cis-Acting Element-specific Transcriptional Activity of Differentially Phosphorylated Nuclear Factor-κB*

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Phosphorylation of nuclear factor-κB (NF-κB) subunits emerges as a mechanism by which transcriptional activity of nuclear NF-κB complexes is regulated in an inhibitor κB-independent fashion. As the main transactivator, the p65 subunit of NF-κB has an outstanding position in the hierarchy of NF-κB proteins. p65 is a multiply phosphorylated protein with phosphorylation sites in the C-terminal transactivation domain and the N-terminal Rel homology domain (RHD). In this study, we describe two previously non-reported phosphorylation sites within the p65 RHD. We show that differential phosphorylation of serine residues within the RHD modulates transcriptional activity in a cis-acting element and promoter-specific context, thus leading to a phosphorylation state-dependent gene expression profile. RelA−/− mouse embryonic fibroblasts reconstituted with wild-type p65 or p65 phosphorylation-deficient mutants showed a distinctive expression profile of synthetic κB-dependent reporters as well as endogenous genes. Hypophosphorylated p65 did not display cis-acting element-specific changes in DNA binding or dimerization behavior. This study shows for the first time that site-specific phosphorylation can target a transcription factor to a particular subset of genes.

Protein phosphorylation is used in many different ways to control the activity of transcription factors. It directs subcellular localization (e.g. nuclear factor of activated cells (1)), selectively controls binding of dimerization partners (e.g. signal transducers and activators of transcription (2)), or alters transcriptional activity by facilitating the interaction with components of the transcriptional machinery (e.g. cyclic AMP-responsive element binding protein (3), p53 (4), and nuclear factor-κB (NF-κB))(5). NF-κB is a key transcription factor in regulating expression of pro-inflammatory, immune-modulatory, and anti-apoptotic genes (6). Cellular activation by a broad array of stimuli, including cytokines, bacterial lipopolysaccharides, viruses, and radiation, results in liberation of dimeric NF-κB from cytoplasmic inhibitory molecules (IκBs). Upon nuclear import and binding to specific decameric recognition motifs, which are reflected by the consensus GGGRHTYYCC (R, purine; Y, pyrimidine; H, not G), NF-κB dimers function as trans-acting elements in the promoter region of NF-κB-dependent genes. Transcriptional activity of nuclear NF-κB complexes is controlled by posttranslational modifications including acetylation (7) and phosphorylation (8). The p65 subunit, which is the prototypical NF-κB activator, is a multiple phosphorylated protein. Two serine residues within the C-terminal transactivation domain are phosphorylated by casein kinase II (9, 10), IκB kinases (11, 12), Ca2+/calmodulin-dependent protein kinase IV (13), and ribosomal S6 kinase 1 (14). Two serines within or adjacent to the N-terminal Rel homology domain (RHD) have been identified to be substrates for protein kinase A, mitogen and stress-activated protein kinase (MSK) (Ser-276 (15, 16)), and protein kinase Cε (Ser-311 (17)). Although the role of individual phospho-serines is not fully determined, it has been shown that they regulate p65 interaction with nuclear co-activator cyclic AMP-responsive element binding protein binding protein/p300 (5, 13, 17). Here we identify two additional serine residues within p65 RHD that are targeted for phosphorylation. We address the functional importance of these residues for NF-κB transcriptional activity. We show that differential phosphorylation of NF-κB p65 RHD modulates transcriptional activity in a cis-acting element and promoter-specific context, leading to phosphorylation state-dependent gene expression profiles. RelA−/− mouse embryonic fibroblasts reconstituted with wt p65 or p65 phosphorylation-deficient mutants showed a distinctive expression profile of synthetic κB-dependent reporters as well as endogenous genes.

MATERIALS AND METHODS

Cell Culture and Reagents—Bovine aortic endothelial cells were obtained from VEC Technologies (Rensselaer, NY) and used between passages four and nine. Mouse embryonic fibroblasts (MEFs) isolated from RelA−/− mice were kindly provided by Dr. A. Beg (Columbia University, New York, NY) and have been described previously (18). Cells were grown at 37 °C in Dulbecco’s modified Eagle’s medium (MediaTech Inc., Herndon, VA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin B (all Atlanta Biologicals, Norcross, GA) in a humidified atmosphere containing 5% CO2. Lipopolysaccharide (LPS) was purchased from Sigma (L-7261) and recombinant murine interferon-γ was from Calbiochem (San Diego, CA).

Plasmid Constructs—All p65 mutants were constructed by primer overlap extension as described (19). All p65 constructs were expressed from the pcDNA3 vector (Invitrogen, Carlsbad, CA) and feature an N-terminal c-myc tag. The 3xκB-Luc construct has been described previously (20). Other κB reporter constructs were generated by replacing the minimal SV40 promoter in the pGL3-promoter vector (Promega, Madison, WI) with a minimal promoter 5′-GAT CTG GGT ATA TAA TGG ATC CCC GGG TAC GCA GCT CAA GCT-3′ that was obtained by annealing complementary oligonucleotides that feature BglII (5′-end) and HindIII (3′-end) compatible overhangs. Double-stranded synthetic

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1 The abbreviations used are: NF-κB, nuclear factor-κB; RHD, Rel homology domain; MSK, mitogen and stress-activated protein kinase; wt, wild type; MEF, mouse embryonic fibroblast; LPS, lipopolysaccharide; PCR, polymerase chain reaction; qPCR, quantitative PCR; VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; IL, interleukin; MnSOD, manganese superoxide dismutase; MIP, monocyte inflammatory protein; EMSA, electrophoretic mobility shift assay; TNF, tumor necrosis factor.
oligonucleotides coding for a tandem \( eB \) site were cloned upstream of this minimal promoter using KpnI and Sacl restriction sites. All oligonucleotides were designed to differ only in their decameric consensus sequence: 5'-GCT-decamer-CTG AGC TCC T-decamer-CTG AGC T-3'. The biestronic retroviral vector pLXIIH, which uses the 5'-long terminal repeat to drive expression of the transgene and the resistance gene was obtained by replacing the neomycin resistance gene of the pLXII vector (Clontech, Palo Alto, CA) with a hygromycin resistance gene cassette. \( p65 \) wt and mutants were cloned into pLXIIH by standard procedures. All constructs were verified by partial DNA sequencing using Dye Terminator chemistry. More detailed information on plasmids and cloning procedures can be obtained from the authors.

**Reporter Gene Assays**—Bovine aortic endothelial cells were transfected as described (20). RelA \( ^{-/-} \) MEFs were grown in 12-well plates and transfected at 70–80% confluency. Cells were exposed to 400 ng of DNA (200 ng of reporter plasmid, 40 ng of wt or mutants, 120 ng of pcDNA3, and 40 ng of cytomegalovirus- \( \beta \)-galactosidase control plasmid) and 1.6 \( \mu \)l of Lipofectamine (Invitrogen) in Dulbecco's modified Eagle's medium for 6 h. Following harvesting of fetal bovine serum to a final concentration of 10%, cells were allowed to recover for 40 h. Cells were lysed with 0.075\% Triton X-100 in 0.1M \( \text{KH}_2\text{PO}_4\), pH 7.8, and supernatants were assayed for luciferase and \( \beta \)-galactosidase activity as described (20).

**Stable Transfectants**—Stable RelA \( ^{-/-} \) MEFs expressing wt \( p65 \) or \( p65 \) mutants were generated by transfecting cells with pcDNA3 carrying wt \( p65 \) or mutants along with pcDNA3.Hygro (Invitrogen) in a 5:1 ratio. Single colonies were isolated by limiting dilution and selected in growth medium containing 300 \( \mu \)g/ml Hygromycin B. Cells clones were screened by Western blot analysis for \( p65 \) expression. At least two different clones for each experiment were used. Virus supernatants were obtained after transiently transfecting the EcoPack2-293 ectopic packaging cell line (Clontech) using Lipofectamine. Virus-containing supernatants were harvested at 48 and 72 h after transfection.

**Gene Expression Analysis**—Probes for Northern blot analysis were generated by polymerase chain reaction (PCR) from reverse transcribed RNA isolated from murine MEFs. Primers for generating a 700-bp fragment of the H2-K MHC class I gene product were 5'-CGC GAT CTT CAA ACC GAG C-3' and 5'-CTG TCA CCA AGT CCA CTC CAG-3', respectively. A 489-bp fragment of the \( \beta \)-actin gene was amplified using the primers 5'-ACC GTG AAA AGA TGA TCC ACC AGC TC-3' and 5'-Tag TTT CTA CCA TCG CAC AG-3'. Northern blots were performed as described (20). Semi-quantitative RT-PCR was performed on single-stranded cDNA generated by reverse transcription of oligo-dT primers (Invitrogen) using 1 \( \mu \)l of DNase I-treated total RNA as template. Reverse transcription was carried out with Superscript II reverse transcriptase and oligo-dT primers (Invitrogen) using 1 \( \mu \)l of template DNA (200 ng of reporter plasmid, 40 ng of wt or mutants, 120 ng of pcDNA3, and 40 ng of cytomegalovirus- \( \beta \)-galactosidase control plasmid) and 1.6 \( \mu \)l of Lipofectamine (Invitrogen) in Dulbecco's modified Eagle's medium for 6 h. After addition of fetal bovine serum to a final concentration of 10%, cells were allowed to recover for 40 h. Cells were lysed with 0.075\% Triton X-100 in 0.1M \( \text{KH}_2\text{PO}_4\), pH 7.8, and supernatants were assayed for luciferase and \( \beta \)-galactosidase activity as described (20).

**Identification and Characterization of p65 RHD Phospho-acceptor Sites**—We used reporter gene assays together with a site-directed mutagenesis approach to identify potentially phosphorylated serine residues within \( p65 \) RHD. Eighteen single Ser-to-Ala mutants and one double mutant (\( p65 \) S238/240A) were expressed in bovine aortic endothelial cells and monitored for transcriptional activity using a NF- \( \kappa B \)-dependent reporter construct that carries three NF- \( \kappa B \) binding sites derived from the porcine \( E \)-selectin promoter (20). This approach revealed seven putative serine phosphorylation sites in \( p65 \). Phosphorylated serine residues were identified by Southern blotting of EcoPack2-293 lysate or with 20 \( \mu \)l of protein A-Sepharose beads. Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were probed with c-myc or p50-specific antibodies as indicated.

**RESULTS**

Identification and Characterization of p65 RHD Phospho-acceptor Sites—We used reporter gene assays together with a site-directed mutagenesis approach to identify potentially phosphorylated serine residues within \( p65 \) RHD. Eighteen single Ser-to-Ala mutants and one double mutant (\( p65 \) S238/240A) were expressed in bovine aortic endothelial cells and monitored for transcriptional activity using a NF- \( \kappa B \)-dependent reporter construct that carries three NF- \( \kappa B \) binding sites derived from the porcine \( E \)-selectin promoter (20). This approach revealed seven putative serine phosphorylation sites in \( p65 \). Phosphorylated serine residues were identified by Southern blotting of EcoPack2-293 lysate or with 20 \( \mu \)l of protein A-Sepharose beads. Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were probed with c-myc or p50-specific antibodies as indicated.
pho-peptide species as compared with the wt protein. All mutants displayed residual phospho-peptides, indicating that p65 is multiply phosphorylated, as expected from previous results (19). It is noteworthy that the S205A mutant showed the largest decrease in phosphorylated peptide species. This finding could point to sequential phosphorylation, where a phospho-serine at position 205 would be required for other phosphorylation reactions to take place. Differences in intensities of separated phospho-peptides imply that not all serines are phosphorylated in the totality of cellular p65 proteins.

Differential p65 Phosphorylation Targets NF-κB Activity to Gene Subsets—We first analyzed expression of three genes in RelA−/− MEFs derived clonal cell lines reconstituted with wt p65 or p65 phosphorylation deficient mutants. We determined LPS-induced mRNA levels for ICAM-1, VCAM-1, and MHC class I genes by Northern blotting and semi-quantitative RT-PCR, respectively. Although MHC class I mRNA was increased by wt p65 as well as all p65 mutants (Fig. 2, a and b), ICAM-1 was detected only in wt p65-expressing cells, although we observed, in two of five independent experiments, ICAM-1 mRNA in cells expressing the p65 S205A mutant. VCAM-1 was efficiently induced by wt p65 and to a lesser extent by p65 S205A. Analysis was extended using qPCR to analyze mRNA levels for ICAM-1, VCAM-1, MHC class I, IL-6, MnSOD, and MIP-2 gene products. For this set of experiments,
retrovirally transduced cell pools rather than single-cell clones were used. Furthermore, cells were activated by combined LPS (1 μg/ml) and interferon-γ (100 units/ml) treatment. All genes tested were induced after LPS/interferon-γ exposure (Fig. 3). Induction in empty vector-transduced cells was limited (ICAM-1, 5-fold; VCAM-1, MHC class I, and MIP-2, 3-fold; IL-6, 70-fold; and MnSOD, 2-fold). In the resting state, wt p65-expressing cells showed higher mRNA levels of all genes analyzed than cells transduced with p65 serine mutants or empty vector. After LPS/interferon-γ stimulation, expression profiles for ICAM-1, VCAM-1, and MHC class I genes were comparable with the ones obtained with LPS stimulation alone (Fig. 2). IL-6 was expressed in a p65 phosphorylation-dependent manner showing highest induction in wt p65 and lowest in p65 S281A-expressing cells. S205A and S276A mutants induced MnSOD as efficiently as wt p65, whereas the S281A mutants showed reduced expression levels. Similar to ICAM-1, the mouse GROα analog MIP-2 was only efficiently induced by wt p65 and to a much lesser extent by the p65 S205A mutant.

The genomic organization of the respective enhancers is presented in Fig. 4. IL-6, MIP-2, and MnSOD intronic enhancer feature a single NF-κB consensus site, whereas ICAM-1, VCAM-1, and MHC class I H2-K enhancers carry two κB sites, one which is located far upstream (~1390) in the ICAM-1 promoter. Individual consensus sequences are highly conserved between mouse and human. In fact, only the κB consensus in the MnSOD enhancer is changed at position +3 (G for C) relative to the mouse sequence. Other cis-acting elements include the interferon responsive element in ICAM-1, VCAM-1, and MHC genes, Sp-1 consensus sites in ICAM-1, VCAM-1, and MIP-2 genes, CAAT/enhancer binding protein binding sites in ICAM-1, IL-6, and MnSOD, whereas IL-6 and MHC genes feature binding sites for members of the ATF/cyclic AMP-responsive element binding protein family of transcription factors.

Transcriptional Activity of Differentially Phosphorylated p65 Is cis-Acting Element Restricted—The most likely explanation for different p65 phosphorylation status-dependent gene transcription is that the sequence of a κB consensus site within a promoter will dictate the requirement for p65 phosphorylation. To test this hypothesis, we investigated transcriptional activity of differentially phosphorylated p65 proteins on an array of decameric κB consensus sequences. To evaluate different

![Graphical representation of gene expression](image-url)
NF-κB consensus sites, reporters were constructed in a way that only the decameric NF-κB consensus site was altered. When reporters containing two copies of a given κB consensus site were expressed alone or together with wt p65 or mutants, they could be divided into three groups according to their expression characteristics. Transcription from the first group (Fig. 5, a–e) of reporter constructs was only efficiently induced by wt p65. There was little or no induction when the reporters where co-transfected with p65 serine mutants. This group included highly asymmetric κB sites that fit a GGRWWWYYYY consensus. Similarly, reporter constructs of the second group (Fig. 5, f–j) gave a characteristic expression profile. They were activated strongest by p65 wt, followed by the serine 205, 276, and 281 mutants with decreasing activity and are represented by a KGRAHWTYCC consensus. The third group consisted of binding sites that harbor four guanine bases in the 5′-half of the consensus sequence or represented a complete palindrome (Fig. 5, k–m). These constructs were induced by wt p65 and p65 mutants to a similar extent, with p65 S276A mutant showing the highest induction and which fit a GGGRATTYCC consensus. There was no transcriptional activity from a reporter construct harboring scrambled decamers, underlining that transcriptional activation is indeed achieved through the respective κB binding sites and not through other sequences within the reporter construct (Fig. 5 h).

NF-κB Transcriptional Specificity

**FIG. 4.** Genomic organization of regulatory elements in enhancers of mouse genes under investigation. Sequences of corresponding κB consensus sites in human enhancers are shown in italic lettering. Positions are relative to translational (ICAM-1, VCAM-1, MHC class I H-2K) or transcriptional (IL-6, MnSOD, MIP-2) start site.
showed, with the exception of ICAM-1, an adherence to 5'-GGRA-3' at the 5'-end and to 5'-TYCC-3' at the 3'-end, while showing substantial heterogeneity at positions 1 and 1.

Group III sequences had a preference for four guanidines at the 5'-end (two of three) and featured a conservative TTYCC sequence in the 3'-half site. It is noteworthy that a single T-for-C substitution at the 3'-end can completely alter the transcriptional profile when engaged by differentially phosphorylated p65 proteins. Although transcription from the pig ELAM-1 xB site (GGGAATTCCT) was only driven by wt p65, all mutant proteins where equally capable of driving transcription from a palindromic GGGAATTCCT consensus sequence.

DNA Binding and Dimerization Behavior of Differentially Phosphorylated p65 Mutants—For DNA binding studies, we used one sequence of each group of xB binding sites showing characteristic expression profiles when positioned upstream of a reporter gene. Thus, we studied NF-xB binding to the IL-2 receptor-α xB (GGGAATTCCT, group I), Ig κ light chain xB (GGGAATTCCT, group II), and human ELAM-1 xB (GGGAATTCCT, group III) sites. Several DNA protein complexes with different electrophoretic mobility could be resolved (Fig. 6). The complex with the greatest mobility was composed of p50 homodimers as identified by specific removal of this complex by p50-specific antiserum (Fig. 7a). This complex was found in all p65 and empty vector-transfected MEF extracts. RelA<sup>−−</sup> MEFs...
transfected with wt p65 or p65 serine mutants but not empty vector-transfected cells showed two additional complexes that were identified as p50/p65 heterodimers and p65 homodimers by supershift analysis (Fig 7a). We could not detect any c-Rel-containing complexes. The relative abundance of p50 homodimers resulted most likely from the experimental design and limitations imposed by the transient transfection model, which results in 20–30% transfected cells as assayed by flow cytometry using transfected green fluorescent protein as a marker. Because p50 homodimers are a major NF-kB species in RelA−/− MEFS transiently transfected with empty vector (vector), wt p65 (wt), or p65 serine mutants are indicated. Nuclear extracts that were incubated with three double-stranded oligonucleotides coding for different NF-kB sites as indicated, +, a p65 homodimer complex that is a proteolytic product. Lower panel, Western blot analysis of nuclear extracts used for EMSA experiments using p65-specific antibody (p65 input).
fection with p50 (lane 1), wt p65 (lane 2), p65 S205A (lane 3), p65 S276A (lane 4), or p65 S281A (lane 5) and equal amounts of p50. Cell extracts were subjected to immunoprecipitation of p50/p65 complexes. Cells were transfected with empty vector (lane 1), wt p65 (lane 2), p65 S205A (lane 3), p65 S276A (lane 4), or p65 S281A (lane 5) and equal amounts of p50. Cell extracts were subjected to immunoprecipitation with p50 (b) or c-myc (c) specific antibodies. Proteins were detected by Western blotting with antibodies directed against the c-myc tag (b) or p50 (c).

The mechanism by which phosphorylation modulates p65 transcriptional activity in a cis-acting element-specific context remains elusive. Several models have to be considered. First, p65 phosphorylation could regulate its DNA-binding specificity. Although we did not see alterations in DNA binding that could explain the observed differences in transcriptional activity, there is still the possibility that in a cellular context there might be preferential recruitment of differentially phosphorylated p65 isoforms to a given promoter. Second, p65 phosphorylation could change the dimerization behavior of the protein. Therefore, phosphorylation might determine which other Rel protein is drawn into the dimeric NF-κB complex. Different NF-κB heterodimers have been shown to exhibit different transactivation potentials and favor different binding sites (29). However, we were unable to detect a phosphorylation-dependent change in NF-κB dimer composition. All DNA-binding complexes could be removed with p50- or p65-specific antibodies in supershift assays, whereas c-Rel-specific antibodies had no effect. Furthermore, p50/p65 dimerization was independent of p65 phosphorylation status. Third, association with compo-
nents of the transcriptional and/or chromatin modifying machinery could be regulated by phosphorylation. In this model, DNA binding and dimerization would not be altered, but interaction with co-activators, components of the polymerase II holoenzyme or histone-modifying proteins, could be modulated in a DNA-binding, site-specific context.

After submission of this report, Leung et al. (30) reported that a single nucleotide substitution within a βB consensus site can alter the requirement for co-activators necessary to initiate NF-κB-dependent transcription. It is speculated that a βK consensus sequence induces specific conformational changes in the βB dimer configuration and determines thereby which co-activator will interact with NF-κB. Our results are consistent with that model and extend it to include an additional regulatory mechanism at the level of p65 phosphorylation. Thus, interaction of NF-κB with certain co-activators might not only be dictated by DNA sequence but also by RHD phospho-serines. If βB dimers bound to different consensus sites require different co-activators to initiate transcription, we speculate that the groups of consensus sites defined in our experiments will require different co-activators. Although the co-activator recruited to NF-κB dimers bound to group I consensus sites can only be bound efficiently if p65 RHD is fully phosphorylated, a different co-activator that is recruited to dimers bound to group III consensus sites can bind to p65 in a phosphorylation-independent manner.

One additional possibility is that phosphorylation might change p65 acetylation status, as first hypothesized by Chen and Greene (31). Signal-induced acetylation has emerged as an important mechanism to regulate p65 subcellular localization, interaction with inhibitor βB, DNA binding, and transcriptional activity (32, 33). It is thus possible that site-specific phosphorylation dictates the acetylation pattern of the protein and thereby regulates its function.

In summary, we propose a p65 “phosphorylation code” that targets NF-κB activity to distinct subsets of genes. Cells and organs are continuously exposed to a vast variety of extracellular signals, which have to be integrated to achieve specific transcriptional programs. In this study we show, with the example of NF-κB, that transcriptional selectivity can be achieved not only by activating different signaling cascades leading to the engagement of distinct transcriptional activators or repressors but also at the level of the transcription factor itself, which is targeted to specific gene subsets by altering its phosphorylation status. Future studies will show whether this regulatory system applies to other transcription factors known to be regulated by phosphorylation.

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REFERENCES
1. Rao, A., Luo, C., and Hogan, P. G. (1997) Annu. Rev. Immunol. 15, 707–747
2. Horvath, C. M. (2000) Trends Biochem. Sci. 25, 496–502
3. Chrivia, J. C., Kook, R. P., Lamb, N., Hagioara, M., Montminy, M. R., and Goodman, R. H. (1993) Nature 365, 855–859
4. Leung, M. F., Frick, M., and Greene, W. C. (1997) J. Biol. Chem. 272, 32606–32612
5. Wang, D., Westerheide, S. D., Hanson, J. L., and Baldwin, A. S. (2000) J. Biol. Chem. 275, 32592–32597
6. Das, A. K., Zlotogora, J., and Kline, R. J. (1999) Nat. Rev. Mol. Cell. Biol. 1, 1313–1324
7. Liu, W., and Kline, R. J. (2000) Trends Biochem. Sci. 25, 7780–7793
8. Li, J. J., and Kline, R. J. (2001) J. Biol. Chem. 276, 21128–21133
9. Anrather, J., Csizmadia, V., Soares, M. P., and Winkler, H. (1999) J. Biol. Chem. 274, 13594–13603
10. Brostjan, C., Anrather, J., Csizmadia, V., Natarajan, G., and Winkler, H. (1997) J. Immunol. 158, 3836–3844
11. Leung, M. F., Frick, M., and Greene, W. C. (1997) J. Biol. Chem. 272, 16767–16770
12. Leung, M. F., Frick, M., and Greene, W. C. (1997) EMBO J. 16, 4597–4607
13. Chen-Park, F. E., Huang, D. B., Noro, B., Thanos, D., and Ghosh, G. (2002) Nat. Cell Biol. 4, 778–7793
14. Sheng, P., Voss, R. E., and Ghosh, S. (1998) Mol. Cell 1, 661–671
15. Naumann, M., and Scheidereit, C. (1994) EMBO J. 13, 4597–4607
16. Chen, L. F., and Greene, W. C. (2004) EMBO J. 23, 561–568
17. Shimizu, H., Mitomo, K., Watanabe, T., Okamoto, S., and Yamamoto, K. (1990) Science 252, 32606–32612
18. Ballard, D. W., Bohnlein, E., Lowenthal, J. W., Wano, Y., Franza, B. R., and Benhar, R. (1989) Trends Biochem. Sci. 14, 211–218
19. Horvath, C. M. (2000) Annu. Rev. Immunol. 18, 683–703
20. Bird, T. A., Schooley, K., Dower, S. K., Hagen, H., and Virca, G. D. (1997) J. Biol. Chem. 272, 32606–32612
21. Wang, D., Westerheide, S. D., Hanson, J. L., and Baldwin, A. S. (2000) J. Biol. Chem. 275, 32592–32597
22. Livak, K. J., and Schmittgen, T. D. (2001) Methods 25, 402–408
23. Hou, S., Guan, H., and Ricciardi, P. J. (2003) J. Biol. Chem. 278, 45994–45998
24. Taylor, A. L., Rasmussen, H., and Malik, A. B. (2000) Am. J. Physiol. 279, L302–L311
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