Protein Kinase Cθ Modulates Nuclear Receptor-Corepressor Interaction during T Cell Activation*

Mohammad Ishaq‡, Gerald DeGray, and Ven Natarajan

From the Laboratory of Molecular Cell Biology, Science Applications International Corp., NCI-Frederick, Maryland 21702

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Protein Kinase Cθ modulates nuclear receptor-corepressor interaction during T cell activation. Transcriptional repression by nuclear receptor corepressors plays a critical role in T cell development. However, the role of these corepressors in T cell activation is poorly understood. We report that T cell activation silenced transcription driven by nuclear receptors retinoic acid receptor, retinoid X receptor, and thyroid hormone receptor and induced silencing mediator of retinoic acid and thyroid hormone receptors (SMRT)-receptor interaction. Whereas the expression of a dominant active mutant of protein kinase Cθ (PKCθ) induced strong SMRT-receptor interaction in the absence of T cell activation, a dominant negative mutant of PKCθ decreased the interaction. Loss of PKCθ expression by induction of “RNA interference” resulted in the attenuation of basal and activation-induced SMRT-receptor interaction. We suggest that T cell activation silences nuclear receptor-dependent transactivation in part through PKCθ-dependent enhancement of SMRT-receptor interaction.

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‡ To whom correspondence should be addressed: Laboratory of Molecular Cell Biology, Science Applications International Corp., NCI-Frederick, MD 21702. Tel.: 301-846-1500; Fax: 301-846-6762; E-mail: mishaq@nih.gov.

The abbreviations used are: PKC, protein kinase C; BisTris, 2-[bis(2-hydroxyethyl)aminato]-2-(hydroxyethyl)propane-1,3-1,3-diol; RAR, retinoic acid receptor; RARE, RAR element; RXR, retinoid X receptor; RXRE, RXR element; TR, thyroid hormone receptor; TRE, TR element; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; VDR, vitamin D receptor; IL-2, interleukin-2; N-CoR, nuclear hormone receptor corepressor; ATRA, all-trans-retinoic acid; HA, hemagglutinin; DN, dominant negative; DA, dominant active; RNAi, RNA interference; siRNA, short interfering RNA; WT, wild type; Tc, triiodothyronine; Cis-RAR, 9-cis-retinoic acid; MES, 4-morpholineethanesulfonic acid; RIF, receptor interaction domain; JNK, c-Jun NH₂-terminal kinase.

Experimental Procedures

Cells and Treatments—T lymphocyte leukemia Jurkat cell line (clone E6-1) was obtained from American Type Culture Collection (Manassas, VA). Jurkat cells were maintained in RPMI 1640 medium (BioWhittaker, Frederick, MD) supplemented with 10 mM HEPES buffer, 2 mM l-glutamine, 60 μg/ml gentamicin, 10% fetal bovine serum (Hyclone, U.S.A.).
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Logan, UT). CV-1 cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 9-trans-retinoic acid (9-CRA), all-trans-retinoic acid (ATRA), and triiodo-l-thyronine (T_3) were from Sigma and were used at 1 μM. Vitamin D_3 (Sigma) was used at 100 nM. Rottlerin and Go 6976 (Calbiochem) were used at 15 μM and 0.5 μM/ml, respectively. Anti-CD3 antibodies (BD Biosciences) and anti-CD28 (Sigma) were used at 2 and 1 μg/ml, respectively. Antibodies to SMRT, PKCö, and HA tag were obtained from BD Biosciences.

Western Blot—Protein extracts were electrophoresed in a 10% NuPAGE BisTris gel using NuPAGE MES-SDS running buffer (Invitrogen) and transferred to a polyvinylidene difluoride membrane using XCell Blot Module (Invitrogen). The membrane was blocked with 5% milk overnight at 4°C and incubated with the appropriate antibody diluted in 3% milk. The protein was detected using the ECL Western blotting detection system from Amersham Biosciences.

Plasmids and Transfections—RARE-, RXRE-, TRE-, and VDR element (VDRE)-driven transcriptional activations were studied by transfection using luciferase-based reporter plasmids TKRARE-Luc, TKCRBP-II-Luc, TKD4-Luc, and TKD3-Luc, respectively. Dr. K. Ozato, National Institutes of Health, Bethesda, provided TKCRBP-II-Luc and TKRARE-Luc plasmids. TKD3-Luc and TKD4-Luc were constructed by cloning AAGGTGAGA GGTAGTTCA (sequence from mouse osteopontin promoter) and AGGACTCAAGAAGGGACA sequence, respectively, in KpnI and BglII sites of TK-Luc plasmid. TKCRBP-II-Luc plasmid containing RXRE mutant sequence was constructed similarly to serve as negative control. Plasmids expressing human RARα, RIXα, TRα, and VDR were provided by Dr. P. Chambon (Institut de Genetique et de Biologie Moleculaire et Cellulaire, France), Dr. M. R. Evans (The Salk Institute of Biological Sciences, La Jolla, CA), Dr. M. A. Mahajan (New York University School of Medicine), and Dr. H. Young (National Cancer Institute, Frederick, MD), respectively. NFAT-Luc plasmid containing trimierized human distal IL-2 NFAT site inserted into IL-2 minimal promoter was a gift from Dr. G. R. Crabtree (Stanford University, Stanford, CA).

Mammalian two-hybrid assays were carried out using psV40-Luc reporter plasmid containing SV40 promoter linked to five yeast GAL17′-mer DNA-binding sites. PSG1-GAL4A fusion containing ligand-binding domains of RXRα, RARα, TRα, VDR, and GAL4-DBD fusions of different SMRT regions and GAL4-17′-mer-Luc plasmids were provided by Dr. M. Privalsky (University of California, Davis). Empty vector pCEFPL and HA-tagged wild type PKCö expressing plasmid, pCEFPL-PKCö, were provided by Dr. S. Shaw (National Cancer Institute, Bethesda). Mutations to generate dominant active (DA) (A148E) and dominant negative (DN) (K409R) PKCö-expressing plasmids were performed using QuickChange site-directed mutagenesis kit from Stratagene (La Jolla, CA). DA-PKCa (A25E) expressing plasmid and pEF4 HisA empty vector were provided by A. Altman (La Jolla Institute for Allergy and Immunology, San Diego). Jurkat cells (10⁷) were transfected by electroporation using a Gene Pulser II (Bio-Rad) at 0.250 kV and 975 microfarads as described (31, 32). CV-1 cells were transfected using CalPhos Mammalian Transfection Kit from Clontech (Palo Alto, CA). After transfection cells were treated with the indicated reagents and time periods prior to harvest and determination of luciferase activity using the Luciferase Assay System (Promega, WI). Transfection efficiency was normalized to protein concentrations in the extracts. The values in the figures represent the mean of three independent experiments with standard error calculated for each value.

RESULTS

T Cell Activation by CD3 and CD28 Ligation Induces Loss of RARE-, RXRE-, and TRE-dependent Transactivation—We have reported previously that CD3 cross-linking of Jurkat cells induced loss of RXRE-dependent transcription (31). In this report we extended this work to study the effect of activation by CD3 and costimulatory molecule CD28 on RARE-, RXRE-, and VDRE-mediated transactivation. Jurkat cells were transfected with TKRARE-Luc, TKCRBP-II-Luc, TKD4-Luc, or TKD3-Luc either in the presence or absence of receptor expressing plasmids and the cognate ligands. Twelve hours later the cells were treated as indicated for 24 h and harvested, and luciferase activity was measured as described under “Experimental Procedures.” Jurkat cells were transfected with 2.5 μg of NFAT-Luc plasmid. Twelve hours later the cells were treated as indicated for 24 h and harvested, and luciferase activity was measured.

CD3 and costimulatory molecule CD28 on RARE-, RXRE-, TRE-, and VDRE-mediated transactivation. Jurkat cells were transfected with TKRARE-Luc, TKCRBP-II-Luc, TKD4-Luc, or TKD3-Luc in either the presence or absence of receptor expressing plasmids and the cognate ligands. Twelve hours later the cells were treated with antibodies to CD3 and CD28 for 24 h. Fig. 1A shows that CD3/CD28 ligation resulted in a significant inhibition in transcriptional activation by RAR, RXR, and TR receptors. The inhibition was observed with endogenous as well as exogenously expressed receptors and was independent of the presence or absence of the ligand. Under similar conditions, VDR-mediated activation was only weakly inhibited. Little or no luciferase activity was observed when cells were transfected with TKCRBP-II-M-Luc, a plasmid containing mutations in RXRE site, indicating the specificity of receptor-dependent activation assay. To demonstrate that this transcriptional loss was not a generalized phenomenon, a non-nuclear hormone receptor-based reporter assay was tested under similar conditions. Jurkat cells were transfected with NFAT-Luc plasmid for 12 h and then treated with antibodies to

FIG. 1. (A) CD3 and CD28 ligation inhibits RARE-, RXRE-, and TRE-dependent transactivation in Jurkat cells. A, Jurkat cells were transfected with 5.0 μg of TKCRBP-II-Luc, TKRARE-Luc, TKDR4-Luc, or TKD3-Luc in either the presence or absence of receptor expressing plasmids and the cognate ligands. Twelve hours later the cells were treated as indicated for 24 h and harvested, and luciferase activity was measured as described under “Experimental Procedures.” B, Jurkat cells were transfected with 2.5 μg of NFAT-Luc plasmid. Twelve hours later the cells were treated as indicated for 24 h and harvested, and luciferase activity was measured.
CD3 and CD28 for 24 h. Fig. 1B shows that CD3/CD28 engagement induced a strong induction of NFAT-dependent transcription.

Enhancement of SMRT-Receptor Interaction Upon CD3/CD28 Engagement—In order to understand the mechanism of transcriptional loss induced by CD3/CD28 ligation, we explored the possibility that T cell activation may enhance the level of interaction between the receptors and the corepressor SMRT, thereby explaining the loss of transcriptional activation. Jurkat cells express readily detectable levels of SMRT protein that do not change significantly after ligation by CD3/CD28 cross-linking (Fig. 2A). To study the change in the functional interaction between the SMRT and the receptors during T cell ligation, we used a mammalian two-hybrid system to quantify the levels of interaction in Jurkat cells. A luciferase reporter construct containing yeast GAL4 (17-mer) DNA binding sequence, a mammalian expression vector pSG5 containing porter construct containing yeast GAL4 (17-mer) DNA binding domain, and a plasmid bearing specific regions of human SMRT (751–1495 or 1291–1495 amino acids corresponding to receptor interaction domain (RID) 1 or RID 2, respectively) fused to the GAL4 DNA binding domain (GAL4-DBD-SMRT) in pSG5 (33) were transfected in Jurkat cells. After 12 h of incubation, the cells were treated with antibodies to CD3 and CD28 for 24 h. RARα and TRα showed significant basal level interaction with GAL4-DBD-SMRT construct containing SMRT amino acids from 751 to 1495 (Fig. 2B), whereas a lower interaction was observed with RXRa (Fig. 2C). ATRA and T3, ligands specific for RAR and TRα, respectively, inhibited the interaction. Interaction between RXRa and SMRT was not affected by RXR-specific ligand, 9-CRA, a finding also reported earlier in a non-T cell line (33). A very weak or no interaction was observed with VDR and SMRT construct containing 751–1495 amino acids and served as a negative control (Fig. 2C). SMRT construct containing amino acids from 1291 to 1495 (RID 2) showed very little interaction with RARα, TRα, or VDR, but a slightly higher interaction with RXRa (data not shown).

Interestingly, CD3/CD28 ligation strongly increased the basal level interaction between the SMRT and the ligand binding domains of RARα and TRα (Fig. 2B). There was a relatively lower increase in the interaction between SMRT and ligand binding domain of RXRa in cells treated with anti-CD3 and anti-CD28 antibodies (Fig. 2C). As with the basal interaction, the CD3/CD28-induced interaction of SMRT with RAR and TRα was attenuated by their cognate ligands, whereas the CD3/CD28-induced interaction between RXRa and SMRT was not affected by the RXR-specific ligand 9-CRA. There was no effect of CD3/CD28 ligation on the weak interaction seen between VDR and SMRT (Fig. 2C). Together, these data indicate that T cell engagement via CD3 and CD28 receptors induces a significant interaction between SMRT and the ligand binding domains of RARα, RXRa, and TRα.

T Cell Ligation-induced SMRT-Receptor Interaction Is Dependent on the Activation of PKCθ—In an effort to investigate the signal transduction pathways involved in the activation-induced enhancement of SMRT-receptor interaction, we studied the role of novel PKCθ in the process. Jurkat cells were transfected with GAL4–17-mer-Luc and pSG5-GAL4-AD-RARα, pSG5-GAL4-AD-RXRα or pSG5-GAL4-AD-TRα, and GAL4-DBD-SMRT-(751–1495) in the presence or absence of WT-PKCθ, DA-PKCθ, DN-PKCθ, or empty vector. Expression of DA-PKCθ induced a strong interaction between SMRT and ligand binding domains of all three receptors in the absence of CD3/CD28 ligation (Fig. 3A). Expression of DN-PKCθ decreased the interaction, whereas the transfection with WT-PKCθ or empty vector did not have any effect on the SMRT-receptor interaction. Expression of PKCθ protein from the PKCθ constructs was confirmed by Western blot analysis of
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PKC\(\theta\) Modulates Nuclear Receptor-Corepressor Interaction in a T Cell-dependent Manner—PKC\(\theta\) is not expressed ubiquitously and is found predominantly in hematopoietic cells and muscle (37). We next investigated whether PKC\(\theta\)-dependent induction of SMRT-receptor interaction was a feature specific to T cells or could also be observed in PKC\(\theta\) non-expressing cells. CV-1, a kidney fibroblast cell line which is not known to express PKC\(\theta\), was transfected with GAL4–17-mer-Luc, 2 \(\mu\)g of PSG5-GAL4AD fusion constructs containing ligand-binding domains of RAR\(\alpha\), RXR\(\alpha\), or TR\(\alpha\), and 2 \(\mu\)g of GAL4-DBD-SMRT-(751–1495) either in the presence or absence of empty vector or different PKC\(\theta\) constructs. Cells were harvested 36 h later, and luciferase activity was measured as described under "Experimental Procedures."

PKC\(\theta\) Induces SMRT-Receptor Interaction in a T Cell-dependent Manner—PKC\(\theta\) is not expressed ubiquitously and is found predominantly in hematopoietic cells and muscle (37). We next investigated whether PKC\(\theta\)-dependent induction of SMRT-receptor interaction was a feature specific to T cells or could also be observed in PKC\(\theta\) non-expressing cells. CV-1, a kidney fibroblast cell line which is not known to express PKC\(\theta\), was transfected with GAL4–17-mer-Luc, 2 \(\mu\)g of GAL4-DBD-SMRT-(751–1495), and pSG5GAL4-AD-RAR\(\alpha\), pSG5GAL4-AD-RXR\(\alpha\), or pSG5GAL4-AD-T\(\alpha\)R in the presence or absence of empty vector or different PKC\(\theta\) constructs. Cells were harvested 36 h later, and luciferase activity was measured as described under "Experimental Procedures."
show any increase in the presence of WT- or DA-PKCθ (Fig. 5). CV-1 cells also exhibited significant interaction between SMRT and ligand binding domains of RXRα, RXRα, or TRα that did not increase in the presence of WT- or DA-PKCθ. As expected from an earlier report (33), the interaction of SMRT with RAR and RXRα, or TRα, respectively, whereas SMRT interaction with RXRα did not decrease in the presence of 9-CRA (data not shown). Together, these data provide evidence that PKCθ, which is uniquely expressed in T cells, induces SMRT-receptor interaction involving factors that are T cell-specific.

**Partial Loss of PKCθ Expression by PKCθ-specific RNAi Attenuates Basal and T Cell Activation-mediated SMRT-Receptor Interaction**—siRNA duplexes have been shown to silence gene expression in a sequence-specific manner by a process called RNA interference (RNAi) (38–44). We tested three double-stranded siRNAs, directed at nucleotides 477–497, 802–822, and 1954–1974 of human PKCθ mRNA, for silencing PKCθ expression. Transfection of Jurkat cells with these siRNAs showed varying degrees of inhibition of PKCθ expression within 24–36 h of transfection. After 36 h, the levels of PKCθ slowly regained and reached normal levels within 72 h after transfection (data not shown). Of the three, siRNA directed against nucleotides 802–822 was the most effective in inhibiting PKCθ expression (50–70% inhibition, Fig. 6A) and was used in further studies. Transfection of a nonspecific scrambled RNA duplex did not significantly alter the levels of PKCθ.

We next studied the effect of siRNA-mediated silencing of PKCθ expression on basal and CD3/CD28-induced SMRT-receptor interaction. Jurkat cells were transfected with GAL4–17-mer-Luc, GAL4–DBD-SMRT-(751–1495), and pSG5GAL4-AD-RARα, pSG5GAL4-AD-RXRα, or pSG5GAL4-AD-TRα in the presence or absence of 5 μM siRNA corresponding to nucleotides 802–822 of PKCθ or a nonspecific scrambled sequence. After 12 h, the cells were treated with anti-CD3 and CD28 antibodies and incubated for an additional 24 h. Fig. 6B demonstrates that expression of siRNA specific for PKCθ attenuated both basal as well as CD3/CD28-induced interaction between SMRT and all three receptors. Expression of a nonspecific scrambled RNA duplex did not significantly alter the SMRT-receptor interaction. Together these results indicate that PKCθ expression is essential for the basal as well as T cell activation-induced SMRT-receptor interaction.

**DISCUSSION**

Nuclear hormone receptors RAR, RXR, and TR play an important role in the development and homeostasis both by ligand-dependent activation and active repression of target genes. SMRT and a closely related corepressor N-CoR are involved in repression by binding to the receptors and forming complexes with various members of histone deacetylases, a key event in the deacetylation of histones and subsequent transcriptional repression (25). The role of nuclear receptor-driven active re-
pression in T cell function is not known. A recent report (30) that T lymphocytes in N-CoR−/− mouse embryos are arrested at the double-negative stage emphasizes the importance of corepressors and active repression in T cell development.

In this paper we have provided evidence that T cell cross-linking by CD3 and CD28 surface molecules induces loss of RAR-, RXR-, and TR-driven transcriptional activation, indicating that T cell activation signals mediate active repression by these nuclear receptors. In an attempt to investigate the mechanism that would explain this inhibition, we have identified the role of SMRT in the activation-induced transcriptional repression and demonstrated that CD3/CD28 ligation induced SMRT interaction with all the three (RAR, RXR, and TR) receptors. CD3/CD28 ligation of Jurkat cells did not increase the levels of SMRT protein, excluding the possibility that an induced expression of SMRT may account for an enhanced SMRT-receptor interaction. However, this raised the possibility that T cell activation signals induce interaction indirectly by post-translational modifications of either the components of the repressional machinery or the factors that regulate their activity.

Phosphorylation and dephosphorylation are key post-translational events that are known to regulate the functional outcome of T cell activation and IL-2 production. Recently, PKCθ, due its highly T lymphocyte-specific expression, has emerged as one of the foremost protein kinases in the study of T cell function. We have reported previously (32) that PKCθ enhances calcineurin-dependent transactivation of RXR-dependent promoters suggesting the role of this PKC isoform in nuclear hormone receptor-dependent signal transduction. By utilizing a number of different experimental approaches, we have identified PKCθ as a key regulator of SMRT-receptor interaction during T cell activation. One of these was a "reverse genetics" approach in which PKCθ expression was silenced in Jurkat cells by inducing a novel PKCθ-specific RNAi pathway. RNAi, a recently described phenomenon, is emerging as a powerful tool to study the function of genes. Together, these observations support a model in which CD3/CD28 ligation, or signals that activate PKCθ, induce a strong interaction between SMRT and nuclear receptors, a phenomenon that may in part be responsible for the observed transcriptional repression following T cell activation. Although we have not studied the effect of T cell ligation on N-CoR-nuclear receptor interactions, a similar increase may be speculated because of a close resemblance between SMRT and N-CoR proteins in structure and function.

The mechanism that explains CD3/CD28-induced and PKCθ-dependent SMRT-receptor interaction remains unknown. Direct phosphorylation of SMRT or the receptor protein by PKCθ, if it occurs, may stabilize interactions between the interacting partners. Alternatively, an unknown auxiliary protein may act as a substrate for PKCθ and directly or indirectly activate or stabilize the SMRT-receptor interaction. In an earlier study (31), we reported that activation of the JNK pathway resulted in the attenuation of RXRE-driven transcription in Jurkat cells. T cell activation by CD3/CD28 cross-linking or expression of DA-PKCh is known to activate JNK pathway in T cells. Is the induction of PKCθ-mediated SMRT-receptor interaction a direct consequence of PKCθ-dependent JNK activation? Earlier data have shown that activation of JNK pathways inhibits SMRT-receptor interaction in CV-1 cells (45). Expression of DA-PKCh or CD3/CD28 ligation in T cells may induce SMRT-receptor interaction involving alternate pathways. Whatever is the mechanism of PKCθ-mediated induction of SMRT function following T cells activation, it is noteworthy that this PKC isoform uniquely functions in T cells in modulating SMRT function. The failure of DA-PKCh to induce SMRT-nuclear receptor interaction in CV-1 cells emphasizes this specificity and also indicates that PKCθ may not function alone in T cells but needs active participation of other unknown T cell-specific effectors. A robust basal level SMRT-receptor interaction observed in CV-1 cells that does not respond to PKCθ also indicates that PKCθ-independent regulation of receptor-corepressor interaction is operative in cells that may not express PKCθ.

Our data suggest that T cell activation-induced inhibition of RAR, RXR, and TR-driven transactivation may involve the participation of active repressional machinery in which SMRT-receptor interaction is strongly enhanced by PKCθ-dependent signals. In such a scenario, an increased interaction between receptors and the SMRT may stabilize the components of repressional apparatus on the receptor-bound promoters and induce repression. SMRT and N-CoR, although initially known to mediate repression by RAR, RXR, and TR, have also been found to inhibit transcription driven by a number of other unrelated transcription factors (25). Of particular interest is SMRT-mediated suppression of AP-1 and NFκB (46), factors known to be involved in the activation of the IL-2 promoter, and interaction with BCL-6, a mediator of apoptosis in T cells (47). Whether PKCθ plays a role in modulating SMRT interaction with these factors is not known but remains a possibility. It is clear that active repression mediated by corepressors like SMRT and N-CoR may have important physiological consequences not only in the previously described T cell development (30), but also in antigen-driven T cell activation function. Characterization of corepressor function in determining T cell responses to various signals is an area that remains open for investigation.

To summarize, the studies reported in this paper represent an attempt to bridge the gap between our understanding of the phenomenon of nuclear receptor-mediated active transcriptional repression and its role in T cell activation function. We have shown a direct link between the two processes and also identified PKCθ, a T cell-specific PKC isoform, as a key modulator of nuclear receptor-mediated active repression. The data presented should help to unravel the role of active repressional machinery in T cell activation function.

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REFERENCES

1. Lin, J., and Weiss, A. (2001) J. Cell Sci. 114, 243–244
2. Kane, L. P., Lin, J., and Weiss, A. (2000) Curr. Opin. Immunol. 12, 242–249
3. Crabtree, G. R. (1999) Cell 96, 611–614
4. Kuo, C. T., and Leiden, J. M. (1999) Annu. Rev. Immunol. 17, 149–189
5. Clements, J. L., Boerth, N. J., Lee, J. R., and Karetzky, G. A. (1999) Annu. Rev. Immunol. 17, 89–108
6. Russell, J. H. (1995) Curr. Opin. Immunol. 7, 382–387
7. Arendt, C. W., Albrecht, B., Soos, T. J., and Littman, D. R. (2002) Curr. Opin. Immunol. 14, 223–230
8. Isakov, N., and Altman, A. (2002) Annu. Rev. Immunol. 20, 761–784
9. Bi, K., Tanaka, Y., Coudercniere, N., Sugie, K., Hong, S., von Stipdonk, M. J., and Altman, A. (2001) Mol. Immunol. 38, 556–560
10. Altman, A., Isakov, N., and Goffeart, B. (2000) Immuno. Today 21, 567–573
11. Sun, Z., Arendt, C. W., Elmeier, W., Smacher, E. M., Sunshine, M. J., Gandhi, L., Annes, J., Petzikla, K., Kupfer, A., Schwartzberg, P. L., and Littman, D. R. (2000) Nature 404, 402–407
12. Bader-Bittlicher, G., Uherl, F., Bauer, F., Fresser, F., Wachter, H., Grunick, H., Utermann, G., Altman A., and Bader, G. (1996) Mol. Cell. Biol. 16, 1842–1850
13. Lin, X., O’Mahony, A., Mu, Y., Gelezianas, R., and Greene, W. C. (2000) Mol. Cell. Biol. 20, 2933–2940
14. Coudercniere, N., Villalba, M., Englund, N., and Altman, A. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 3394–3399
15. Ghaffari-Tabriri, N., Bauer, B., Villunger, A., Bauer-Bittlicher, G., Altman A., Utermann, G., Uherl, F., and Bader, G. (1999) Eur. J. Immunol. 29, 132–142
16. Werlen, G., Jacinto, E., Xia, Y., and Karin, M. (1998) EMBO J. 17, 3101–3111
17. Villalba, M., Kasabhatla, S., Genestier, L., Mahboubi, A., Green, D. R., and Altman, A. (1999) J. Immunol. 163, 5813–5819
18. Sasahara, Y., Rachid, R., Byrne, M. J., de Fuente, M. A., Abraham R. T., Ramesh, N., and Geha, R. S. (2002) Mol. Cell 10, 1269–1281
