Numerous cytokines, growth, and differentiation factors elicit their intracellular responses via Janus tyrosine kinases (Jaks) and transcription factors of the STAT (signal transducer and activator of transcription) family. Additionally, environmental stress (UV light, heat, aniso-osmolarity, and radicals) has recently been shown to activate intracellular signaling cascades such as the stress-activated protein kinases and nuclear factor-κB. In this study, we demonstrate that in different cell lines a particular stress, namely hyperosmolarity, results in tyrosine phosphorylation of the Janus kinases Jak1, Jak2, and Tyk2 and in the activation of STAT1 and/or STAT3. Both transcription factors are phosphorylated at a specific tyrosine residue and translocation to the nucleus was demonstrated by the use of a STAT3/green fluorescent protein fusion protein. A prominent role for Jak1 in the activation of STATs by hypertonicity was demonstrated by the use of Jak-deficient cell lines. Stress-activated STAT1 and STAT3 transactivate a reporter gene containing the acute-phase response elements of the rat α2-macroglobulin promoter. Experiments using a diffusible solute suggest that not the increase in intracellular osmolarity but the resultant cell shrinkage is the trigger for Jak/STAT activation.

Growth factors and cytokines activate intracellular signal transduction pathways, which ultimately lead to proliferation, differentiation, and/or altered gene expression. In recent years, it has become evident that physicochemical stress such as UV irradiation, heat, free radicals, hypoxia, and aniso-osmolarity can also trigger some of these signal transduction cascades and thereby change cell function. Thus, it was demonstrated that UV irradiation and \( \text{H}_2\text{O}_2 \) activate transcripion of the immediate early gene c-jun via a pathway involving SRC tyrosine kinases, Ras, Raf-1, and the c-Jun N-terminal kinase (JNK)\(^1\). Additionally, transcription factor NF-κB is activated by UV irradiation, oxygen radicals, and hypoxia (4–6). Alterations in cellular hydration induced by either aniso-osmotic environments or under the influence of hormones, oxidative stress, or cumulative substrate uptake represent independent signals, which may modulate cell function (for reviews, see Refs. 7 and 8). In some cells, hyper-osmolarity induced elevation of intracellular calcium concentrations (9, 10) and led to a rapid activation of the mitogen-activated protein kinases Erk-1 and Erk-2 (11–14). In contrast, hyper-osmotic stress mainly triggered activation of the JNKs and p38, the mammalian homologue to the yeast mitogen-activated protein kinase high osmolality glycerol response 1 (HOG1) (15–17).

Recently, it was demonstrated that UV light and high osmolality induce clustering and internalization of receptors for epidermal growth factor (EGF), interleukin-1 (IL-1), and tumor necrosis factor α, finally resulting in an activation of JNK-1 (18).

Another important signal transduction cascade, the Jak/STAT pathway, is activated by numerous cytokines (19), growth (20–24), and differentiation factors (25–29). After ligand-induced dimerization of receptor subunits, cytoplasmic tyrosine kinases of the Jak family become autophosphorylated and thereby activated. Substrates for these kinases are the receptors themselves and transcription factors of the STAT family, which dimerize, translocate to the nucleus, and bind to enhancers within the regulatory regions of target genes resulting in their increased transcription (19, 30).

In this study, we have raised the question whether osmotic shock leads to an activation of the Jak/STAT pathway. We show that in different cell lines hypertonic treatment results in a rapid phosphorylation of Jak1, Jak2, and Tyk2 and in the activation of STAT1 and/or STAT3.

**Experimental Procedures**

*Materials—Restriction enzymes and T4-DNA ligase were purchased from Boehringer Mannheim (Mannheim, Germany). Oligonucleotides were purchased from MWG Biotech (Ebersberg, Germany). Dulbecco’s modified Eagle’s medium (DMEM), DMEM/F-12 mix, and RPMI 1640 were from Life Technologies, Inc. (Eggenstein, Germany), and fetal calf serum was from Seromed (Berlin, Germany). Recombinant human IL-6 and soluble IL-6 receptor gp80 (sgp80) were prepared as described (31, 32). Reporter gene constructs p\( \times \)xoretkCAT and p\( \times \)xCAT were kindly provided by Dr. F. Horn (RWTH Aachen, Germany).

Cell Culture—COS-7, HeLa, 2TGH parental, and mutant cells (U4A, γ2A, and U1A) (33) were grown in DMEM; HepG2 cells in DMEM/F-12 mix; and rat mesangial cells (RMC) (34) in RPMI 1640 at 5% CO\(_2\) in a water-saturated atmosphere. All cell culture media were supplemented with 10% fetal calf serum, streptomycin (100 mg/liter), and penicillin.

Activation of the Janus Kinase/Signal Transducer and Activator of Transcription Pathway by Osmotic Shock*

(Received for publication, November 17, 1997, and in revised form, May 18, 1998)

Petros Gatsios‡, Lara Terstegen‡‡, Freimut Schliesiß, Dieter Häussinger‡†, Ian M. Kerr‡†, Peter C. Heinrich‡, and Lutz Graevey**

*This work was supported by grants from the Deutsche Forschungsgemeinschaft, by the Interdisciplinary Center for Clinical Research in Biomaterials and Tissue-Material-Interaction in Implants (BIOMAT), and by the Fonds der Chemischen Industrie. The costs of publication of this article were defrayed in part by the payment of page charges. This paper is available on line at http://www.jbc.org
FIG. 1. Activation of STAT factors in different cell lines by osmotic shock. A, HepG2, COS-7, and RMC cells were treated with 600 mM sorbitol in the respective culture medium for 15 min. Control cells received fresh medium. Cells were harvested and nuclear extracts were prepared as described under “Experimental Procedures.” 5 μg of nuclear proteins were mixed with a 32P-labeled oligonucleotide (mutated SIE probe of the c-fos promoter 5′-GAT CCG GGA GGG ATT TAG CCC GAA ATG CTG-3′), and EMSAs were performed. The DNA-protein complexes formed were separated from the free probe by electrophoresis on a native 4.5% polyacrylamide gel. The positions of comigrating STAT1 homodimer, STAT1/STAT3 heterodimer, and STAT3 homodimer from IL-6 stimulated HepG2 cells are indicated by the arrows. B, COS-7 cells were stimulated with either 300 mM NaCl, 600 mM sorbitol, or 100 mM NaCl, 1 mM EDTA). The samples were boiled in gel electrophoresis and autoradiographed.

Electrophoretic Mobility Shift Assay (EMSA)—EMSA-EMSA were performed as described previously (36) using a double-stranded 32P-labeled mutated m67SIE oligonucleotide from the c-fos promoter (m67SIE: 5′-GAT CCG GGA GGG ATT TAG CCC GAA ATG CTG-3′) (37). The protein-DNA complexes were separated on a 4.5% polyacrylamide gel containing 7.5% glycerol in 0.25-fold TBE at 20 V/cm for 4 h. Gels were fixed in 10% methanol, 10% acetic acid, and 80% water for 1 h, dried, and autoradiographed.

Immunoprecipitation—Cells were washed twice with PBS and solubilized in 1 ml of lysis buffer (1% Brij 96, 20 mM Tris/HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA) for 30 min at 4 °C. Nuclei were removed by centrifugation for 10 min at 12,000 × g. The supernatants were incubated with 1 μg of anti-Jak1 (polyclonal), anti-Jak2 (polyclonal), anti-Tyk2 (monoclonal) or anti-gp120 (B-T12; monoclonal) antibodies, respectively, for a minimum of 8 h at 4 °C. After antibody incubation, the lysates were treated with protein A-Sepharose (5 μg/ml in lysis buffer) and washed four times with wash buffer (0.1% Brij 96, 20 mM Tris/HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA). The samples were boiled in gel electrophoresis sample buffer, and the precipitated proteins were separated on a SDS-polyacrylamide (10%) gel.

Western Blotting and Immunodetection—The electrophoretically separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes by the semi-dry Western blotting method. Nonspecific binding was blocked with 10% bovine serum albumin in TBS-N (20 mM Tris/HCl, pH 7.4, 137 mM NaCl, 0.1% Nonidet P-40) for 15 min. The blots were incubated with primary antibodies at 1/1000 dilution in TBS-N for 1 h. After extensive rinsing with TBS-N, the immunoblots were developed with the enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech) system following the manufacturer’s instructions. The following primary antibodies were used in Western blotting experiments: anti-phosphotyrosine mouse monoclonal antibody (4G10; Upstate Biotechnology); anti-Jak1 and -Jak2 rabbit polyclonal antibodies (kindly provided by Dr. Ziemiecki, Bern); anti-Tyk2 (Upstate Biotechnology); anti-gp120 mouse monoclonal antibody (B-P4); phosphospecific STAT1 (Tyr-701), and STAT3 (Tyr-705) rabbit polyclonal antibody (New England Biolabs). Phosphospecific STAT1 antibody (Tyr-701) detects STAT1 only when phosphorylated at Tyr-701. Phosphospecific STAT3 antibody (Tyr-705) detects STAT3 only when phosphorylated at Tyr-705.

Transient Transfections—HepG2 cells were grown on 60-mm dishes to 30% confluence and transfected in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, using standard calcium phosphate precipitates, with 7.5 μg of reporter construct plasmid DNA and 2.5 μg of internal control plasmid DNA PCH110 (Amersham Pharmacia Biotech). Cells were incubated with precipitate for 18 h, washed twice with PBS, and media changed for additional 6 h. Cells were then stimulated for 3 h and protein extracts were prepared for CAT and β-galactosidase assays as described (38).

Construction of the STAT3/Green Fluorescent Protein (GFP) Chimera—Green fluorescent protein cDNA was introduced into the pSBC-2 mammalian expression vector via the EcoRI/HindIII restriction sites. An oligonucleotide (5′-GGC CGC GGA GAT CTG GC-3′) containing the BgII restriction site was cloned into the NotI/EcoRI sites of pSBC-2-GFP expression vector. A BgII site was introduced at the stop-codon of STAT3 cDNA via polymerase chain reaction. The STAT3 cDNA was subcloned into the pSBC-2-GFP expression vector via NotI/BgII restriction sites resulting in a fusion protein of STAT3 with GFP at its C terminus (pSBC-2-STAT3-GFP).

HeLa Cell Transfections and Fluorescence Studies—HeLa cells were transfected using the DNA/calcium phosphate precipitation method. Approximately 105 cells were seeded on coverslips and cultured for 48 h. Cells were washed twice with PBS and fixed with 2% paraformaldehyde and coverslips were mounted on slides with Mowiat4M 4–88 (Calbiochem Corp., La Jolla, CA). Cells were analyzed using a Nikon Eclipse E600MTM fluorescence microscope at a magnification of ×400.

RESULTS

Activation of STAT Factors by Osmotic Shock in Different Cell Lines—In order to study whether hypertonicity (osmotic shock) results in an activation of the Jak/STAT signaling pathway, human hepatoma HepG2 cells were serum-deprived for 18 h and subsequently incubated with serum-free medium containing 600 mm sorbitol for 15 min. Such a treatment was recently shown to activate the JNK cascade in HeLa cells (18). Nuclear extracts were incubated with a 32P-labeled oligonucleotide specific for STAT1 and STAT3 (37, 39) and analyzed in an EMSA. Upon sorbitol treatment a prominent band was detected in the gel shift assay that migrated at a position typical for the STAT3 homodimer (Fig. 1A, left panel). When the same experiment was repeated in the SV40-transformed simian kidney cell line COS-7, a prominent band migrating somewhat faster was observed (Fig. 1A, middle panel). This band represents the STAT1 homodimer, which is also the major STAT forms activated in these cells upon stimulation with IL-6 and the soluble form of the IL-6 receptor (ssp80) (40). A rat mesangial cell line (RMC) showed two bands after osmotic shock, a STAT1 homodimer and a STAT1/STAT3 heterodimer (Fig. 1A, right panel). These results suggest that hypertonicity leads to a rapid activation of STAT factors independent of the cell type used. However, the extent of stimulation is different.
between these cell lines being most prominent in COS-7 cells. Therefore, these cells were used for the majority of the experiments. To confirm that the observed STAT activation is due to the hypertonic shock and not a specific result of sorbitol treatment, we treated COS-7 cells with medium containing 300 mM NaCl, which corresponds to a hyperosmolarity of 600 mosM. It was apparent that in these cells both STAT3 and STAT1 were activated, however with different kinetics (Fig. 2C). In the RMC cells, a similar time course of STAT factor activation could be shown (Fig. 2D). These data suggest that activation of the Jak/STAT pathway by osmotic shock does not require phosphorylation of gp130.

Osmotic Shock Induces Nuclear Translocation of a STAT3/Green Fluorescent Protein Chimera—In order to study whether activation of STATs via osmotic shock also results in their nuclear translocation, we transiently expressed a STAT3/green fluorescent protein chimera in HeLa cells. In control cells, a uniform staining of the cytoplasm and nucleus was observed upon fluorescence microscopy (Fig. 3A). In contrast, in HeLa cells that were stimulated with IL-6 and sgp80 or treated with hyperosmotic medium for 30 min, an exclusive staining of the nuclei was detected (Fig. 3, B and C). Thus, hyperosmolarity leads to a recruitment of STAT3 to the nucleus.

Osmotic Shock Leads to the Tyrosine Phosphorylation of STATs—Activation of STATs usually requires phosphorylation of specific tyrosine residues (Tyr-701 in STAT1 and Tyr-705 in STAT3), which then mediate the dimerization of STATs via neighboring Src homology 2 domains (39, 41). In order to demonstrate that osmotic shock induces the tyrosine phosphorylation of STAT1, COS-7 cells were treated with 600 mM sorbitol for different times and nuclear extracts were prepared and analyzed by 10% SDS-PAGE. Proteins were transferred to a PVDF membrane and incubated with an antisera specific for Tyr-701-phosphorylated STAT1. After osmotic shock, a protein migrating at a position corresponding to STAT1 was detected (Fig. 4A). The extent of phosphorylation correlates well with the DNA binding capacity shown in Fig. 2B. In HepG2 cells, a similar phosphorylation pattern was seen for STAT1 as well as for STAT3 (Fig. 4B). Note that the phosphorylation of STAT3 precedes that of STAT1, which is also reflected in the EMSA (Fig. 2C).

Jak Kinases Are Involved in Signaling after Hypertonic Shock—The Jak1 kinase was demonstrated to be essential for the Jak/STAT signaling via the IL-6 signal transducer gp130 (33). To find out whether osmotic shock leads to an activation of Jak1, cell lysates from COS-7 cells treated with 600 mM sorbitol for 15 min were immunoprecipitated with a Jak1-specific antiserum. Western blotting was performed with a phosphotyrosine-specific antibody and after stripping with an Jak1 antibody. Hypertonicity resulted in an increased Jak1 phosphorylation, which was almost as prominent as after stimulation with IL-6/sgp80 (Fig. 5A). Interestingly, after IL-6/sgp80 treatment, an additional band migrating at about 150 kDa was immunoprecipitated. This band most likely corresponds to the phosphorylated signal transducer gp130 to which Jak1 is constitutively bound (data not shown). Phosphorylation of gp130, however, was hardly detectable after osmotic shock. When gp130 was directly immunoprecipitated from sorbitol- or IL-6-treated HepG2 cells, the phosphotyrosine antisera only detected gp130 in cells that were stimulated with IL-6 (Fig. 5B). These data suggest that activation of the Jak/STAT pathway by osmotic shock does not require phosphorylation of gp130.
In order to assess whether also other Jak kinases are phosphorylated upon hypertonic treatment, COS-7 and HepG2 cells were treated as above and immunoprecipitations were performed with antisera against Jak2 and Tyk2. Both kinases showed an increased tyrosine phosphorylation within 10 or 20 min after addition of sorbitol, although the Jak2 signal in COS-7 cells was very faint (Fig. 6).

To analyze whether the activation of Jaks is a prerequisite for STAT activation by osmotic shock, we made use of the Jak1-deficient cell line, U4A (33). Parental fibrosarcoma cells 2TGH, U4A cells, and U4A cells in which the Jak1 kinase has been stably reintroduced were treated with sorbitol for 15 min, and nuclear extracts were analyzed in an EMSA (Fig. 7A). In 2TGH cells, a prominent STAT1 activation was detected that was markedly inhibited in Jak1-deficient cells. This was even more clearly seen when IL-6/gp80 was used as a stimulus. Reconstitution of Jak1 in U4A cells resulted in a cell line that upon stimulation with IL-6/gp80 or sorbitol displayed an en-
enhanced STAT activation when compared with the parental cells (Fig. 7). These findings suggest that Jak1 is involved in STAT activation after osmotic shock. However, it does not seem to be as essential as after IL-6 stimulation (33), inasmuch as in Jak1-deficient cells a residual activation by sorbitol remained.

We also analyzed γ2A and U1A cells, which lack Jak2 and Tyk2, respectively, and compared them with clones in which the respective kinase had been reconstituted (Fig. 7B) (33). In contrast to the Jak1-deficient cell line, γ2A and U1A cells displayed a normal responsiveness to osmotic shock, suggesting that Jak2 and Tyk2 are not crucial for STAT activation after hypertonicity.

**Activation by Osmotic Shock of a Reporter Construct Containing STAT Binding Sites**—In order to study whether activation of STATs by osmotic shock also results in an increased gene activation, we made use of a reporter construct p7xcore/tk/CAT in which an element containing seven identical STAT binding sites (the proximal core element of the α2-macroglobulin promoter) was cloned in front of the basal thymidine kinase promoter followed by the chloramphenicol acetyltransferase gene. A vector only containing the thymidine kinase promoter (ptk/CAT) was used as a control. These constructs were transiently expressed in HepG2 cells, and cells were treated with IL-6 or 600 mM sorbitol for 3 h after which reporter activity was determined (Fig. 8). IL-6 stimulation of cells that were transfected with the p7xcore/tk/CAT construct resulted in an 4.6-fold increased CAT activity when compared with ptk/CAT cells (Fig. 8). Osmotic shock resulted in a decrease to about 25% of the basal reporter activity in ptk/CAT cells (data not shown). However, in osmotically shocked p7xcore/tk/CAT cells, the reporter activity was decreased to a much lesser extent resulting in a relative induction of about 2.4-fold (Fig. 8). Thus, hyperosmotically activated STATs are capable of trans-activating a respective reporter gene.

**Hypertonicity-induced Cell Shrinkage Is Crucial for STAT Activation**—In order to assess whether hyperosmolarity by itself or the resultant cell shrinkage is the trigger for Jak/STAT activation, we increased the hypertonicity of the medium with 600 mM urea, which is known to be a rapidly permeating solute that does not affect cell volume (42). When nuclear extracts from these cells were analyzed in an EMSA, no STAT activation was detected (Fig. 9A). However, when these cells were “super-induced” with either IL-6/sgp80 or increasing concentrations of sorbitol again, a prominent STAT activation was seen (Fig. 9B). From these results, we conclude that hypertonicity itself is not sufficient for activation of the Jak/STAT pathway and that urea does not inhibit STAT activation.

**DISCUSSION**

In this study, we demonstrate that hyperosmotic shock activates the Jak/STAT pathway. This pathway is known to mediate the signaling of numerous cytokines, growth, and differentiation factors (19, 30). Thus, we and others have recently shown that IL-6 activates three Jak tyrosine kinases, namely Jak1, Jak2, and Tyk2, which then phosphorylate the IL-6 signal transducer gp130 and the latent cytoplasmic transcription factors STAT1 and STAT3 (43–45). Activated STAT factors homo- and heterodimerize via an Src homology 2 domain/phosphotyrosine interaction, are transported to the nucleus, and
Osmotic Shock Activates the Jak/STAT Pathway

Stress-activated STATs could stimulate gene transcription of reporter gene constructs containing well characterized STAT1/3 binding sites as enhancers. When compared with the activity of the basal thymidine kinase promoter, both IL-6 and osmotic stress resulted in induction of reporter gene activity. However, it should be pointed out that under hypertonicity the basal CAT activity was strongly reduced. This could be due to a general suppression of transcription and/or to an increased degradation of the chloramphenicol acetyltransferase. An increased protein catabolism after cell shrinkage was observed in several cell systems.

Dimerization of surface receptors is currently believed to be the trigger for activation of associated JakS. However, we have no evidence for an involvement of the signal transducer gp130 in the hyperosmotic signal cascade since tyrosine phosphorylation of gp130 was absent after osmotic shock. Thus, stress activation of the Jak/STAT is either triggered by a mechanism located downstream of gp130 or other cytokine/growth factor receptors are involved. Recently, it was shown that hyperosmolarity and UV light lead to a clustering and phosphorylation of the receptors for EGF, IL-1, and tumor necrosis factor α (18).

The EGF receptor has been described to be an activator of STAT1 and STAT3 (20, 39, 50), and EGF stimulates the tyrosine-phosphorylation of Jak1, a function that is dependent on the intrinsic kinase activity of the EGF receptor (41). However, phosphorylation of STATs by EGF does not require Jak1 (51), and therefore the EGF receptor is probably not involved in stress activation of STATs.

It is currently unknown whether the initial trigger for activation of intracellular signal cascades is the hyperosmolarity itself or the resulting cell shrinkage. When COS-7 cells were treated with 600 mM urea, no STAT activation was observed. Since urea readily diffuses into the cell, it does not lead to gross changes in cell volume (42), although a slight shrinkage of liver parenchymal cells due to opening of K⁺ channels in the plasma membrane was observed (52). Thus, raising the intracellular osmolarity is obviously not sufficient for signaling. Additionally, the pathways leading to activation of JNKs and p38 were insensitive to hyper-osmotic urea (53). However, addition of a non-permeating solute (600 mM sorbitol) or the IL-6/sgp80 complex to cells equilibrated in 600 mM urea still resulted in a STAT activation, suggesting that the cell shrinkage itself is the trigger for Jak/STAT activation. In human neutrophils, osmotic cell shrinkage induces tyrosine phosphorylation of several proteins and activation of the Na⁺/H⁺ exchanger (42).

**Fig. 8.** Hypertonically activated STATs transactivate a reporter construct containing STAT binding sites. HepG2 cells were transfected with the ptk/CAT and the p7xcore/tk/CAT construct, respectively. After 24 h, cells were treated with 100 units/ml IL-6 or 600 mM sorbitol for 3 h and processed for reporter assays. CAT activities were normalized to β-galactosidase activities, and the ratio p7xcore/tk/CAT:ptk/CAT was calculated. The data presented are the mean of six values obtained from three independent experiments. The bars indicate the standard deviation.

**Fig. 9.** STAT activation is induced by cell shrinkage but not by an increase in osmolarity. COS-7 cells were treated with either 600 mM sorbitol or 600 mM urea for 15 min (A) or pretreated with 600 mM urea for 15 min and then incubated with increasing concentrations of sorbitol or 100 units/ml IL-6 plus 0.5 μg/ml sgp80 for another 15 min (B). Nuclear extracts and EMSAs were performed as described in the legend to Fig. 1. The positions of the activated STATs are indicated by arrows.
hamster ovary cells, an osmotically induced decrease in cell volume resulted in the tyrosine phosphorylation of three proteins of about 42, 85, and 120 kDa (54). The identity of the two larger proteins was not determined; however, it is conceivable that they may represent a STAT factor and a Jak kinase, respectively.

The physiological relevance of the activation of the Jak/STAT pathway by osmotic stress is currently unknown. Jak1/Jak2 and anti-gp130 antibodies, respectively.

**REFERENCES**

1. Devary, Y., Gottlieb, R. A., Smeal, T., and Karin, M. (1992) Science 258, 2804–2811.
2. Devary, Y., Gottlieb, R. A., Smeal, T., and Karin, M. (1992) Cell 71, 1081–1091.
3. Derijard, B., Hibi, M., Wu, I. H., and Davis, R. J. (1994) Science 265, 1422–1445.
4. Derijard, B., Hibi, M., Wu, I. H., and Davis, R. J. (1994) Cell 76, 1025–1037.
5. Derijard, B., Rosette, C., Di Donato, J. A., and Karin, M. (1993) Science 262, 1442–1445.
6. Schreck, R., Rieber, P., and Baeuerle, P. A. (1991) EMBO J. 10, 2247–2258.
7. Kool, R. C., Chen, E. Y., Mivechi, N. F., Denko, N. C., Stambrook, P., and Giaccia, A. J. (1994) Cancer Res. 54, 5273–5279.
8. Burg, M. B., Kwon, E. D., and Kultz, D. (1997) Annu. Rev. Physiol. 59, 437–455.
9. Haussinger, D. (1996) Biochem. J. 313, 697–710.
10. Haussinger, D. (1996) Biochem. J. 320, 167–171.
11. Haussinger, D. (1996) Gastroenterology 110, 868–865.
12. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
13. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Annu. Rev. Biochem. 63, 661–669.
14. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2285–2289.
15. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
16. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
17. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
18. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
19. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
20. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
21. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
22. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
23. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
24. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
25. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
26. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
27. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
28. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
29. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
30. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
31. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
32. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
33. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
34. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
35. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
36. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
37. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
38. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
39. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
40. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
41. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
42. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
43. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
44. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
45. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
46. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
47. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
48. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
49. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.
50. Schlessinger, J., Hunt, T., and Nebreda, A. R. (1994) Science 263, 89–92.