Human Monocytes Use Rac1, Not Rac2, in the NADPH Oxidase Complex*

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Phagocyte NADPH oxidase is critical for defense against pathogens and contributes to inflammatory tissue injury. One component of the NADPH oxidase complex is the small GTP-binding protein Rac. There are two isoforms of Rac, and Rac2 is the predominant isoform in neutrophils and has been shown to be essential for NADPH oxidase activity. In primary human monocytes we report that in contrast to neutrophils, Rac1 is the predominantly expressed isoform. Upon monocyte activation by a variety of agents, we found that Rac1 dissociates from Rho GDP dissociation inhibitor (RhoGDI) and translocates to the membrane. We also found that Rac1 interacts with two other NADPH oxidase components, p67phox and p47phox, upon monocyte activation. These data indicate that Rac1, and not Rac2, is a component of the activated NADPH oxidase in monocytes. This finding suggests that it may be possible to selectively interfere with either monocyte or neutrophil NADPH oxidase activity, thereby selectively targeting chronic versus acute inflammatory processes.

Professional phagocytes, such as neutrophils and monocytes, are uniquely capable of generating large amounts of superoxide anion by the multicomponent NADPH oxidase in response to a variety of stimuli. Superoxide anion plays a key role in the defense against invading pathogens. The NADPH oxidase complex is comprised of several phox proteins: membrane-bounded gp91phox and p22phox and cytosolic p40phox, p47phox, and p67phox. Apart from the above core components, a low molecular weight GTP-binding protein, Rac, in its GTP-binding form, is required for the assembly and activation of NADPH oxidase in the amphiphile-activated cell-free system (1, 2). Rac is also involved in superoxide anion production in intact phagocytes as demonstrated by using Rac-specific antisense oligodeoxyribonucleotides (3), by selective defects of superoxide anion production by neutrophils derived from Rac2-deficient mice (4), and by defective neutrophils from a patient with an inhibitory mutation in Rac2 (5).

Three different Rac isoforms have been described in humans. Rac1, Rac2, and Rac3 are highly related GTPases belonging to the Rho subfamily of Ras proteins. In resting neutrophils, Rac2 is complexed with Rho GDP dissociation inhibitor (RhoGDI)‡ in the cytosol forming a heterodimer. Superoxide production induced by phorbol 12-myristate 13-acetate (PMA) or formylmethionyl-leucyl-phenylalanine (fMLP) was accompanied by the dissociation of Rac2 from RhoGDI and its movement to the membrane (6). Among the three Rac isoforms, Rac1 and Rac3 are expressed in many different cell types, whereas Rac2 is primarily expressed in myeloid cells (7). Rac1 and Rac2 isoforms are 92% identical and share GTP binding and GTP hydrolysis motifs. Rac3 also has a high degree of amino acid identity with human Rac1 and Rac2 (92 and 89%, respectively). Human neutrophils express both Rac1 and Rac2 proteins; however, about 96% of the Rac protein is Rac2 (8). The distinct difference between the highly homologous Rac isoforms is at their C terminus, particularly in a region that constitutes a polybasic domain. The polybasic domain in Rac1 has six contiguous basic amino acids, whereas in Rac2 three of these six residues are neutral amino acids. It is not clear whether Rac isoforms are activated by different stimuli, stimulate different targets, or regulate distinct signal pathways. Both Rac1 and Rac2 are capable of stimulating the respiratory burst in vitro with a semi-recombinant system (9), but their effects in vivo may be different (10).

Although it has been reported that Rac2 expression is much lower in macrophage and U937 cells relative to neutrophils (6), there is little information pertinent to Rac isoform expression and the role of Rac isoforms in the activation of NADPH oxidase in primary human monocytes. There are also no reports to date describing the association or dissociation state of Rac with RhoGDI in human monocytes. Activation-induced superoxide anion production by NADPH oxidase in monocytes contributes to the pathogenesis of chronic inflammatory diseases such as atherosclerosis. In contrast, neutrophils contribute little to this process and participate predominantly in acute inflammatory responses. Therefore, a better understanding of the regulation of the monocyte NADPH oxidase activity is an important step toward controlling chronic inflammatory diseases such as atherosclerosis and rheumatoid arthritis. In this study, we evaluated the expression of Rac isoforms in freshly isolated human monocytes and found that Rac1, rather than Rac2, is the predominant Rac isoform and that Rac1 is utilized to assemble the activated NADPH oxidase complex upon monocyte activation.

EXPERIMENTAL PROCEDURES

Materials—Recombinant Rac1 and Rac2 were obtained from Calbiochem (San Diego, CA). Mouse monoclonal antibodies against Rac1 were from Upstate Biotechnology (Lake Placid, NY). Rabbit polyclonal antibodies for Rac2, RhoGDI, and goat anti-mouse pre-adsorbed secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal antibodies for p67phox and p47phox were purchased previously (11). A protease inhibitor mixture and phenylmethylsulfonyl fluoride were from Sigma. Zymosan, which was from ICN Biomedicals (Cleveland, OH), was opsonized (12) and used at a concentration of 2 mg/ml. PMA and fMLP were purchased from Sigma.

Isolation of Human Monocytes and Neutrophils—The process for isolation of human monocytes was described previously (3). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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§ The abbreviations used are: RhoGDI, Rho GDP dissociation inhibitor; PMA, phorbol 12-myristate 13-acetate; fMLP, formylmethionyl-leucyl-phenylalanine; RT, reverse transcriptase; PAK, p21-activated kinases; ZOP, opsonized zymosan.

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cell lysates was also described previously (13) except the centrifugation fractions was described previously (13). The method for preparation of monocyte fractionation to isolate the membrane and cytosol from untreated or ZOP-treated cells were incubated with the indicated mixture followed by release in sample buffer. Immune-complexes were released with 5 mM EDTA and rested for 2 h in Dulbecco’s modified Eagle medium with 10% bovine calf serum before use in experiments. For activation, monocytes were treated with ZOP (2 mg/ml) for 1 h, PMA (100 ng/ml), or fMLP (1 µM) for 10 mins, respectively. The method for preparation of cell lysates—The process of monocyte fractionation to isolate the membrane and cytosol fractions was described previously (13). The method for preparation of cell lysates was also described previously (13) except the centrifugation after cell lysis was at 500 × g for 5 min at 4 °C.

**Immunoprecipitation/Western Blotting**—Human monocyte lysates from untreated or ZOP-treated cells were incubated with the indicated antibodies for 2 h at 4 °C with constant rotation, then mixed with protein G-agarose beads and further rotated at 4 °C overnight. Immune-complexes were collected and washed three times with lysis buffer (1% Triton X-100, 150 mM NaCl, 50 mM NaF, 50 mM Tris, pH 7.4, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 500 µM phenylmethylsulfonyl fluoride, and 1:100 diluted protease inhibitor mixture) followed by release in sample buffer. Immune-complexes were separated by 12% SDS-PAGE and transferred to polyvinylidene difluoride membranes (0.2 µm, Bio-Rad). After blocking overnight with 5% milk in phosphate-buffered saline containing 0.1% Tween 20, the membranes were probed with the indicated antibodies and corresponding secondary antibodies and finally developed using enhanced chemiluminescence (Pierce).

**RNA Extraction and Real-time RT-PCR**—Total cellular RNA was extracted from freshly isolated human monocytes using the RNeasy mini kit from Qiagen (Valencia, CA). One microgram of total RNA was reverse transcribed using the TaqMan Reverse Transcription kit (PE Applied Biosystem, Foster City, CA). The cDNAs were subjected to real-time PCR using SYBR Green PCR core reagents. The PCR reactions were carried out in an ABI Prism sequence detector 7700 (PE Applied Biosystem). The thermal cycle conditions included 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of amplification at 95 °C for 15 s and 60 °C for 1 min. The threshold cycle (Ct) values obtained through the experiments indicate the fractional cycle numbers at which the amount of amplified target reach a fixed threshold. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal reference. Oligodeoxyribonucleotides used for real-time RT-PCR are listed in Table I.

### TABLE I

| Designation     | Sequence                                      | Description     |
|-----------------|-----------------------------------------------|-----------------|
| Rac1 sense      | 5′-AGG AAG AGA AAA TGC CTG-3′                 | Bases 691–708   |
| Rac1 antisense  | 5′-AGC AAA GCG TAC AAA GGT-3′                 | Bases 755–772   |
| Rac2 sense      | 5′-TAG ATG GGT CTG ATC TCT-3′                 | Bases 736–753   |
| Rac2 antisense  | 5′-AGA AAA GAC CAT CAA CGA-3′                 | Bases 841–858   |
| Rac3 sense      | 5′-AGG AAG TGC ACC GTG TTC-3′                 | Bases 666–683   |
| Rac3 antisense  | 5′-AGC ATC ATC CCC ATG CT-3′                  | Bases 808–825   |
| GAPDH sense     | 5′-TCG GAG TCA ACG GAT TTG GTC GTA-3′         | Bases 120–143   |
| GAPDH antisense | 5′-TGG CAT GGA CTG TGG TCA TGA GTC-3′         | Bases 644–621   |

**RESULTS**

**Expression of Rac1 and Rac2 Protein in Human Monocytes**—To evaluate the expression of Rac proteins in human monocytes, we performed Western blotting analysis using human monocyte lysates and detected Rac proteins with specific antibodies for Rac1 and Rac2. As shown in Fig. 1A, antibodies specific for Rac1 recognized recombinant Rac1 but not recombinant Rac2, whereas Rac2-specific antibodies reacted solely with recombinant Rac2 rather than recombinant Rac1 (a specific Rac3 antibody is not available). Repeated experiments indicated that the molecular weight of monocyte Rac1 is about 20 kDa, and it migrated faster than the recombinant Rac1 (22.15 kDa). There is a clear band of Rac1 from monocyte lysates detected with Rac1 antibodies, whereas only a very weak Rac2 band was detected in the same amount of monocyte lysate. To further confirm the specificity of Rac1 antibodies, we pre-incubated the antibody with recombinant Rac1 at a molar ratio of 1:10 (antibody to recombinant protein) and found that such a pre-incubation blocked the Rac1 antibody reaction with both recombinant Rac1 and monocyte lysates (Fig. 1B, right panel), demonstrating the specificity of this antibody. Western blotting analysis (Fig. 1C) with 50 µg cell lysates derived from freshly isolated monocytes or neutrophils demonstrated that Rac2 was the predominant iso-
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Quantitative Western blot analysis of Rac1/Rac2 expression in human monocytes (A and B) and human neutrophils (C and D). Recombinant Rac1/2 or cell lysates were separated by 12% SDS-PAGE and Western blotting was done with antibodies (1:2000) specific for Rac1 (A and C) or Rac2 (B and D). The integrated densities of each band were determined by analysis of lightly exposed films using the software program NIH image.

Quantitation of mRNA and Protein Levels of Rac Isoforms in Human Monocytes—To further evaluate the expression of Rac isoforms in human monocytes, we performed real-time RT-PCR to analyze the mRNA levels of the three Rac isoforms and quantitative Western blotting to assess the levels of Rac1 and Rac2 proteins. Fig. 2 is a representative amplification plot of real-time RT-PCR for the three Rac isoforms. The threshold cycle values ($C_T$) for Rac1, Rac2, and Rac3 were 17.35, 30.53, and 37.13, respectively, whereas the $C_T$ value for glyceraldehyde-3-phosphate dehydrogenase RNA control was 18.49. These data indicate that the mRNA level of Rac1 is remarkably higher than that of Rac2 in human monocytes, whereas the mRNA level of Rac2 was much higher than that of Rac3. The mRNA level of Rac3 in monocytes was extremely low based on the real-time RT-PCR data. Quantitative Western blots were performed to determine the level of Rac1 and Rac2 protein expressed in monocytes and neutrophils. Fig. 3A shows the quantitative Western blotting of Rac1 using human monocyte lysates as compared with different doses of recombinant Rac1. The two plots in the lower panel of Fig. 3A give the integrated density calculated with NIH image software using lightly exposed films. According to these results, it is estimated that the expression level of Rac1 protein in human monocytes is about 1.21 ng/μg lysate. A similar experiment was performed in parallel to quantify the expression level of Rac2 protein in human monocytes (Fig. 3B). It is −0.13 ng/μg lysate. Because it is known that Rac2 is the predominate isoform of Rac in neutrophils we needed to confirm this finding using the same antibodies used in Fig. 3, A and B. Our results, shown in Fig. 3, C and D, indicated that Rac2 has a much higher expression level than Rac1 in neutrophils (5.3 ng/μg lysate versus 0.08 ng/μg lysate). Our results are consistent with previous results (8). Rac1 is therefore the predominant isoform in human monocytes, accounting for about 90% of Rac expression with Rac2 accounting for the other 10%. These results are opposite that of human neutrophils.

Dissociation of Rac1 with RhoGDI and Translocation of Rac1 from Cytosol to Membrane upon Activation of Human Monocytes—As Rac1 is the predominant Rac isoform expressed in human monocytes, we next examined its association state with RhoGDI and its movement to the membrane upon activation of monocytes. Immunoprecipitation/Western blotting experiments demonstrated that immune-complexes derived from precipitation with antibodies specific for RhoGDI contained more detectable Rac1 in untreated monocyte lysates as compared with ZOP-activated lysates (Fig. 4A), suggesting the association of Rac1 with RhoGDI in resting monocytes and dissociation of these two proteins upon monocyte activation by ZOP. Stripping and reprobing the same membrane with Rac2-specific antibodies showed no detectable band (data not shown). Although it appears that no Rac1 is present in the immunoprecipitate of RhoGDI from ZOP-activated monocytes, a weak band of Rac1 was detected upon over-exposure. We further isolated the membrane fraction from untreated or ZOP-activated (1 h) human monocytes and found that there is about 4–5-fold more Rac1 protein in the membrane fraction from ZOP-activated monocytes than in the unactivated membrane fraction (Fig. 4B, upper panel). These results suggest that the translocation of Rac1 occurs upon monocyte activation by ZOP. There was no detectable Rac2 in either untreated or ZOP-activated membrane fractions (Fig. 4B, middle panel). The lower panel of Fig. 4B is the result of a dot-blot with recombinant Rac2 in the same membrane used in the middle panel of Fig. 4B to confirm the reactivity of Rac2 antibodies. To examine the translocation of Rac isoforms upon monocyte activation with different stimuli, we triggered monocytes with two additional activators. As shown in Fig. 4C, upper panel, in addition to ZOP, activation of monocytes with PMA or fMLP also caused an increase in Rac1 in the membrane fraction; however, there was no detectable translocation of

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It is well documented that the cytosolic components of NADPH oxidase like p67phox, p47phox, and Rac translocate to the membrane upon stimulation, where they form a complex with the transmembrane flavocytochrome b558 to assemble the activated enzyme complex. To evaluate the involvement of Rac1 in the assembly of NADPH oxidase in human monocytes, we next performed immunoprecipitation/Western blotting experiments to examine whether membrane-associated Rac1 could form a complex with p67phox or p47phox upon monocyte activation. The latter two proteins have been demonstrated to translocate from the cytosol to the membrane when monocytes were activated with ZOP (13). As shown in Fig. 5A, we found that neither Rac1 nor Rac2 could be detected in immune-complexes derived from immunoprecipitates of untreated or ZOP-treated monocyte cytosol fractions using specific antibodies for p67phox or p47phox. In membrane fractions, there was no detectable Rac2 associated with p67phox or p47phox; however, it is clear that in the ZOP-treated membrane fraction, Rac1 was detected in the immune-complexes precipitated with either p67phox- or p47phox-specific antibodies. In the reverse experiments, Rac1-specific antibody was used for immunoprecipitation. These immune-complexes contained both p67phox and p47phox, but only in ZOP-treated membrane samples (Fig. 5B). Taken together, these results suggest that Rac1 forms a complex with p67phox and p47phox in the membranes of ZOP-activated monocytes.

**DISCUSSION**

Rac2 is a Rho-related guanine nucleotide-binding protein, which is essential for activation of the NADPH oxidase in human neutrophils. In this study, we demonstrated the novel finding that Rac1 is the predominant Rac isoform, at both the mRNA and protein levels, in primary human monocytes. This finding is in sharp contrast to Rac isoform expression and function in human neutrophils where Rac2 is the predominant isoform (Fig. 3 and Ref. 8). Upon activation of monocytes with ZOP, Rac1, which exists as a heterodimer with RhoGDI in resting cells, dissociates from its partner and translocates from the cytosol to the membrane (Fig. 4). Translocation of Rac1, not Rac2, from cytosol to membrane upon monocyte activation by PMA or fMLP were also observed. Furthermore Rac1 associates with p67phox and p47phox in membranes of activated monocytes. These data indicate that human monocytes use Rac1, rather than Rac2, to assemble the activated NADPH oxidase. This is the first report to describe the expression of Rac isoforms and examine their role in the assembly of the NADPH oxidase complex in primary human monocytes.

In addition to its role in NADPH oxidase complex formation and activity, Rac has been reported to have distinct cellular functions including the modulation of the actin-based cytoskel-
Alton (15), activation of various serine/threonine kinases (e.g., p21-activated kinases (PARs)) (16), and participation in signaling events related to cell proliferation (17, 18), suggesting a wide diversity of signaling pathways cross-linked to Rac. Few reports have discriminated between the roles of specific isoforms of Rac in those signal pathways. Studies on the role of Rac in controlling NADPH oxidase assembly and activity have mainly focused on its interaction with other components of this enzyme complex, especially p67phox. Several groups have demonstrated Rac1 or Rac2 binding to p67phox in vitro or in yeast two-hybrid system (10, 19, 20). Further studies indicated that the Rac effector domain (amino acids 26–45) is critical for binding with the N-terminal tetra trico repeat region of p67phox because specific point mutants in this domain abrogated Rac interaction with p67phox and NADPH oxidase activity in cell-free assays (21, 22). Rac1 and Rac2 share 100% homology in their effector domain. This may explain why both Rac1 and Rac2 are capable of reconstituting superoxide anion production in the cell-free NADPH oxidase system (1, 2, 9). A Ser/Thr kinase PAK is a prime target protein for mediating some of the cellular effects of Rac, and a recent report indicated that a PAK-controlled metabolic switch up-regulates phagocyte NADPH oxidase (23). Interestingly, studies related to structural requirements for PAK activation by Rac suggested that Rac1 binds to and stimulates the kinase activity of PAK –2 and –4– 5-fold, respectively, better than Rac2. Polybasic domain differences, the most notable difference in Rac isoforms, account for the disparate abilities of Rac1 and Rac2 to activate PAK (24). Therefore the polybasic domain of Rac is believed to be a novel effector domain that may allow the two Rac isoforms to activate distinct effector targets. A recent report indicated that another potentially significant difference between Rac1 and Rac2 is the notably different predicted sites of phosphorylation (25). Our data indicate that human monocytes and neutrophils have different expression patterns of Rac isoforms and differential participation of Rac isoforms in the NADPH oxidase complex. Further studies will explore the impact of this finding on NADPH oxidase function.

In this study we also found that Rac1 associates with p47phox, in addition to p67phox, in ZO-P-activated membrane fractions of monocytes. It is not clear whether this binding between Rac1 and p47phox is direct or mediated through other components of the NADPH oxidase complex such as p67phox. We previously reported the increased association of p67phox with p47phox in ZO-activated human monocytes (13). Although studies on the interactions between Rac and other components of the NADPH oxidase complex have mainly focused on p67phox, there are also reports describing the interaction of Rac2 with p47phox. For example, two reports suggested that Rac2 translocation and subsequent association with the membrane were diminished in chronic granulomatous disease neutrophils deficient in p47phox but were not affected in chronic granulomatous disease neutrophils lacking p67phox (26, 27). In contrast, others demonstrated that Rac2 translocates independently of the other cytosolic factors and can associate with the membrane in the absence of either p67phox or p47phox in human neutrophils (8, 28). Recent studies in a cell-free system indicated that prenylated Rac1 translocates to the membrane and serves as a carrier or as a membrane anchor for p67phox (29). This has not been investigated in intact monocytes. Further studies in primary monocytes will give us more insight toward a better understanding of the role of Rac isoforms in the assembly and activity of the NADPH oxidase complex.

In summary, we report that Rac1 is the predominant isoform of Rac in primary human monocytes and that monocytes use Rac1, not Rac2, for assembly of the NADPH oxidase complex. Taken together, these data suggest that different Rac isoforms in the NADPH oxidase complex of human neutrophils and monocytes may contribute to the altered kinetics, responsiveness, and overall regulation of the formation of the NADPH oxidase complex. The differential usage of Rac isoforms in monocytes and neutrophils may provide novel approaches for selectively interfering with chronic versus acute inflammatory responses.

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REFERENCES
1. Abo, A., Pick, E., Hall, A., Totty, N., Teahan, C. G., and Segal, A. W. (1991) Nature 353, 668 – 670
2. Knaus, U. G., Heyworth, P. G., Evans, T., Curnutte, J. T., and Bokoch, G. M. (1991) Science 254, 1512–1515
3. Dorseuil, O., Vazquez, A., Lang, P., Bertoglio, J., Gacon, G., and Leca, G. (1992) J. Biol. Chem. 267, 20540–20542
4. Roberts, A. W., Kim, C., Chen, L., Lowe, J. B., Kapur, R., Petryniak, B., Spaetti, A., Pollock, J. D., Borneo, J. B., Bradford, G. B., Atkinson, S. J., Dinauer, M. C., and Williams, D. A. (1999) Immunity 10, 183–196
5. Ambrosio, D. R., Knall, C., Abell, A. N., Paspodino, J., Kurthkhubashe, A., Thurman, G., Gonzalez-Alle, C., Hiestet, A., deMoor, M., Harbeck, R. J., Oyer, R., Johnson, L. G., and Roos, D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4654–4659
6. Abo, A., Webb, M. R., Grogan, A., and Segal, A. W. (1994) Biochem. J. 298, 585–591
7. Matos, P., Skaug, J., Marques, B., Beck, S., Verissimo, F., Gespach, C., Boavida, M. G., Scherrer, S. W., and Jordan, P. (2000) Biochem. Biophys. Res. Commun. 277, 741–751
8. Heyworth, P. G., Bohl, B. P., Bokoch, G. M., and Curnutte, J. (1994) J. Biol. Chem. 269, 30749–30752
9. Heyworth, P. G., Knaus, U. G., Xu, X., Uihlinger, D. J., Conroy, L., Bokoch, G. M., and Curnutte, J. (1994) Mol. Cell Biol. 14, 261–269
10. Dorseuil, O., Reibel, L., Bokoch, G. M., Camonis, J., and Gacon, G. (1996) J. Biol. Chem. 271, 83–88
11. Forbes, L. Y., Moss, S. J., and Segal, A. W. (1999) FEBS Lett. 449, 229–229
12. Johnston, R. B. (1981) J. Clin. Invest. 67, 817–825
13. Zhao, X., Bley, E. A., Wientjes, F. B., and Cathcart, M. K. (2002) J. Biol. Chem. 277, 25393–25399
14. Stossel, T. P., Pollard, T. D., Mason, R. J., and Vaughan, M. (1971) J. Clin. Invest. 50, 1745–1747
15. Nokes, C. D., and Hall, A. (1995) Cell 81, 53–62
16. Mancus, E., Leung, T., Salibullah, H., Zhao, Z. S., and Lim, L. (1994) Nature 367, 40–46
17. Peppenbenc, M. P., Qu, R. G., de Vries-Smits, A. M., Tetersole, L. G., de Laat, S. W., McCormick, F., Hall, A., Simmons, M. H., and Bro, J. (1995) Cell 81, 849–856
18. Irani, K., Xia, Y., Zweier, J. L., Sollott, S. J., Der, C. J., Pearson, E. R., Sundaresan, M., Finkel, T., and Goldschmidt-Clermont, P. J. (1997) Science 275, 1649–1652
19. Diekmann, D., Abo, A., Johnston, C., Segal, A. W., and Hall, A. (1994) Science 265, 531–533
20. Nisimoto, Y., Freeman, J. L., Motalebi, S. A., Harirbberg, M., and Lambeth, J. D. (1997) J. Biol. Chem. 272, 18834–18841
21. Lapouge, K., Smith, S. J., Walker, P. A., Gamblin, S. J., Smerdon, S. J., and Spaetti, A. (2000) Mol. Cell 6, 899–907
22. Kwong, C. H., Adams, A. G., and Leto, L. T. (1995) J. Biol. Chem. 270, 19685–19672
23. Sbalom-Burak, T., and Knaus, U. G. (2002) J. Biol. Chem. 277, 40659–40665
24. Knaus, U. G., Wang, Y., Reilly, A. M., Warnock, D., and Jackson, J. H. (1998) J. Biol. Chem. 273, 21512–21518
25. Yaffe, M. B., Leparc, G. G., Lai, J., Ohta, T., Volinia, S., and Cantley, L. C. (2001) Nature 41, 348–353
26. de Bonna, J., Ruest, J. M., and Babi, M. B. (1994) J. Biol. Chem. 269, 6729–6734
27. Dusi, S., Donini, M., and Rossi, F. (1995) Biochem. J. 308, 991–994
28. Dorseuil, O., Quinn, M. T., and Bokoch, G. M. (1995) J. Leukoc. Biol. 58, 108–113
29. Goralzanky, Y., Sigal, N., Itan, I., Lotan, O., and Pick, E. (2000) J. Biol. Chem. 275, 40073–40081