Prostaglandin E$_2$ Activates HPK1 Kinase Activity via a PKA-dependent Pathway*3

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Hematopoietic progenitor kinase 1 (HPK1)2 is a hematopoietic cell-restricted member of the Ste20 serine/threonine kinase super family. We recently reported that the immunosuppressive eicosanoid, prostaglandin E$_2$ (PGE$_2$), is capable of activating HPK1 in T cells. In this report, we demonstrate that unlike the TCR-induced activation of HPK1 kinase activity, the induction of HPK1 catalytic activity by PGE$_2$ does not require the presence of phosphorytosine-based signaling molecules such as Lck, ZAP-70, SLP-76, and Lat. Nor does the PGE$_2$-induced HPK1 activation require the intermolecular interaction between its proline-rich regions and the SH3 domain-containing adaptor proteins, as required by the signaling from the TCR to HPK1. Instead, our study reveals that PGE$_2$ signal to HPK1 via a 3′-5′-cyclic adenosine monophosphate-regulated, PKA-dependent pathway. Consistent with this observation, changing the serine 171 residue that forms the optimal PKA phosphorylation site within the "activation loop" of HPK1 to alanine completely prevents this mutant from responding to PGE$_2$-generated stimulation signals. Moreover, the inability of HPK1 to respond to PGE$_2$ stimulation in PKA-deficient S49 cells further supports the importance of PKA in this signaling pathway. We speculate that this unique signaling pathway enables PGE$_2$ signals to engage a proven negative regulator of TCR signal transduction pathway and uses it to inhibit T cell activation.

Hematopoietic progenitor kinase 1 (HPK1)2 is a member of the GCK sub-family of the Ste20 kinases (1). HPK1 is expressed ubiquitously in all embryonic tissues examined, but this expression profile shifts to a hematopoietic cell-restricted pattern postpartum at neonatal day 1, leading to speculation that it may perform a specialized function in hematopoietic cells (2). The role of HPK1 in biological processes is characterized best in T cells, where HPK1 has emerged as an important negative regulator of T cell antigen receptor (TCR)-induced interleukin-2 gene transcription (3, 4). In addition to controlling interleukin-2 gene transcription, overexpression studies indicate that HPK1 also plays a role in activation-induced cell death upon TCR engagement (5, 6). Although some of these overexpression studies produced conflicting data as to the role that HPK1 plays in these T cell functions (4, 7–9), a recent study reveals that T cells from HPK1$^{−/−}$ mice proliferate more robustly in response to TCR engagement (10). HPK1$^{−/−}$ mice also exhibited a more severe autoimmune phenotype in the experimental model of autoimmune encephalomyelitis. These findings firmly validate the earlier biochemical findings that HPK1 functions as a negative regulator of T cell activation.

The catalytic activity of HPK1 is elevated upon ligand engagement of a variety of cell surface receptors present on hematopoietic cells. In addition to TCR and B cell antigen receptor engagement (3, 9, 11, 12), ligand binding to transforming growth factor-β receptor (TGF-βR) (13, 14), the erythropoietin receptor (15), Fas (16), and E prostanoid receptors (17) can also induce HPK1 kinase activity. With the exception of TCR-mediated signal transduction where some mechanisms controlling HPK1 activation have been delineated, the exact biochemical mechanisms utilized by these receptors to activate HPK1 remain poorly understood. However, the signaling mechanisms utilized by these receptors can be grouped into three general categories: 1) Activation that depends on protein tyrosine kinases (PTKs), their substrates, and the Src homology (SH) domain-containing adapter proteins that couple these molecules to HPK1 (3, 18, 19); 2) Activation that relies on caspase activation and the subsequent cleavage of HPK1; 3) Activation that is presumed to utilize protein serine/threonine kinases (PS/TK) to conduct signals to HPK1 (13, 14, 17). Of these three categories, the PS/TK-dependent mechanism is the only mode of activation that has not been well characterized.

We recently reported that exposing T lineage or myeloid lineage cell lines to physiological concentrations of prostaglandin E$_2$ (PGE$_2$) would induce robust HPK1 kinase activity (17). Because the mechanisms that control PGE$_2$-induced activation have not been delineated, we decided to characterize this signaling pathway by comparing it with the signaling mechanisms used by well characterized mechanism downstream of the TCR.

EXPERIMENTAL PROCEDURES

Cell Lines—JE6.1 Jurkat and its mutants were grown in RPMI 1640 complete medium (RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM l-glutamine, and 100 units of penicil-
lin/streptomycin). Lck-deficient JCaM 1.6 and the ZAP-70-deficient p116 Jurkat were obtained from ATCC (Mannasus, VA). The Lat-deficient ANJ Jurkat was a kind gift from Dr. Samelson (National Cancer Institute, National Institutes of Health), and the SLP-76-deficient J14 Jurkat was a kind gift from Dr. Koretzky (University of Philadelphia, PA). Wild type S49 pre T cells and the PKA-deficient mutant, kin− S49, were kind gifts from Dr. Insel (University of California, San Diego, CA).

Antibodies and Other Reagents—The horseradish peroxidase-coupled anti-phosphotyrosine antibody (RC20H) was purchased from Transduction Laboratories (Lexington, KY). The anti-human HPK1 rabbit polyclonal antibody 47 (17) was used to immunoprecipitate and immunoblot human HPK1. The anti-murine HPK1 antisera 5, 6, and 7 were generous gifts from Dr. Kiefer (Max-Planck Institute for Molecular Biomedicine, Münster, Germany). Immunoprecipitation and Western blotting of the ectopically expressed, hemagglutinin (HA)-tagged murine HPK1 were performed with the 12CA5 anti-HA mAb and anti-murine HPK1 rabbit polyclonal antibody 7 (2), respectively. Both the 12CA5 and the anti-human CD3ε (OKT3.14) mAbs used in TCR cross-linking experiments were purified from hybridoma supernatants in our laboratory. γ-[32P]ATP was obtained from PerkinElmer Life Sciences (Boston, MA). Anti-phospho-PKD (ser-916), clone MC29, and anti-PKD polyclonal antibody followed by horseradish peroxidase-coupled antimouse antibody use Lck to transduce signals via PTK-dependent pathways leading to HPK1 activation are the best understood among all known receptors capable of activating HPK1. Studies using mutant cell lines lacking Lck and ZAP-70 revealed that the presence of these PTKs is required for HPK1 activation by the TCR (3). Because some rhodopsin-like G protein-coupled receptor use Lck to transduce signals via PTK-dependent pathways (21), we determined whether PGE2 receptors in T cells must also engage these PTKs to activate HPK1 kinase activity. First, we assessed whether PGE2 stimulation would induce general tyrosine phosphorylation in the Jurkat T cell line. Cells were left untreated or stimulated either with 10 nm PGE2 or by anti-CD3ε (OKT3.14) mAb-mediated TCR cross-linking. Anti-phosphotyrosine immunoblotting of whole cell lysates revealed no detectable change in global tyrosine phosphorylation levels upon PGE2 stimulation, whereas robust tyrosine phosphorylation was observed upon TCR cross-linking (Fig. 1A). Identical phosphorylation pattern was observed when cells were stimulated for varying time ranging from 2 to 10 min, but a modest tyrosine phosphorylation was detected at 20 min time point - a substantial amount of time after HPK1 was activated (supplemental Fig. S1, A and B). Anti-phosphotyrosine blotting of immunoprecipitated HPK1 confirmed that, unlike prominent tyrosine phosphorylation induced by TCR cross-linking (Fig. 1B, lane 3), stimulation by PGE2 did not induce detectable tyrosine phosphorylation of HPK1 (lane 2). Western blot analysis using anti-human HPK1 antibody demonstrated that comparable amounts of HPK1 were present in all lanes (Fig. 1C). It also revealed that PGE2 stimulation did not lead to activation-induced cleavage of HPK1, thus ruling out the involvement of caspase-mediated activation. The immune complex in vitro kinase (IVK) assay revealed that as in the previously reported studies (3, 17), both TCR and PGE2 receptors could activate HPK1 (Fig. 1D, lanes 2 and 3).

It has been shown that Jurkat somatic mutant cell lines that lack Lck and ZAP-70, J.CaM1 and p116, respectively, cannot
activate HPK1 upon TCR engagement (3). Through the use of these mutant cell lines, we assessed whether HPK1 would catalytically respond to stimulation by PGE$_2$. Wild type or mutant Jurkat cell lines were left untreated or stimulated with either 10 nM PGE$_2$ or by antibody-mediated TCR cross-linking. These cells were lysed, and the immunoprecipitated HPK1 was subjected to IVK analysis. We observed that the absence of Lck or ZAP-70 did not interfere with the ability of HPK1 to respond to PGE$_2$ stimulation (Fig. 2A, lanes 5 and 8). As previously reported, the presence of these PTKs is required for an HPK1 response to TCR cross-linking (2A, lanes 6 and 9). Western blot analysis using an anti-HPK1 antibody indicated that comparable amounts of immunoprecipitated HPK1 were used in all IVK reactions (Fig. 2B, lanes 1–3 and 4–9). We conclude from these studies that PGE$_2$ utilizes a PTK-independent pathway to induce HPK1 kinase activity.

**HPK1 Is Responsive to PGE$_2$ Stimulation in Lat and SLP-76-deficient Jurkat T Cell Lines**—Scaffolding proteins play a critical role in transducing activation signals from the TCR to HPK1. It has been shown that the presence of Lat, and to a lesser extent SLP-76, is required for TCR-induced HPK1 activation (3). To assess the role of these scaffolding proteins in PGE$_2$-induced HPK1 activation, we evaluated the mutant Jurkat T cell lines, ANJ3 and J14, which lacked the expression of Lat and SLP-76, respectively, for their ability to activate HPK1 in response to PGE$_2$ stimulation. A wild type Jurkat T cell line and the Lat and SLP-76 mutants were stimulated with 10 nM PGE$_2$ or by TCR cross-linking. Endogenous HPK1 was immunoprecipitated, and its catalytic activity was assessed by an IVK assay. Analysis of the receptor-induced HPK1 kinase activity revealed that all Jurkat cell lines could robustly induce HPK1 kinase activity upon PGE$_2$ stimulation (Fig. 2A, lanes 2, 11, and 14), whereas the mutant cell lines failed to activate HPK1 in response to PGE$_2$ stimulation. A wild type Jurkat T cell line and the Lat and SLP-76 mutants were stimulated with 10 nM PGE$_2$ or by TCR cross-linking. Endogenous HPK1 was immunoprecipitated, and its catalytic activity was assessed by an IVK assay. Analysis of the receptor-induced HPK1 kinase activity revealed that all Jurkat cell lines could robustly induce HPK1 kinase activity upon PGE$_2$ stimulation (Fig. 2A, lanes 2, 11, and 14), whereas the mutant cell lines failed to activate HPK1 in response to TCR engagement (lanes 12 and 15). Western blot analysis using an anti-HPK1 antibody indicated that comparable amounts of immunoprecipitated HPK1 were used in all IVK reactions (Fig. 2B, lanes 1–3 and 10–15). We conclude that, unlike TCR-induced signaling to HPK1, Lat and SLP-76 are not involved in PGE$_2$-induced HPK1 activation.

**Proline-rich Regions of HPK1 Are Not Required for PGE$_2$-induced HPK1 Activation**—The interaction between SH3 domain-containing adapter proteins and the proline-rich motifs of HPK1 is critical for TCR-mediated signaling to HPK1 (3, 8, 9, 11, 22–24). Three of the four proline-rich regions of HPK1 (P1, P2, and P4) conform to the class II consensus sequence for an SH3 protein interacting domain (11) and are known interaction sites for SH3 domain-containing proteins (25). To assess whether the proline-rich motifs in HPK1 are required in PGE$_2$-induced HPK1 activation, we transfected constructs that encoded either the HA-tagged wild type HPK1 or a mutant form in which P1, P2, and P4 proline-rich motifs (HA-ΔP-HPK1) had been deleted. Trans-
amounts of HPK1 were present in all IVK reactions (Fig. 3A). Western blot analysis indicated that comparable amounts of HPK1 proteins were immunoprecipitated with an anti-HA mAb and subjected to IVK assay. The autoradiographic bands depicted 32P-incorporated histone H2A catalyzed by HPK1 kinase activity. The dotted line indicates where images were joined to form the figure. B, PVDF membrane containing the electrophoretically resolved proteins from IVK reactions was Western blotted with the anti-human HPK1 antibody 47. Data represent a reproducible trend observed in three out of three experiments.

Elevation of cAMP Levels Induces HPK1 Activation—Prostaglandin E2 can bind with high affinity to each of the four E prostanoid receptors. However, EP2 and EP4, the receptors that couple with and signal through the stimulatory Gα (GαS), are the dominant receptors expressed in primary hematopoietic cells and hematopoietic cell lines (26–29). The GTP-bound Gαs subunit interacts with adenyl cyclase and potentiates cAMP production upon PGE2 stimulation. To test whether elevation of intracellular cAMP levels would activate HPK1 in Jurkat T cells, we stimulated cells with cholera toxin, cell permeable cAMP analogues, and forskolin, a potent adenyl cyclase activator, to elevate cAMP levels and assess the catalytic activity of HPK1 in response to these stimulations. Analysis by an IVK assay revealed that, despite our initial belief that HPK1 might use cAMP-independent signaling pathways to activate HPK1, our current data showed that HPK1 responded to the stimulation by cholera toxin, N6-O2'-dibutyryl-cAMP, 8-bromo-cAMP, and forskolin (Fig. 5A, lanes 2–5). Western blot analysis revealed that comparable amounts of HPK1 were present in all lanes (Fig. 5B).
PKA-dependent HPK1 Activation

Because PKA is the major effector molecule whose catalytic activity is regulated by the direct binding of cAMP, we tested whether PKA activity was required for PGE$_2$-induced HPK1 activation. To test the involvement of PKA in this process, we stimulated the S49 pre-T cell line and its PKA-deficient mutant, the kin$^{-}$ S49 cell line, with PGE$_2$ and compared the ability of HPK1 to respond to PGE$_2$ stimulation. Similar to the response found in Jurkat cells, the catalytic activity of HPK1 in wild type S49 cells was elevated in response to stimulation by either PGE$_2$ or forskolin (Fig. 5C, lanes 2 and 3). The response of HPK1 could be blocked by pretreating the S49 cells 30 min before stimulation with 10 $\mu$M H-89, an isoquinoline sulphonamide drug capable of specifically inhibiting PKA (Fig. 5C, lanes 4 and 5). In support of the critical role PKA plays in PGE$_2$-induced HPK1 activation, the PKA-deficient kin$^{-}$ S49 mutant cell line was not able to activate HPK1 when stimulated by either PGE$_2$ or forskolin (Fig. 5C, lanes 7 and 8), confirming the critical role that PKA plays in this signaling pathway. Western blot analysis using an anti-HPK1 antibody indicated that comparable amounts of immunoprecipitated HPK1 were used in IVK reactions (Fig. 5D, lanes 1–8). To verify that HPK1 is catalytically active and is capable of responding to PKA-independent signals, we stimulated kin$^{-}$ S49 cells with pervanadate, a potent inhibitor of protein tyrosine phosphatase and a robust activator of HPK1. Our analysis indicated that HPK1 in kin$^{-}$ S49 cells responded catalytically to pervanadate (Fig. 5E), despite its inability to respond to PGE$_2$ stimulation (D). This finding suggests that HPK1 is catalytically active and is responsive to non-PKA-dependent signal transduction pathway. To further strengthen the evidence supporting the role of PKA in PGE$_2$-induced activation of HPK1, we stimulated Jurkat cells with PGE$_2$ for 5 min, in the presence or absence of H-89, and Western blotted the immunoprecipitated HPK1 with anti-phospho PKA substrate antibody. Analysis revealed that PGE$_2$-activated HPK1 was recognized by anti-phospho PKA substrate antibody (Fig. 5F). This recognition was abrogated by H-89 pre-treatment (Fig. 5F), suggesting that PKA directly phosphorylated HPK1. The dependence on PKA for PGE$_2$-induced HPK1 activation sets it apart from TCR-induced HPK1 activation where PKA does not play a role in signal transduction (30).

Serine 171 Is Required for PGE$_2$-induced HPK1 Activation—The susceptibility of PGE$_2$-induced HPK1 activation to a PKA inhibitor, in conjunction with the inability of the PKA-deficient kin$^{-}$ S49 mutant cell line to activate HPK1 upon PGE$_2$ stimulation, strongly suggest that PKA is a critical upstream regulator of PGE$_2$-induced HPK1 activation. These findings led us to analyze the HPK1 amino acid sequence for the presence of the optimal consensus PKA motif, the amino acid sequence RXRXS/T, where X represents any amino acid (31). Sequence analysis identified serine 171, located within the “activation loop” of the kinase domain (the region flanked by the conserved “DFG” and “APE”) amino acid sequences in the kinase subdomain VII and VIII), as the only optimal PKA site in HPK1 (Fig.

**FIGURE 5.** HPK1 is catalytically responsive to intracellular cAMP concentration. A, 10 million Jurkat cells were treated with the indicated cAMP-elevating agents at 37 °C for 5 min (30 min for stimulation by cholera toxin) at the following concentrations: 10 ng/ml cholera toxin; 10 μM N6; O$_3$-dibutyryl-cAMP; 10 μM 8-bromo-cAMP; and 50 μM forskolin. CTX, DB, 8BM, and Fors denote cholera toxin, N6, O$_3$-dibutyryl-cAMP, 8-bromo-cAMP, and forskolin, respectively. B, PVDF membrane containing the electrophoretically resolved proteins from IVK reactions was Western blotted with the anti-murine HPK1 antibody 7. C, 10 million S49 cells or the PKA-deficient kin$^{-}$ S49 mutant line was stimulated with 10 nM PGE$_2$ or 50 μM forskolin, 10 million Jurkat cells were left unstimulated, stimulated with 10 nM PGE$_2$ (P) or 1 μM pervanadate (Per) at 37 °C for 5 min. Immunoprecipitated HPK1 isolated from these cells was subjected to an in vitro immune complex kinase assay. Data represent a reproducible trend observed in three out of three experiments. F, Jurkat cells were left unstimulated, stimulated by PGE$_2$ (P), or pretreated with 10 μM H-89 PKA inhibitor for 30 min prior to stimulation by PGE$_2$ for 5 min at 37 °C.
PKA-dependent HPK1 Activation

6A). Further analysis revealed that, whereas the arginine residue at −2 position relative to the serine 171 (arginine 169) was conserved in all KHS family members, only HPK1 possessed an arginine at the −3 (arginine 168) position relative to serine 171. The conserved double arginine sequence was also found in the murine HPK1 sequence, but not in the majority of Ste20 orthologues (data not shown). To assess the importance of arginine 168 and 169 to direct its substrate phosphorylation (35). In the case of MST3B, however, the conserved serine or threonine residue among Ste20 family members at residue 171 as one of the critical events necessary for TCR-induced activation of HPK1 (30). Because PKD is not activated by PGE2 stimulation (supplemental Fig. S1, C and D), the requirement for serine 171 phosphorylation in two receptor systems that utilizes two different kinases highlights the importance of this phosphorylation in HPK1 activation. It is important to note here that, whereas our data and that of others indicate that serine 171 is indispensable for the activation of HPK1, we believe that the phosphorylation is a necessary, but not sufficient event that controls HPK1 kinase activity. Phosphorylation of immunoprecipitated HPK1 in vitro by purified PKA does not render HPK1 more catalytically active (data not shown). Thus, we conclude that other post-translational modifications, most likely additional phosphorylations by other PS/TK, are required to fully activate HPK1.

The PGE2-induced phosphorylation of HPK1’s serine 171 represents a novel PKA-dependent activation mechanism not described previously for HPK1 or for other KHS family members. Among mammalian Ste20 orthologues, only MST-3B, the brain-specific splice variant isoform of MST3 has been previously shown to be catalytically responsive to PAKA-mediated phosphorylation (35). In the case of MST-3B, however, the unique peptide segment that contains the PKA substrate motif requires both arginine 168 and 169 to direct its substrate specificity.

DISCUSSION

We report here that TCR and PGE2 receptors utilize distinct signaling mechanisms to activate HPK1. Whereas TCR relies on PTK-dependent signal transduction pathways and the intact proline-rich regions of HPK1 to transmit activation signals to HPK1, PGE2 signals via PKA to activate HPK1. The reliance on PKA as the activator of HPK1 is consistent with the existing belief that PS/TK can signal to HPK1. This belief is based in part on the fact that ligand engagement of TGF-βR, a receptor PS/TK, results in the activation of HPK1 (13, 14). However, because TGF-β signaling pathways for HPK1 activation have not been characterized, the exact PS/TK pathway utilized by TGF-βR to activate HPK1 remains unknown. With our observation that PKA plays a prominent role in PGE2 signaling to HPK1, coupled with the recent report that TGF-β stimulation activates PKA in fetal skin fibroblasts (32), these findings support the possibility that the TGF-βR may also use PKA to activate HPK1.
is located N-terminal to the kinase domain. Thus, the inducible phosphorylation of HPK1's serine 171 represents the only report of a PKA-mediated inducible phosphorylation event that occurred within the activation loop of the mammalian Ste20 kinase domain. Perhaps this is a reflection of the infrequent occurrence of the double arginine motif in the activation loop of the Ste20 orthologues. Among the 31 human Ste20 orthologues identified by Sugn's genome analysis of human kinase genes, only MYO3, PAK4, SLK, and all MSN sub-family members (HGK, TNIK, MINK, and NRK) possess the double arginine motif at the location analogous to the double arginine residues of HPK1 (data not shown). We are currently investigating whether these kinases would respond catalytically to PKA.

Most tumors overexpress cyclooxygenase-2 and consequently produce high level of PGE2. Although PGE2 stimulation favors tumor growth by activating the T cell factor/lymphotocyte enhancer-binding factor signal transduction pathway in epithelial cells (36), it functions paradoxically as a potent inhibitor of T cell activation (37–40). We propose here that HPK1, due in part to its hematopoietic cell-restricted expression, may lead to the development of a novel therapeutic approach that could overcome tumor-mediated immune suppression by inhibiting the catalytic activity of HPK1.

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REFERENCES
1. Dan, I., Watanabe, N. M., and Kusumi, A. (2001) Trends Cell Biol. 11, 220–230
2. Kiefer, F., Tibbles, L. A., Anafi, M., Janssen, A., Zanke, B. W., Lassam, N., Pawson, T., Woodgett, J. R., and Iscove, N. N. (1996) EMBO J. 15, 7013–7025
3. Liu, J., Kiefer, F., Dang, A., Hashimoto, A., Cobb, M. H., Kurosaki, T., and Weiss, A. (2000) Immunity 12, 399–408
4. Yu, J., Riou, C., Davidson, D., Minhas, R., Robson, J. D., Julius, M., Arnold, R., Kiefer, F., and Veillette, A. (2001) Mol. Cell. Biol. 21, 6102–6112
5. Schulze-Luehrmann, J., Santner-Nanan, D., Jha, M. K., Schimpl, A., Avots, A., and Serfling, E. (2002) Blood 100, 954–960
6. Brenner, D., Golks, A., Kiefer, F., Krämer, P. H., and Arnold, R. (2005) EMBO J. 24, 4279–4290
7. Hu, M. C., Qiu, W. R., Wang, X., Meyer, C. F., and Tan, T. H. (1996) Genes Dev. 10, 2251–2264
8. Ling, P., Yao, Z., Meyer, C. F., Wang, X. S., Oehrl, W., Feller, S. M., and Tan, T. H. (1999) Mol. Cell. Biol. 19, 1359–1368
9. Ma, W., Xia, C., Ling, P., Qiu, M., Luo, Y., Tan, T. H., and Liu, M. (2001) Oncogene 20, 1703–1714
10. Shui, J. W., Boomer, J. S., Han, J., Xu, J., Dement, G. A., Zhou, G., and Tan, T. H. (2007) Nat. Immunol. 8, 84–91
11. Liu, S. K., Smith, C. A., Arnold, R., Kiefer, F., and McGlade, C. J. (2000) J. Immunol. 165, 1417–1426
12. Ling, P., Meyer, C. F., Redmond, L. P., Shui, J. W., Davis, B., Rich, R. R., Hu, M. C., Wange, R. L., and Tan, T. H. (2001) J. Biol. Chem. 276, 18908–18914
13. Zhou, G., Lee, S. C., Yao, Z., and Tan, T. H. (1999) J. Biol. Chem. 274, 13133–13138
14. Wang, W., Zhou, G., Hu, M. C., Yao, Z., and Tan, T. H. (1997) J. Biol. Chem. 272, 22771–22775
15. Nagata, Y., Kiefer, F., Watanabe, T., and Todokoro, K. (1999) Blood 93, 3347–3354
16. Chen, Y. R., Meyer, C. F., Ahmed, B., Yao, Z., and Tan, T. H. (1999) Oncogene 18, 7370–7377
17. Sawasdkisol, S., Russo, K. M., and Burakoff, S. J. (2003) Blood 101, 3687–3689
18. Sauer, K., Liou, I., Singh, S. B., Yablonski, D., Weiss, A., and Perlmutter, R. M. (2001) J. Biol. Chem. 276, 45207–45216
19. Tsuji, S., Okamoto, M., Yamada, K., Okamoto, N., Goitsuka, R., Arnold, R., Kiefer, F., and Kitamura, D. (2001) J. Exp. Med. 194, 529–539
20. Chang, J. H., Pratt, J. C., Sawasdkisol, S., Kapeller, R., and Burakoff, S. J. (1998) Mol. Cell. Biol. 18, 4986–4993
21. Gu, C., Ma, Y. C., Benjamin, J., Litmann, D., Chao, M. V., and Huang, X. Y. (2000) J. Biol. Chem. 275, 20726–20733
22. Anafi, M., Kiefer, F., Gish, G. D., Mbandu, G., Iscove, N. N., and Pawson, T. (1997) J. Biol. Chem. 272, 27804–27811
23. Oehrl, W., Kardinal, C., Ruf, S., Adermann, K., Groffen, J., Feng, G. S., Blenis, J., Tan, T. H., and Feller, S. M. (1998) Oncogene 17, 1893–1901
24. Ensenat, D., Yao, Z., Wang, X. S., Kori, R., Zhou, G., Lee, S. C., and Tan, T. H. (1999) J. Biol. Chem. 274, 33945–33950
25. Boomer, J. S., and Tan, T. H. (2005) J. Cell. Biochem. 95, 34–44
26. Arakawa, T., Laneuville, O., Miller, C. A., Lakkides, M. K., Wingard, B. A., DeWitt, D. L., and Smith, W. L. (1996) J. Biol. Chem. 271, 29569–29575
27. Mori, K., Tanaka, I., Kotani, M., Miyaoaka, F., Sando, T., Muro, S., Sasaki, Y., Nakagawa, O., Ogawa, Y., Usui, T., Ozaki, S., Ichikawa, A., Narumiya, S., and Nakao, K. (1996) J. Mol. Med. 74, 333–336
28. Blaschke, V., Jungermann, K., and Puschel, G. P. (1996) FEBS Lett. 394, 39–43
29. Takayama, K., Garcia-Cardena, G., Sukhova, G. K., Comander, J., Gimbrone, M. A., Jr., and Libby, P. (2002) J. Biol. Chem. 277, 44147–44154
30. Arnold, R., Patzkaz, I. M., Neuhaus, B., Vancanuwenbergh, S., Veillette, A., Van Lint, J., and Kiefer, F. (2005) Mol. Cell. Biol. 25, 2364–2383
31. Pearson, R. B., and Kemp, B. E. (1991) Methods Enzymol. 200, 62–81
32. Giannouli, C. C., and Kletsas, D. (2006) Cell. Signal. 18, 1417–1429
33. Bossemeyer, D. (1995) FEBS Lett. 369, 57–61
34. Johnson, L. N., Noble, M. E., and Owen, D. J. (1996) Cell 85, 149–158
35. Zhou, T. H., Ling, K., Guo, J., Zhou, H., Wu, Y. L., Jing, Q., Ma, L., and Pei, G. (2000) J. Biol. Chem. 275, 2513–2519
36. Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M., and Gutkind, J. S. (2005) Science 310, 1504–1510
37. Chouaib, S., Welte, K., Mertelsmann, R., and Dupont, B. (1985) J. Immunol. 135, 1172–1179
38. Anastassiou, E. D., Paliogianni, F., Balow, J. P., Yamada, H., and Boumpas, D. T. (1992) J. Immunol. 148, 2845–2852
39. Betz, M., and Fox, B. S. (1991) J. Immunol. 146, 108–113
40. Paliogianni, F., and Boumpas, D. T. (1996) Cell Immunol. 171, 95–101