloheximide cannot be blocking the dsRNA effect. Thus de novo protein synthesis cannot be required for the induction of apoptosis by dsRNA, a result that is consistent with the recent observations of others (37). Inclusion of the caspase inhibitor ZVAD (38) in the induction mixture prevented DNA laddering (Fig. 1) and completely blocked the observed cell death (data not shown).

HeLa cells contain two splice variant forms of STAT1, referred to as STAT1α (91 kDa) and STAT1β (84 kDa), respectively (Fig. 2A). While investigating the role of STAT1 in the induction of β-IFN expression we discovered that exposure of HeLa cells to dsRNA for 2 h generated a novel form of STAT1α (STAT1α γ) migrating with slightly faster mobility than the 84-kDa form (STAT1β; Fig. 2A). Since the production of STAT1γ occurs with similar kinetics to the activation of apoptosis, we investigated whether STAT1γ generation was associated with the activation of caspases. To determine whether the production of STAT1γ required de novo protein synthesis, we repeated the induction of HeLa cells with dsRNA in the presence of cycloheximide. Although cycloheximide alone could also gener-
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Fig. 2. A post-translationally modified form of STAT1 (STAT1* is generated in response to apoptosis. A, HeLa cells were treated with dsRNA for the times indicated and cell extracts analyzed for STAT1 using Western blotting. In addition to STAT1α and STAT1β, a novel form of STAT1 (STAT1*) is detected in dsRNA-treated cells. By probing similar blots with additional STAT1-specific antisera, STAT1* can be shown to lack the C terminus of STAT1α and STAT1β (data not shown). B, HeLa cells were treated with dsRNA or cycloheximide (CHX) alone or in combination for 3 h and cell extracts analyzed for STAT1 using Western blotting. C, HeLa cells were treated with dsRNA and cycloheximide in combination for the indicated times and cell extracts analyzed for STAT1, STAT2, STAT3, or PARP using Western blotting. D, HeLa cells were treated with dsRNA and cycloheximide in combination for 3 h in the presence of ZVAD or TPCK (left panel), or were treated with etoposide (right panel) and cell extracts analyzed for STAT1 using Western blotting.

ate STAT1* to some degree, the combined treatment of HeLa cells with dsRNA and cycloheximide led to enhanced production of STAT1* (Fig. 2B) and was accompanied by the loss of both STAT1α and -β. These results suggested that STAT1* is derived from STAT1α and -β by post-translational modification. To determine whether other members of the STAT family are similarly affected we examined the state of STAT2 and STAT3 (Fig. 2C) and STAT6 (data not shown) in extracts from dsRNA- and cycloheximide-treated HeLa cells without seeing equivalent changes in mobility. By contrast, the well defined caspase substrate, PARP, is cleaved under the same conditions (Fig. 2C).

Fig. 2D shows that STAT1* generation in cells treated with dsRNA and cycloheximide could be completely blocked by treating cells with the caspase inhibitor ZVAD, whereas the serine protease inhibitor, TPCK, was unable to affect cleavage. By contrast, TPCK completely inhibited dsRNA-dependent activation of NF-κB under the same conditions (39), while ZVAD had no effect (data not shown). The cleavage of PARP was similarly blocked by ZVAD but not by TPCK (data not shown). To test whether STAT1* could be generated by other inducers of apoptosis, we treated HeLa cells with the DNA topoisomerase II inhibitor etoposide (40). This treatment also resulted in the production of STAT1*, although we note that this treatment reproducibly leads to preferential cleavage of STAT1β over STAT1α (Fig. 2D). Taken together, these results indicate that the dsRNA/cycloheximide induction mixture triggers apoptosis in HeLa cells and that during this process STAT1α and -β are converted to a shorter form, presumably by proteolytic cleavage.

Because STAT1α and -β differ at their C termini, but are converted to a single product, it seemed likely that STAT1* is generated by a single cleavage event occurring near the C terminus of the protein. By using a panel of antibodies we were able to show that STAT1* lacks the C-terminal end of STAT1α and -β (data not shown). Since a caspase-mediated cleavage was strongly implicated by the above data, we inspected the C-terminal end of the human STAT1 protein sequence for an appropriate motif. We noticed a potential cleavage site for caspases at the aspartic acid residue at position 694 (Fig. 3A). Cleavage at this site in STAT1 would produce a species with a predicted molecular mass of 81 kDa, in line with the observed size of STAT1*. To verify that this sequence was indeed a target for cleavage, we created an epitope-tagged wild type STAT1 expression vector, and ones in which the aspartic acid residue at position 694 was changed to alanine (D694A) or glutamic acid (D694E). When the epitope-tagged wild type form of STAT1α was expressed in HeLa cells, a protein with mobility similar to that of the endogenous form of STAT1α (91 kDa) could be observed (Fig. 3B). A small amount of a protein with mobility similar to that of the endogenous form of STAT1α (81 kDa, Fig. 3B) could also be observed in untreated cells; this product is generated as a result of a low level of apoptosis induced by the transfection conditions, and can be blocked with ZVAD (data not shown). When cells were treated with dsRNA and cycloheximide the conversion of the 91- to the 81-kDa truncation form was much enhanced (Fig. 3B). The 81-kDa form migrated with the same mobility as a protein produced by expression of a form of STAT1α truncated after amino acid 694 (G695/STOP) (Fig. 3B), as expected if STAT1* is generated by cleavage at this site. In contrast to the wild type form, the D694A and D694E mutant forms of STAT1α were not cleaved upon induction (Fig. 3B). Reprobing the Western blot with the STAT1 antibody showed that the endogenous STAT1α and -β were cleaved in each case (Fig. 3B). These results confirmed the presence of a cleavage site at aspartic acid 694 in STAT1, and demonstrated that this site is cleaved in both STAT1α and -β during apoptosis. To confirm that STAT1 can be cleaved by a caspase, we tested whether recombinant STAT1 can be cleaved by recombinant caspase-3 since the cleavage site in STAT1 is similar to that seen in several caspase-3 substrates (Fig. 3A). Fig. 4 demon-
strates that the 91-kDa STAT1 protein is cleaved by caspase-3 to give an 81-kDa product that has the same mobility as wild-type STAT1 (91 kDa) and the 81-kDa cleavage product are indicated to the left of the panel.

maximal activity, serine 727 (53). Once phosphorylated, STAT1 can homo- or heterodimerize to produce complexes capable of binding to DNA and activating transcription (19). Clearly, cleavage of STAT1 after amino acid 694 would produce a truncated form of the protein that cannot be phosphorylated on tyrosine 701, and thus should not be activated by α- or γ-IFN. To test this, we examined the ability of STAT1* to rescue responses to α- and γ-IFN in U3A cells which lack functional STAT1 (54). Responses to α-IFN were assessed using a reporter promoter containing a 15-mer “core” sequence that is a strong binding site for the type I-IFN-activated transcription factor ISGF3 (IFN stimulatable response element (55, 56)), while responses to γ-IFN were assessed using a reporter promoter containing a GAS site from the IRF-1 promoter that is a strong binding site for the γ-IFN-activated factor (GAF). In contrast to wild-type STAT1, the truncated form (G695/STOP) was completely unable to rescue either α-IFN (Fig. 5A) or γ-IFN (Fig. 5B) responses. Altering amino acid 694 from an aspartic acid to an alanine (D694A) did not interfere with either signal transduction pathway (Fig. 5, A and B). These results demonstrate that the effect of the proteolytic cleavage of STAT1 during apoptosis would be to impair the ability of cells undergoing apoptosis to respond to a variety of cytokine- and growth factor-mediated signals.

**DISCUSSION**

The data we have presented indicate that dsRNA-sensitive switch which triggers a pre-established program of both apoptosis and β-IFN production. In neither case is concurrent protein synthesis required, and as a result there must be a means by which dsRNA can activate the caspases. Studies on the Fas and tumor necrosis factor receptors indicate that upon activation the receptors recruit adaptor molecules through cytoplasmic “death domains” (57–60); these adaptors in turn recruit additional molecules which contain caspase motifs (61–63). Once recruited these molecules activate the downstream caspase cascade. One possible candidate for the mediation of the dsRNA signal is the cellular enzyme protein kinase R (PKR). This kinase is known to function to downregulate protein synthesis in virally infected cells by phosphorylating the translational initiation factor eukaryotic initiation factor-2α (2α), but has also been shown to play a role in the activation of NF-κB by dsRNA (65–68). It is tempting to speculate that PKR may also signal to the apoptotic execution machinery. Consistent with this it has been shown that overexpression of PKR can cause apoptosis in cell culture (69), while mouse embryonic fibroblasts lacking PKR are unable to undergo apoptosis in response to dsRNA (37). Interestingly,
PKR deficiency also impairs responses to other inducers of apoptosis (37, 70). The identification of a link between PKR and the caspases represents a significant future goal.

In these studies we have also demonstrated that the transcription factor STAT1, which is required for the ability of cells to respond to a wide variety of signals, is cleaved during apoptosis by a caspase. The protease inhibitor ZVAD used in our studies is a general caspase inhibitor (38), and we have yet to identify which specific member(s) of the caspase family is involved in generating STAT1* in vivo, although we have demonstrated that caspase-3 can cleave STAT1 efficiently in vitro. Although the cleavage site in STAT1 has a number of similarities to the sites known to be substrates for caspase-3 (Fig. 3A), we note that it also has some similarity with substrates for caspase-1, especially the minor caspase-1 cleavage product of pro-IL-1β (49, 50). While we have yet to test it directly, we speculate that STAT1 may also be a caspase-1 substrate, since caspase-1 appears to be rather promiscuous in its preferences (71–73). It is also interesting to note that STAT1 is not cleaved at amino acid 143 despite containing an ELDS motif in this region, and that STAT3 and STAT6 are also refractory to cleavage despite containing possible caspase cleavage sites (Fig. 3A). We speculate that cleavage by caspases could be prevented by the tertiary structure of some proteins.

The C-terminal cleavage product of STAT1 has lost the tyrosine residue at position 701 and thus cannot be activated by α- and γ-IFN as shown here, or presumably by the other ligands that activate by promoting this phosphorylation. It is pertinent to ask what role STAT1 inactivation could play in the apoptotic response. It has previously been observed that many cell types, including fibroblast (74) and hematopoietic cells (75) can undergo apoptosis as a result of becoming depleted of growth factors or cytokines and can be rescued by the addition of defined survival factors, which include STAT1 activators such as platelet-derived growth factor (74), colony-stimulating factor (76), and γ-IFN (77). It is interesting to speculate that one consequence of apoptosis is to down-regulate the ability of a cell to respond to such factors. Since multiple growth factors can protect cells against apoptosis, we think it unlikely that STAT1 cleavage alone would be sufficient to render cells insensitive to protection by survival factors. Nevertheless, inactivation of STAT1 function may be an example of a class of modifications that ensures that the commitment phase of apoptosis is irreversible. An alternative role for STAT1 cleavage is suggested by the recent demonstration that STAT1 can play a role in apoptosis by up-regulating the level of caspase-1 in response to cytokines (78). Consistent with this we have determined that the degree of apoptotic death we observe in response to dsRNA and cycloheximide is much enhanced if cells are primed by a prior exposure to either type I or type II IFN (data not shown). When cells are challenged with an apoptosis-inducing signal, the inactivation of STAT1 would lower the levels of caspase, and thus might play a role in limiting the propensity of a cell to undergo apoptosis. The resolution of this issue awaits further study.

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**REFERENCES**

1. Fraser, A., and Evan, G. (1996) Cell 85, 781–784
2. Takahashi, A., and Earnshaw, W. C. (1996) Curr. Opin. Genet. Dev. 6, 50–55
3. Martin, S. J., and Green, D. R. (1995) Cell 82, 349–352
4. Hardwick, J. M. (1997) Adv. Pharmacol. 41, 295–336
5. Savill, J., Fadok, V., Henson, P., and Haslett, C. (1993) Immunol. Today 14, 121–136
6. Ray, C. A., Black, R. A., Krunen, S. R., Greenstreet, T. A., Sleath, P. R., Salvesan, G. S., and Pickup, D. J. (1992) Cell 69, 597–604
7. Clem, R. J., and Miller, L. K. (1994) Mol. Cell. Biol. 14, 5212–5222
8. Tewari, M., and Dixit, V. M. (1995) J. Biol. Chem. 270, 3255–3260
9. Bump, N. J., Hackett, M., Hugunin, M., Seshagiri, S., Brady, K., Chen, P., Ferenz, C., Franklin, S., Ghayur, T., and Li, P. (1995) Science 269, 1896–1888
10. Teodoro, J. G., and Branton, P. E. (1997) J. Virol. 71, 1739–1746
11. Uren, A. G., and Vaux, D. L. (1997) Vitam. Horm. 53, 175–193
12. Hinshaw, V. S., Olsen, C. W., Dybdahl-Sissoko, N., and Evans, D. (1994) J. Virol. 68, 3667–3673
13. Mori, I., Komatsu, T., Takeuchi, K., Nakakuki, K., Sudo, M., and Kimura, Y. (1995) J. Gen. Virol. 76, 2769–2773
14. Takizawa, T., Matsuakawa, S., Higuchi, Y., Nakamura, S., Nakanishi, Y., and Fukuda, R. (1993) J. Gen. Virol. 74, 2347–2355
15. Takizawa, T., Fukuda, R., Miyawaki, T., Ohashi, K., and Nakanishi, Y. (1995) Virology 209, 288–296
16. Takizawa, T., Ohashi, K., and Nakanishi, Y. (1996) J. Virol. 70, 8128–8132
17. Goodbourn, S., and King, P. (1996) in Eukaryotic Gene Transcription, (Hames, D., ed) pp. 132–146, Oxford University Press, Oxford
18. Briand, B. B., Fusich, D., and Muller, L. (1994) Curr. Biol. 4, 1033–1035
19. Schindler, C., and Darnell, J. E., Jr. (1995) Annu. Rev. Biochem. 64, 621–651
20. Ihle, J. N. (1996) Cell 84, 331–334
21. King, P., and Goodbourn, S. (1994) J. Biol. Chem. 269, 30609–30615
22. McKendry, R., John, J., Flavell, D., Muller, M., Kerr, I. M., and Stark, G. R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11455–11459
23. Whiteside, S. T., Visvanathan, K. V., and Goodbourn, S. (1992) Nucleic Acids Res. 20, 1514–1518
24. Laemmli, U. K. (1970) Nature 227, 680–685
25. Hanke, T., Szawadowski, P., and Randall, R. E. (1992) J. Gen. Virol. 73, 653–660
26. Dalton, S., and Treisman, R. (1992) Cell 68, 597–612
