ERK and the F-box Protein βTRCP Target STAT1 for Degradation*

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The transcription factor STAT1 has roles in development, and phosphorylation of both residues appears to be required for homeostasis. STAT1 activity is carefully regulated to prevent susceptibility of STAT1 to degradation via distinct F-box-associated E3 ligases (11). Overexpression of STAT1 can promote tumorigenesis. Indeed, STAT1/p53-deficient mice have an increased incidence of spontaneous tumors compared with single p53-deficient mice (10), strongly suggesting that STAT1 and p53 cooperate to promote tumorigenesis.

This article has been withdrawn by Paul A. Townsend, Richard A. Knight, Sean P. Barry, David S. Latchman, and Anastasis Stephanou. An investigation at University College London determined that a duplicated blot in Fig. 1, A and B, the GAPDH blot is the same, flipped horizontally. The withdrawing authors sincerely apologize to the scientific community for any confusion or adverse consequences resulting from the publication of the article.

The signal transducers and activators of transcription (STATs) are a family of latent cytoplasmic transcription factors. After ligation of cytokine receptors, STATs become phosphorylated by receptor kinases and dimerize and translocate to the nucleus where they modulate expression of STAT-responsive genes (1, 2). STAT1 is the classical mediator of the effects of interferon-γ (IFN-γ), and binding of IFN-γ to its receptor results in Janus kinase-mediated phosphorylation of a specific tyrosine (Tyr701) residue found in the C-terminal transcriptional domain of STAT1 (2, 3). STAT1 is also phosphorylated, at least in part by the p38 MAPK (4) and ERK pathway (5), on a serine (Ser727) residue, again located in the transcriptional domain.

The ubiquitin-proteasome pathway plays a central role in regulating many cellular processes such as cell cycle progression by targeting phosphorylated regulatory proteins for degradation via distinct F-box-associated E3 ligases (11). Overactivity in the degradation of tumor suppressor proteins is an underlying mechanism that promotes tumorigenesis (11). Several reports have identified E3 ligases that specifically target STAT1 for degradation. This has been particularly well investigated in the context of viral infection. For example, the simian viral protein SV5 enables the virus to circumvent the interferon-mediated host cell anti-viral response by degrading STAT1 (12, 13). The SV5 protein interacts with the damaged DNA-binding protein-1 (DBD1) and Cul4A to form an active E3 ligase. More recently, the STAT-interacting LIM protein has also been shown to form an E3 ligase that targets STAT1 and STAT4 proteolysis via the ubiquitin-proteasome pathway (14). However, E3 ligases that specifically target phosphorylated forms of STAT1 for proteolysis in mammalian cells have not yet been described.

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2. The abbreviations used are: STAT, signal transducers and activators of transcription; IFN, interferon-γ; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; E3, ubiquitin-protein isopeptide ligase; MEF, mouse embryonic fibroblast; HA, hemagglutinin; GST, glutathione S-transferase; siRNA, short interfering RNA; pERK, phospho-ERK.
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been characterized. In this study, we provide evidence that the F-box E3 ligase βTRCP interacts with and promotes STAT1 proteasomal degradation in an ERK-dependent manner.

**EXPERIMENTAL PROCEDURES**

**Plasmids, Antibodies, siRNA, and Chemicals—**STAT1-pGEX5X-2 was a gift from Dr. I. Behrmann (Institut für Biochemie, Universitätshochschule Düsseldorf, Germany). Mammalian expression plasmids for HA-tagged F-box proteins βTRCP (Fbw1a), Fbw2, Fbw4, and Fbw7 (15) were gifts from Dr. D. Nakayama (Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Higashi-ku, Japan). STAT1α/MYC-pCDNA6A, STAT1β/pRc/CMV, STAT1Y701F/pRc/CMV, STAT1S727A/pRc/CMV, and mouse STAT3/pCDNA3 expression plasmids were a generous gift from Dr. J. Darnell (Rockefeller University, New York). The HA-tagged ubiquitin expression vector was provided by Dr. P. Salomoni (MRC Toxicology Unit, University of Leicester, UK). Mammalian expression plasmids for constitutively active mutated MEK-1 (pMEK-1-HA) and wild-type mouse ERK2 (pWT.ERK2-HA) were PCR-cloned using the template plasmid pCAMEK/ERK2–14 (a bicistronic plasmid encoding constitutively active Xenopus MEK-1 and mouse ERK2). Coding sequences were fused to the HA tag by cloning into pCDNA3.1 +/HA. Anti-βTRCP antibody was purchased from Invitrogen, and anti-20 S proteasome was purchased from Invitrogen, and anti-20 S proteasome (kindly provided by Uwe Vinkemeier) proteins incubated at 25 °C for 20 min. Reactions were terminated by addition of SDS sample buffer, and the 32P-incorporated proteins were fractionated by SDS-PAGE and visualized by autoradiography. Membranes were subsequently blotted with the anti-STAT1 (E-23) antibody to reveal STAT1 quantities.

**Confocal Microscopy—**STAT1−/− MEF cells were seeded on glass coverslips at a density of 10^3 cells per 13-mm coverslip and transfected the following day using FuGENE 6 and incubated for another 24 h. Cells were fixed in paraformaldehyde, permeabilized in paraformaldehyde, 0.1% v/v Triton X-100, and stained using antigen-specific antibodies as outlined (8). Alexa Fluor 488 donkey anti-rabbit IgG, Texas Red goat anti-rat conjugates were used as secondary antibodies (10 μg/ml) and were purchased from Invitrogen. Coverslips were mounted in mounting mix containing 4',6-diamidino-2-phenylindole (Vecta Laboratories, UK), visualized with an LCS SP2 laser scanning confocal microscope, with objectives, and analyzed using LCS Lite (Leica) and Adobe Photoshop 6.0 software.

**ERK Phosphorylates and Modulates STAT1 Levels—**During ongoing studies on STAT1 activation, we had observed that levels of unphosphorylated or phosphorylated STAT1 are reduced over time following various stressful stimuli, suggesting that STAT1 is modulated during cellular stimulation. To understand the mechanism for this rapid removal of activated STAT1, we examined the effects of H2O2-mediated oxidative stress in mouse embryonic fibroblasts (MEFs). H2O2 resulted in phosphorylation of STAT1 Tyr-701 and STAT1 Ser-727, and this was accompanied by a reduction in the levels of both total STAT1 and phospho-STAT1 (Fig. 1A). Similar observations were also observed following exposure to the DNA-damaging agent cisplatin (data not shown). Treatment with the proteasomal inhibitor, lactacystin (Fig. 1A), reduced STAT1, phospho-STAT1 Tyr-701, and STAT1 Ser-727 degradation (Fig. 1A). These results imply that STAT1 and phosphorylated STAT1 levels are regulated by the proteasomal pathway. We also confirmed that STAT1 becomes ubiquitylated, and this is enhanced following H2O2 treatment in MEF cells as assessed by co-immunoprecipitation assays (Fig. 1B). We also show that ubiquitylated STAT1 co-localizes with the 20 S proteasome following H2O2 (Fig. 1C). These finding demonstrate that STAT1 levels are modulated by the ubiquitin pathway.

**ERK Phosphorylates and Modulates STAT1 Levels—**Phosphorylation of regulatory proteins can act as a trigger for their degradation by the ubiquitin–proteasomal pathway (11). To determine whether proteasomal degradation of STAT1 also required its prior phosphorylation, and which kinase was

### In Vitro Kinase Assays

Kinase reactions were carried out in 40 μl of kinase buffer (25 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM CaCl₂, 1 mM dithiothreitol, 1.25 μg of phosphatidylserine) containing 10 μCi of [γ-32P]ATP (3000 Ci mmol⁻¹; PerkinElmer Life Sciences) together with recombinant active ERK (1 unit) (New England Biolabs) and either myelin basic protein (Invitrogen) or recombinant wild-type STAT1α or STAT1β, mutant STAT1αY701F, or mutant STAT1βS727A (kindly provided by Uwe Vinkemeier) proteins incubated at 25 °C for 20 min. Reactions were terminated by addition of SDS sample buffer, and the 32P-incorporated proteins were fractionated by SDS-PAGE and visualized by autoradiography.
involved, we studied the effects of various kinase inhibitors on STAT1 degradation following oxidative stress. The MEK-ERK inhibitor U0126, but not the p38 kinase inhibitor SB203580, reduced STAT1 degradation, suggesting that ERK-mediated STAT1 phosphorylation was important for its proteasomal breakdown following oxidative stress. The effectiveness of these MAPK kinase inhibitors was confirmed by reduced levels of activated phospho-ERK (pERK) and phospho-p38 respectively (Fig. 2A).

Next we investigated the mechanism of ERK-mediated STAT1 proteolysis and examined whether activated pERK is able to interact with and directly phosphorylate STAT1/H9251. Co-immunoprecipitation assays using a specific anti-STAT1 antibody confirmed a weak STAT1 and pERK interaction following oxidative stress (Fig. 2B) and not under normal conditions (data not shown). Moreover, co-localization of pERK and STAT1 was confirmed in vivo by confocal microscopy following oxidative stress (Fig. 2C). A conserved STAT1 serine at position 727 has a
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FIGURE 3. STAT1 is phosphorylated directly with active ERK on serine 727. A recombinant myelin basic protein (MBP), or STAT1 wild-type (ST1a), or STAT1β (ST1b), or STAT1Y701F (ST1Y701) mutant, or STAT1S727A (ST1S727) mutant were incubated with recombinant active ERK with γ-ATP in a kinase assay followed by autoradiography. The panel below is a Western blot (WB) of the recombinant STAT1 proteins following autoradiography with the indicated antibodies, STAT1 (E-23) (ST1), -β and the phosphoserine 727 STAT1 (pST1 727). B, constitutively active ERK reduces STAT1 levels. MEF cells were transfected with a constitutively active MEK1 expression construct (HA-cMek1), and lysates were analyzed by Western blotting with the indicated antibodies, anti-STAT1 (a antibody (C-24) (ST1) or anti-HA (HA), GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Similar results were observed in three independent experiments.

Possible ERK consensus phosphorylation motif. Recombinant active ERK readily phosphorylated recombinant wild-type STAT1 (Fig. 3A) in an in vitro kinase assay. In contrast, recombinant active pERK was not able to phosphorylate STAT1S727A mutant but was still able to phosphorylate STAT1Y701F mutant (Fig. 3A). In addition, pERK was unable to phosphorylate C-terminal truncated STAT1 that retains tyrosine 701.

Next, to further examine the role of the kinase cascade in STAT1 degradation, we expressed constitutively active MEK1 to promote ERK activation. Cells expressing constitutively active MEK1 exposed to oxidative stress indeed showed enhanced STAT1 degradation, suggesting that STAT1 levels are inversely correlated with enhanced pERK activity (Fig. 3B). These experiments suggest that STAT1 is efficiently phosphorylated by ERK and confirms that STAT1S727 is the major ERK substrate.

βTRCP Interacts Specifically with Phospho-STAT1 Serine 727—The targeting of specific proteins for proteasomal degradation is governed by the F-box protein subunit of the SCF (SKP1-CUL-F-box) complex that directly recruits specific substrates (11). HA-tagged F-box proteins were transfected into MEF cells for 48 h. The transfected cells were then treated with H2O2 for 4 h to activate ERK. The cells were lysed and the lysates incubated with GST-STAT1. Fig. 4A shows that the GST-STAT1 (which will be serine-phosphorylated by the active endogenous ERK in the lysate) interacts with Fbw2 and Fbw1/βTRCP but not Fbw4. No interactions were seen in lysates treated with the ERK inhibitor U0126 (data not shown).

Because Fbw1/βTRCP has been well documented to play a role in targeting key checkpoint cell cycle regulators, and we have also implicated STAT1 in checkpoint regulation (11), we focused the rest of our studies on the role of the STAT1/βTRCP association. To investigate whether endogenous STAT1 and βTRCP interact, we immunoprecipitated βTRCP from the same cell lysates used in the experiments shown in Fig. 4A (IP-βTRCP) and blotted with an anti-total STAT1 antibody (Fig. 4B). The data show that IP with the anti-βTRCP antibody (but not the control preimmune antibody) results in pull down of STAT1. βTRCP and STAT1 were also co-localized, and at least a portion of the co-localized proteins was associated with a proteasomal marker (Fig. 4C).

Next, we examined whether the STAT1/βTRCP interaction is dependent on STAT1 phosphorylation. Wild-type STAT1 or mutant STAT1Y701F immunoprecipitated efficiently with βTRCP, but STAT1β or a STAT1S727A mutant did not (Fig. 4D). This indicates that serine 727 is essential for this interaction and suggests that serine 727 may be phosphorylated in vivo and mediate the binding of STAT1 with βTRCP.

To confirm that βTRCP inhibition stabilizes STAT1, we next tested whether direct inhibition of βTRCP expression by siRNA also inhibited degradation of STAT1. As shown in Fig. 5A, human fibrosarcoma cells (3T3GHT) transfected with βTRCP siRNA but not control siRNA showed more abundant STAT1 protein levels following treatment with cycloheximide and H2O2. In MEF cells, knock down of ERK1 or ERK2 by siRNA also inhibited degradation of STAT1. As shown in Fig. 5B. Moreover, βTRCP siRNA knockdown of its known Cdc25A substrate resulted in increased STAT1 but not ERK levels (Fig. 5B). These results further support the suggestion that STAT1 degradation is mediated by βTRCP.

To further investigate the importance of serine 727 in STAT1 degradation, we re-expressed STAT1 wild-type or STAT1S727A mutant forms into STAT1−/− MEF cells. Compared with wild-type STAT1, the half-life of the STAT1S727A mutant was increased following treatment with cycloheximide and H2O2 (Fig. 6A). Similar results were also obtained in human STAT1-deficient U3A fibrosarcoma cells stably expressing wild-type STAT1 or the STAT1S727A mutant (data not shown). These results confirm that the phosphorylation state of serine 727 is an important determinant of STAT1 stability by triggering its proteasomal degradation.

STAT1 and ERK Kinase Activity Inversely Correlate in Leukemic Cell Lines—The finding that ERK is frequently activated in a variety of cancers, including acute myeloid leukemias (16), prompted us to test whether STAT1 levels and ERK activity are inversely correlated in leukemic cell lines. We have preliminary data showing that levels of constitutive phospho-STAT1 serine 727 varied significantly (compared with phospho-STAT3 serine 727) in a panel of leukemic cell lines (data not shown). We next examined whether the activity of the ERK pathway influences STAT1 levels in two of the cell lines, Ramos, which was a low STAT1 expressor, and RL, a high STAT1 expressor. Constitutive ERK activity was significantly higher in Ramos compared with the RL cell line (Fig. 6B). Treating the cells with the MEK1-ERK pharmacological inhibitor U0126 not only reduced pERK activity and decreased constitutive STAT1 serine 727 phosphorylation but also enhanced STAT1 levels, suggesting...
that pERK activity is associated with reduced levels of STAT1 (Fig. 6B).

Furthermore, MEF cells subjected to serum deprivation followed by addition of serum (which contains numerous growth factors) enhanced phospho-ERK, and this was also associated with reduced expression of STAT1 (Fig. 6C). Importantly, this effect was abrogated with the MEK1-ERK pharmacological inhibitor U0126. In addition, the cytokine urocortin, which we have studied extensively in the past (16), is also able to enhance phospho-ERK and reduce STAT1 expression (Fig. 6C). This effect of urocortin on STAT1 is also reversed by the MEK-ERK inhibitor. Thus, this is further evidence that activation of the ERK pathway is linked to STAT1 turnover.

DISCUSSION

STAT1 plays important roles in the interferon-γ response and also following various stressful stimuli that induce apoptotic or cell cycle checkpoint responses (2, 3, 5, 7, 9). However, the molecular mechanisms that govern the stability of activated/phosphorylated STAT1 have not been fully elucidated. Here we demonstrate that STAT1 phosphorylated on Ser727 is predominantly degraded by the RING finger domain E3 ligase TRCP.

As has been previously shown to target substrates such as IkB (17), our data indicate that endogenous phosphorylated STAT1 and constitutively tyrosine-phosphorylated STAT1 interacts with TRCP in proteasomal degradation is dependent on its phosphorylation status. Many TRCP substrates, such as IκB (17), have a DSGXXS degron motif that allows binding following serine phosphorylation. However, other TRCP substrates, including Wee1 (14), have been found to lack such a consensus site. The interaction of Wee1 with TRCP requires phosphorylation of two serine residues, Ser53 and Ser123 by polo-like kinase 1 (Plk1) and CDK, respectively (18). Although the sequence surrounding phosphorylated Ser53 (DpSAFQE) is similar to the conserved TRCP-binding motif, the role of Ser123 phosphorylation (EEFGSSpSPVK) in TRCP binding to Wee1 is unclear.

In this study we demonstrate that the STAT1 Ser727 site, which also lacks the DSGXXS degron motif, is required for binding to TRCP, because the STAT1 S727A mutant failed to bind the ligase (Fig. 3D). However, because a region less than 20 residues downstream of serine 727 (744DpSMMN748) in STAT1 is comparable with a TRCP consensus sequence, serine 727 phosphorylation may play a similar role in targeting TRCP to this downstream sequence in STAT1 as serine 123 phosphorylation in Wee1. In addition to Wee1, Cdc25A, a checkpoint cell cycle regulator, and Emi1, a negative regulator of the E3 ligase APC, are also degraded by the TRCP pathway (19, 20), suggesting that TRCP-mediated degradation may be important for mitotic progression by regulating protein levels of several key cell cycle regulators.

FIGURE 4. βTRCP interacts specifically with phospho-STAT1 serine 727. A, various HA epitope-tagged F-box proteins βTRCP (FBOX1A), Fbw2 (FBOX2), and Fbw4 (FBOX4) were transfected into MEF cells for 48 h and exposed to H2O2 (200 μM). B, STAT1 and βTRCP interact endogenously following exposure to H2O2 (200 μM) for 4 h. MEF cell lysates were immunoprecipitated with GST-STAT1, followed by GST pulldown and Western blotting (WB) with anti-HA antibody (upper panel). Other panels show control for GST alone pulldown (2nd panel) and inputs for GST-STAT1 (3rd panel) and HA-tagged F-box proteins (lower panel). WCL, whole cell lysate. B, STAT1 and βTRCP interact endogenously following exposure to H2O2 (200 μM) for 4 h. MEF cell lysates were immunoprecipitated with a specific anti-βTRCP antibody (IP-βTRCP) or a preimmune control antibody (PI), followed by Western blotting with the indicated antibodies. C, STAT1 and βTRCP co-localize with the proteasome in MEF cells. MEF cells were plated on coverslips and transfected with Myc-STAT1 and HA-βTRCP and exposed to H2O2 (200 μM) for 4 h in presence of the proteasome inhibitor MG132.

Cells were stained with anti-Myc (ST1) blue, anti-HA (βTRCP) red, and anti-20 S proteasome (20 S) green. D, STAT1 and βTRCP interaction is dependent on STAT1 serine 727. Wild-type STAT1 or mutant STAT1S727A or STAT1Y701P were transfected together with HA-tagged βTRCP into STAT1-deficient MEF cells. Cell lysates were immunoprecipitated with anti-HA antibody (IP-HA) followed by Western blotting (WB) with the indicated antibodies.
in eIF4B and Ser123 in Cdc25A, have also been shown to be phosphorylated by more than one kinase (22, 23). In STAT1, mutation of serine 727 to alanine results in loss of roughly 80% of IFN-γ responsiveness (21) but has much less effect on the response to type I interferon. These differential effects may also depend on how serine 727 phosphorylation affects recruitment of co-activators/co-repressors, because phosphoserine 727 is required for the interaction between STAT1 and MCM5 (24), thus coupling the transcriptional activity of STAT1 to cell cycle regulation. Moreover, recruitment of the co-activator CBP is strongly reduced at the interferon-responsive GBP promoter in cells expressing mutant STAT1S727A following treatment (25). STAT1 is expressed as two isoforms STAT1α and STAT1β, which lack the last 38 residues of the C-terminal domain, including the serine 727 residue. Therefore, the biological outcome of STAT1α serine phosphorylation by different kinases may depend on the cell and stimulus context. However, because STAT1β still contains the tyrosine 701 residue, we cannot rule out that this site may also be regulated by post-translational processing.

We have previously demonstrated that the cardioprotective agent urocortin mediates its protective effects against ischemia/reperfusion-induced apoptotic cell death via activation of the MAPK-ERK pathway. Thus, pretreatment with a pharmacological phospho-ERK inhibitor inhibited the survival-promoting effect of urocortin (16). Moreover, apoptosis in cardiac myocytes exposed to ischemia/reperfusion injury is, at least in part, STAT1-dependent (26). Thus, these data, together with this study, suggest that the cardioprotective effects of urocortin may be mediated via ERK-STAT1 turnover and reduction in pro-apoptotic STAT1 levels.

It is known that serine 727 phosphorylation of STAT1 can be mediated by at least two kinase cascades, p38 and ERK (4, 5, 21). The same serine residues in other proteins, such as residue 422

Here we propose that phosphorylation of serine 727 in STAT1 by p38 and ERK leads to opposite effects. p38-mediated phosphorylation has been reported to maximize STAT1 tran-
scriptional activity and may mediate the cell cycle arrest induced by IFNγ through enhancement of the transcription of cell cycle arrest and apoptotic genes. In contrast, proliferative growth factors and other cytokines, which are known to activate ERKs, would target STAT1 for proteasomal breakdown, compromising its transcriptional activity and thus promoting cell cycle progression. This may be reflected in the molecular pathology of some tumors, where constitutively active ERKs lead to STAT1 degradation, and thus allow unregulated cell proliferation.

Our present findings describe a novel pathway for the regulation of STAT1 stability mediated via its phosphorylation and proteasomal degradation in an ERK-βTRCP-dependent manner. Serine 727 phosphorylation by ERKs, although not by p38 MAPK, is necessary for βTRCP binding, although it does not form part of a classic βTRCP degron, because the S727A mutant no longer binds βTRCP. The high levels of active ERK induced by proliferative growth factors, and which are constitutive in certain tumors, may also have a role in promoting STAT1 degradation and reduce its functional activity.

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REFERENCES

1. Levy, D. E., and Darnell, J. E., Jr. (2002) Science 297, 729–736
2. Levy, D. E., and Darnell, J. E., Jr. (2002) Cell 118, 1629–1639
3. Schindler, C., Shuai, K., Prezioso, V. R., and Darnell, J. E., Jr. (1992) Immunity 2, 257, 91–100
4. David, M., Petricoin, E., III, Benjamin, C., Pine, R., Weber, M. J., and Darnell, J. E. (1998) J. Biol. Chem. 273, 25704–25710
5. Kovarik, P., Mangold, M., Ramsauer, K., Heidari, H., Steinborn, R., Zotter, A., Ley, D. E., Muller, M., and Decker, T. (1999) EMBO J. 18, 9928–9933
6. Wen, Z., Zhong, Z., and Darnell, J. E., Jr. (1995) Cell 82, 241–250
7. Townsend, P. A., Scarabeli, T. M., Davidson, S. M., Knight, R. A., Latchman, D. S., and Stephanou, A. (2004) J. Biol. Chem. 279, 5811–5820
8. Soond, S. M., Carroll, C., Townsend, P. A., Sayan, E., Melino, G., Behrmann, I., Knight, R. A., Latchman, D. S., and Stephanou, A. (2007) FEBS Lett. 581, 1217–1226
9. Townsend, P. A., McComick, J., Barry, S., Lawrence, K. M., Knight, R. A., Hubank, M., Chen, P.-L., Latchman, D. S., and Stephanou, A. (2005) J. Cell Sci. 118, 1629–1639
10. Kaplan, D. H., Shankaran, V., Dighe, A. S., Stoker, E., Aguet, M., Old, L. J., and Schreiber, R. D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7556–7561
11. Ang, X. L., and Harper, J. W. (2005) Oncogene 24, 2860–2870
12. Didcock, L., Young, D. F., Goodbourn, S., and Randall, R. E. (1999) J. Virol. 73, 9928–9933
13. Ulaane, C. M., and Horvath C. M. (2002) Virology 304, 160–166
14. Tanaka, T., Soriano, M. A., and Grusby, M. J. (2005) Immunity 22, 729–736
15. Yada, M., Hatakeyama, S., Kamura, T., Nishiyama, M., Tsunematsu, R., Imaki, H., Ishida, N., Okumura, F., Nakayama, K., and Nakayama, K. I. (2004) EMBO J. 23, 2116–2125
16. Brar, B. K., Jonassen, A. K., Stephanou, A., Santilli, G., Railson, J., Knight, R. A., Yellon, D. M., and Latchman, D. S. (2000) J. Biol. Chem. 275, 8508–8514
17. Yaron, A., Hatzubai, A., Davis, M., Lavon, I., Amit, S., Manning, A. M., Andersen, J. S., Mann, R., Ruscito, F., and Ben-Neriah, Y. (1998) Nature 396, 590–594
18. Watanabe, Y., Ozasa, S., Minami, M., Watanabe, N., Hunter, T., and Okumura, F. (2003) Proc. Natl. Acad. Sci. U. S. A. 101, 9293–9298
19. Busino, L., Donzelli, M., Chiesa, M., Guardavaccaro, D., Ganoth, D., Ang, X. L., and Harter, K. P. (2003) Proc. Natl. Acad. Sci. U. S. A. 101, 7601–7606
20. Margottin-Goguet, F., Hsu, J. Y., Loktev, A., Hsieh, H. M., Reimann, J. D., Behrmann, I., Knight, R. A., Latchman, D. S., and Stephanou, A. (2007) J. Biol. Chem. 282, 1629–1639
21. Shahbazian, D., Roux, P. P., Mieulet, V., Cohen, M. S., Raught, B., Taunton, J., Blenis, J., Hershey, J. W., Blenis, J., Pende, M., and Sonenberg, N. (2006) EMBO J. 25, 590–594
22. Andersen, J. S., Mann, M., Mercurio, F., and Ben-Neriah, Y. (1998) Nature 396, 590–594
23. Hassepass, I., Voit, R., and Hoffmann, I. (2003) J. Biol. Chem. 278, 29824–29829
24. Zhang, J. J., Zhao, Y., Chait, B. T., Latham, W. W., Ritz, M., Knippers, R., and Darnell, J. E. (1998) EMBO J. 17, 6963–6971
25. Varinou, L., Ramsauer, K., Karaghiosoff, M., Kolbe, T., Pfeffer, K., Muller, M., and Decker, T. (2003) Immunity 19, 793–802
26. Stephanou, A., Brar, B. K., Scarabeli, T. M., Jonassen, A. K., Yellon, D. M., Marber, M. S., Knight, R. A., and Latchman, D. S. (2000) J. Biol. Chem. 275, 10002–10008