Multiple Signalling Pathways Lead to the Activation of the Nuclear Factor κB by the Rho Family of GTPases*

(Received for publication, December 24, 1997)

Silvia Montaner‡, Rosario Perona, Luisa Saniger§, and Juan Carlos Lacal¶

From the Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas and Department of Biochemistry, School of Medicine, Autonoma University, 28029 Madrid, Spain

Members of the Rho family of small GTPases activate the nuclear factor κB (NF-κB) (Perona, R., Montaner, S., Saniger, L., Sánchez-Pérez, I., Bravo, R., and Lacal, J. C. (1997), Genes & Dev. 11, 463–475). We have investigated whether different members of the family of exchange factors specific for Rho proteins (Dbl family) could activate the transcription factor NF-κB and have explored both their specificity under in vivo conditions and the mechanisms involved. Activated forms of Dbl, Ost, and Vav proteins induce NF-κB activation. While the activation induced by the Vav oncogen was efficiently inhibited by a dominant negative mutant of Rac1, the corresponding mutant of Cdc42Hs was able to block NF-κB activation induced by Rac1 and Cdc42Hs proteins, but not by Ras proteins. These results indicate that, in mammalian cells, multiple pathways coexist for the activation of NF-κB, some of which are mediated by specific members of the Ras and Rho families of small GTPases.

The Rho family of small GTPases are involved in the regulation of critical cellular functions, such as cell growth, apoptosis, invasion, and cytoskeleton organization, and in several aspects of development (2, 3). During the last few years, a growing body of evidence has emerged revealing the implication of RhoA, Rac1, and Cdc42Hs proteins in signal transduction cascades that regulate the activity of different transcription factors. Constitutively activated forms of Rac1 and Cdc42Hs GTPases are able to induce the activation of the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) in COS-7, NIH-3T3, and HeLa cells (4, 5). However, RhoA, RhoB, and Cdc42Hs, but not Rac1, induce the activation of JNK/SAPK in human kidney 293T cells (6). Activated Rac1 and Cdc42Hs mutants have also been shown to activate the p38/Mpk2 pathway, which is induced by different stress conditions (5). Furthermore, Hill et al. (7) have reported the regulation of the transcriptional activity of the serum response factor (SRF) by RhoA, Rac1 and Cdc42Hs-mediated by, at least, two independent mechanisms.

Recently, our group has described the activation of the nuclear factor κB by members of the Rho family of small GTP binding proteins (1). The NF-κB complex is composed of different homodimers and heterodimers of the members of the Rel/NF-κB family of transcription factors. In its inactive state, NF-κB is located in the cytoplasm where it is retained by a third inhibitory protein denominated IκB (8, 9). Activation by multiple external stimuli triggers the phosphorylation and proteolytic degradation of the IκB protein, releasing the NF-κB dimer, which translocates to the nucleus and binds DNA. We have reported that RhoA, Rac1, and Cdc42Hs GTPases induce the transactivation of the NF-κB-dependent HIV promoter in COS-7, NIH-3T3, and Jurkat cells. Rho proteins activate the translocation of NF-κB complexes by inducing the phosphorylation of the IκBα isoform in Ser-32 and Ser-36 residues. Furthermore, RhoA and Cdc42Hs seem to mediate specifically the activation of NF-κB by certain physiological stimuli such as tumor necrosis factor α (TNFα) (1).

The activity of Rho proteins is regulated by guanine nucleotide exchange factors that promote the transition between the inactive, GDP-bound state and the active, GTP-bound state of these GTPases (10). Rho-related exchange factors acquire tumorigenic activity by deletion of their N-terminal region, and in fact, most of them have been identified as potent oncogenes isolated from different transformed cell lines. The ability of catalyzing the GDP/GTP exchange is critically mediated by two conserved regions of these molecules, designated as the Dbl homology (DH) domain and the Pleckstrin homology (PH) domain (11). Because of these features, these exchange factors have been cloned in a rapidly growing family known as the Dbl family, which includes the Dbl protein itself, identified in 1985 from a diffuse B-cell lymphoma (12), Ost, isolated from a rat osteosarcoma (13) and Vav, cloned as a transforming gene from a human hematopoietic cell line (14).

Results derived from in vitro experiments have shown that the members of the Dbl family have a wide specificity regarding their nucleotide exchange activity for the different Rho proteins. Nevertheless, during the last few years, a considerable effort has been made in order to identify which processes these Dbl-related proteins could mediate in vivo. Overexpression of some of these exchange factors induces cytoskeleton arrange-

‡ Fellow from DGICYT.
¶ To whom correspondence should be addressed: Instituto de Investigaciones Biomédicas, Arturo Dupuyier 4, 28029 Madrid, Spain. Tel.: 34-91-585-4607; Fax: 34-91-585-4606; E-mail: jclac@iibi.uam.es.
1 The abbreviations used are: JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; SRF, serum response factor; TNFα, tumor necrosis factor α; HIV, human immunodeficiency virus; MEKK1, c-Jun N-terminal kinase kinase kinase; MEKK1 WT, MEKK1 wild-type.
ments that had previously been related to RhoA, Rac1, or Cdc42Hs (15–18). Tiam1 is able to induce membrane ruffling in fibroblasts and COS cells (19, 20), whereas Lbc is implicated in stress fiber formation (21) and Fgdi1 induces the extension of filopodia (22). On the other hand, there is also abundant information relating activation of Rho proteins with signaling events. Thus, activation of the 70-kDa S6 kinase is induced by overexpression of the Dbl and Fgd1 proteins (22, 23), and several members of the Dbl family such as Dbl, Ost, Vav, Tiam1, and Fgdi1 have been found to activate the JNK/SAPK cascade (4, 20, 22, 24). Moreover, activation of JNK/SAPK induced by Vav and Tiam1 oncogenes seems to be inhibited by the dominant negative mutant of Rac1, Rac1/Asn-17 (20, 24). These results indicate that the Dbl-related guanine nucleotide exchange factors have a limited specificity for Rho proteins in vivo.

Although it is well established that Rho GTPases are regulated by specific nucleotide exchange factors that generally belong to the Dbl family of proteins, little is known about their specificity under physiological conditions. In this study, we have investigated further the pathways leading to the activation of NF-κB where Rho proteins are implicated. Both specificity by some members of the family of exchange factors for Rho proteins and the mechanisms involved are addressed.

EXPERIMENTAL PROCEDURES

Cell Lines and Transfections—Simian COS-7 fibroblast-like cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1 mM glutamine. Cells were transfected by the calcium phosphate precipitation method in 60-mm dishes (25). The total amount of DNA was adjusted to 5–6 μg/plate.

DNA Constructs—(−453/+80) HIV-LUC reporter containing the two NF-κB binding sites of the HIV enhancer region and the same plasmid with 3-base pair substitutions in the κB sites (HIV-LUC) were previously described (26). The 5′ × Gal4-LUC reporter and the plasmids GAL4-c-Jun(1–223) and GAL4-c-Jun(1–223;A63/73) were kindly provided by Dr. M. Karin (University of California, San Diego). GAL4-c-Jun(1–223) and GAL4-c-Jun(1–223;A63/73) expression vectors encode for fusion proteins containing the Gal4 DNA binding domain and the c-Jun activation domain (5). Both phosphorylation sites by JNK/SAPK were substituted by alanine residues in GAL4-c-Jun(1–223;A63/73). These results indicate that members of the family of Rho proteins and the mechanisms involved are addressed.

RESULTS AND DISCUSSION

We have recently described that members of the Rho family of small GTP binding proteins are capable of activating the transcription factor NF-κB (1). Overexpression of either normal or constitutively activated forms of RhoA, Rac1, and Cdc42Hs induced the transactivation of the human immunodeficiency virus (HIV) promoter, which is mostly mediated by the presence of two κB binding sites in its enhancer region (26). Moreover, the activation of NF-κB by Rho proteins implicated the translocation of RelA/p50 and p50/p50 dimers to the nucleus and was observed in a variety of cell types, such as simian COS-7, murine NIH-3T3, and human T-cell lymphoma Jurkat cells.

Overexpression of Rho GTPases has been useful to reveal the possible role of these molecules in the regulation of NF-κB. However, it could also induce artifacts that are not representative of their physiological role due to improper localization or other indirect effects. Activation of Rho proteins is catalyzed under physiological conditions by the activation of certain specific nucleotide exchange factors that belong to the Dbl family of proteins. Thus, we investigated whether some of these Dbl-related molecules, which are known specific exchange factors for Rho proteins, were able to activate NF-κB. COS-7 cells were transfected with the HIV-LUC plasmid as a reporter along with the expression vectors encoding for the constitutively activated forms of Dbl, Ost, and Vav proteins or their corresponding empty vectors. These oncogenic versions contain a deletion in their N-terminal domain that confers transforming potential to these molecules. As shown in Fig. 1A, transient expression of either Dbl, Ost, or Vav oncogenes was able to induce the transactivation of the HIV promoter, as we had observed for the constitutively activated Rho proteins (QL-mutants). This activation was mostly due to the presence of the two κB sites located in the HIV enhancer domain because this effect was blocked when a mutant plasmid HIV-LUC with 3-base pair substitutions in the κB sites was used (data not shown). By contrast, while a significant activation was obtained with the corresponding Rho proteins as previously reported (1), no effect was observed by the overexpression of the PKCζ isoenzyme, in keeping with previous reports (27–29).

Activation of the transcriptional activity of c-Jun induced by all these proteins was also assayed in parallel experiments. The corresponding vectors for the activated forms of Rho GTPases and the truncated versions of Dbl, Ost, and Vav were cotransfected in COS-7 cells along with the 5′ × Gal4-LUC reporter and the plasmid GAL4-c-Jun(1–223) encoding for a fusion protein containing the GAL4 DNA binding domain and the c-Jun activation domain (5). As shown in Fig. 1B, expression of either Rac1 or Cdc42Hs was able to induce the c-Jun transcriptional activity while expression of RhoA was negative as the expression of PKCζ(1), in agreement with previous reports (1, 4, 5). The oncogenic forms of Dbl, Vav, and Ost proteins were also able to induce the activation of the c-Jun transcription factor. This effect was mediated by the phosphorylation of c-Jun at residues serines 63 and 73, since activation was efficiently blocked by cotransfection of the plasmid GAL4-c-Jun(1–223;A63/73). These results indicate that members of the family of specific exchange factors for Rho proteins, in addition to the JNK/SAPK cascade, are able to activate the transcription factor NF-κB. These results provide further evidence to support the concept that Rho proteins may be involved in the physiological regulation of NF-κB.

Using NF-κB activation as a reporter assay, we next investigated whether distinct proteins of the Dbl family of exchange factors had any degree of specificity for different members of the Rho family of small GTPases. This information may be very
useful to identify the physiological pathways where each Rho protein may exert its regulatory function on NF-κB-dependent transcription. The product of the vav proto-oncogen is mostly expressed in hematopoietic cell lines, and this exchange factor has been found to be critical for antigen receptor-mediated activation and proliferation of B and T lymphocytes (14, 30, 31). Recently it has been described that a truncated Vav protein could efficiently activate the JNK/SAPK by a Rac1-dependent pathway since a dominant negative mutant of Rac1, Rac/Asn-17, inhibited the activation induced by this exchange factor (24). Moreover, Rac1 GDP/GTP exchange reaction catalyzed by Vav is dependent on tyrosine phosphorylation (32). Thus, we investigated the specificity for this exchange factor regarding the activation of NF-κB. COS-7 cells were cotransfected with the corresponding empty vector. The same results were obtained in three independent experiments.
Multiple Pathways Coexist for the Activation of NF-κB

Fig. 2. Activation of NF-κB induced by Vav oncogen depends on Rac1 GTPase. COS-7 cells were cotransfected with 0.5 μg of (-453/+80) HIV-LUC and 1 μg of pCMV-β-galactosidase per 60-mm plate along with 1 μg of the plasmid pCEV27 or the derived expression vectors containing the dominant negative mutants RhoA/Asn-19, Rac1/Asn-17, or Cdc42Hs/Asn-17, and 3 μg of pMEX or pMEX-vav. Luciferase activity was determined 24 h after transfection, and data represent the means ± S.D. of a single experiment performed in triplicate. Results are expressed as percentage of maximal induction obtained with the corresponding empty vector. The same results were obtained in three independent experiments.

Fig. 3. Activation of NF-κB induced by Dbl oncogen depends on Cdc42Hs GTPase. COS-7 cells were cotransfected with 0.5 μg of (-453/+80) HIV-LUC and 1 μg of pCMV-β-galactosidase per 60-mm plate along with 1 μg of the plasmid pCEV27 or the derived expression vectors containing the dominant negative mutants RhoA/Asn-19, Rac1/Asn-17, or Cdc42Hs/Asn-17, and 3 μg of pZipNeo or pZipNeo-dbl. Luciferase activity was determined 24 h after transfection, and data represent the means ± S.D. of a single experiment performed in triplicate. Results are expressed as percentage of maximal induction obtained with the corresponding empty vector. The same results were obtained in three independent experiments.

Furthermore, this molecule was able to bind specifically to the Rac1-GTP form, suggesting that Ost could also serve as an effector molecule for this GTPase. In keeping with these results, in vitro experiments have shown that Ost is able to activate the JNK/SAPK cascade in COS-7 cells (4). However, no evidence is available to link Ost to RhoA under in vivo conditions since RhoA is not able to activate the JNK/SAPK in this cell system.
by Rho and Ras have shown to be independent (4, 7). Therefore, we investigated whether the H-Ras/Raf kinase pathway was involved in the activation of NF-κB induced by Rho proteins. As shown in Fig. 5A, we were not able to detect any effect of the overexpression of the dominant negative mutants of H-Ras and Raf kinase (H-Ras/Asn-17 and Raf-C4) in the transactivation of the HIV promoter induced by RhoA, Rac1, or Cdc42Hs proteins. These results indicate that Rho-mediated activation of NF-κB is either independent of the Ras/Raf pathway or that it is located downstream of Ras.

We then studied whether the Rho proteins could affect the activation of NF-κB mediated by the H-Ras protein. To that end, COS-7 cells were cotransfected with the expression vectors of H-Ras-Val12 and the dominant negative mutants of Rho proteins, RhoA/Asn-19, Rac1/Asn-17, and Cdc42Hs/Asn-17 along with the HIV-LUC plasmid. In keeping with our previous results (1), the induction of the HIV-LUC reporter in response to TNFα was efficiently inhibited by the overexpression of the dominant negative mutants of RhoA and Cdc42Hs proteins (Fig. 5B), indicating that these two GTPases are implicated in this pathway. However, neither of the three Rho mutants had any significant effect in the activation of NF-κB induced by the H-Ras-Val12 protein. These results provide strong evidence that the signal transduction cascades regulated by H-Ras and Rho GTPases for NF-κB activation are mutually independent.

It has also been shown that cytokines such as TNFα or environmental stress such as UV irradiation can induce both c-Jun and NF-κB activities simultaneously, a result that could suggest a cross-talking among these two signaling events. Thus, we then explored the possibility of a common link between the Rho-dependent signal transduction pathways that regulate the activity of c-Jun and NF-κB transcription factors. In this regard, it has been reported that overexpression of MEKK1 (JNKKK) is able to induce the transactivation of the HIV and IL-2Rα promoters in Jurkat cells in an NF-κB-dependent manner (44). On the other hand, a kinase-defective, dominant negative mutant of MEKK1 inhibits the transactivation of a β-CAT reporter induced by TNFα in NIH-3T3 cells (45). Lee et al. (29) have also observed that MEKK1 is able to activate the IκB kinase complex, which is responsible of the site-specific phosphorylation of IκB in Ser-32 and Ser-36 residues. Thus, we investigated whether MEKK1 was involved in the activation of the nuclear factor κB induced by members of the Rho family of small GTP binding proteins. To that end,
COS-7 cells were cotransfected with the HIV-LUC plasmid and the derived expression vectors encoding for the wild type form of the kinase MEKK1, along with the constitutively activated forms (QL) of RhoA, Rac1, and Cdc42Hs proteins or their corresponding empty vectors. As shown in Fig. 6A, the MEKK1 wild type protein was capable of activating NF-κB in COS-7 cells, acting in a cooperative manner in the transactivation of the HIV promoter when coexpressed with Rac1 (QL) or Cdc42Hs (QL). By contrast, the activation by the expression of the RhoA (QL) protein was not affected by the MEKK1 cotransfection. Furthermore, when the constitutively activated forms of RhoA, Rac1, and Cdc42Hs were coexpressed with a catalytically inactive, dominant negative mutant of MEKK1, MEKK\(\Delta\) (K432M), the transactivation of the HIV promoter by Rac1 (QL) and Cdc42Hs (QL) was efficiently inhibited, whereas this dominant negative mutant had no effect on the activation mediated by RhoA (QL) (Fig. 6B). These results suggest that Rac1 and Cdc42Hs, but not RhoA, activate the transcription factor NF-κB through a MEKK1-dependent cascade. A further conclusion from these results is that activation of MEKK1 by Rac1 and Cdc42Hs can be a common link between the JNK/SAPK pathway and the NF-κB pathway. Therefore, agonists that activate Rac1 or Cdc42Hs may simultaneously activate these two pathways through MEKK1. On the other side, RhoA seems to follow an MEKK1-independent mechanism for the activation of NF-κB, and in keeping with this, it does not activate the JNK/SAPK pathway in the COS-7 cells (Fig. 1B).

In summary, we have found that constitutively activated forms of Dbl, Ost and Vav proteins, known exchange factors for Rho GTPases, induce the transactivation of the NF-κB-dependent HIV promoter. We have investigated the in vivo specificity of these molecules for the different Rho GTPases and found that Dbl activates NF-κB in a Cdc42Hs-dependent manner while Vav seems to be selective for Rac1. Finally, both RhoA and Cdc42Hs are required for the activation of NF-κB by Ost.
We have also concluded that Rho and H-Ras GTPases activate NF-κB by mutually independent cascades. We report that the wild type form of MEKK1 induces the transactivation of the HIV promoter in COS-7 cells. Activation of NF-κB by Rac1 and Cdc42Hs GTPases, but not that of RhoA, can be efficiently blocked by a kinase-defective mutant of MEKK1, indicating that Rac1 and Cdc42Hs activate the transcription factor NF-κB by a MEKK1-dependent pathway while RhoA follows an MEKK1-independent mechanism. Our study provides evidence for a cross-talking between NF-κB and JNK/SAPK pathways in COS-7 cells where specific members of the Rho family of GTPases are involved. All these results can be summarized in the scheme shown in Fig. 7, where some of the signaling cascades in which the Ras and Rho GTPases participate are depicted. The results shown here open a new perspective in deciphering the relevance of Rho proteins in the regulation of multiple signal transduction pathways that control gene regulation and trigger diverse responses in mammalian cells.

REFERENCES

1. Perona, R., Montaner, S., Sangier, L., Sánchez-Pérez, I., Bravo, R., and Lacal, J. C. (1997) Genes Dev. 11, 463–473
2. Narumiya, S. (1996) J. Biochem. 120, 215–228
3. Van Aelst, L., and D'Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
4. Coso, O. A., Chiariello, M., Yu, J.-C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. S. (1995) Cell 81, 1137–1146
5. Minden, A., Lin, A., Claret, F. X., Abo, A., and Karin, M. (1995) Cell 81, 1147–1157
6. Teramoto, H., Crespo, P., Xu, N., Igishi, T., Xu, N., and Gutkind, J. S. (1996) J. Biol. Chem. 271, 25731–25734
7. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
8. Miyamoto, S., and Verma, I. M. (1995) Adv. Cancer Res. 66, 255–292
9. Baldwin, Jr., A. S., (1997) Annu. Rev. Immunol. 14, 649–683
10. Lacal, J. C., and McCormick, F. (1993) The ras Superfamily of GTPases, pp. 259–282, CRC Press Inc., Boca Raton, FL
11. Cerione, R. A., and Zheng, Y. (1996) Curr. Opin. Cell Biol. 8, 216–222
12. Evt, A., and Aaronson, S. A. (1995) Nature 374, 314–318
13. Horii, Y., Beeler, J. F., Sakaguchi, K., Tachibana, M., and Miki, T. (1994) EMBIO J. 13, 4776–4786
14. Katzav, S., Martin-Zanca, D., and Barbacid, M. (1989) EMBO J. 8, 2283–2290
15. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
16. Ridley, A. J., Paterson, H. P., Johnston, C. L., Diekmann, D., and Hall, A. (1992) Cell 70, 401–410
17. Nobes, C. D., and Hall, A. (1995) Cell 81, 53–62
18. Kozma, R., Ahmed, S., Best, A., and Lim, L. (1995) Mol. Cell. Biol. 15, 1942–1952
19. Michiels, F., Habets, G. G. M., Stam, J. C., van der Kammen, R. A., and Collard, J. G. (1995) Nature 375, 338–340
20. Michiels, F., Stam, J. C., Hordijk, P. L., van der Kammen, R. A., Ruuls-Van Stalle, L., Feltkamp, C. A., and Collard, J. G. (1997) J. Cell. Biol. 137, 387–398
21. Zhang, Y., Olson, M. F., Hall, A., Cerione, R. A., and Tokas, D. (1995) J. Biol. Chem. 270, 9031–9034
22. Zheng, Y., Fischer, D. J., Santos, M. F., Tigi, G., Pasteris, N. G., Gorski, J. L., and Xu, Y. (1996) J. Biol. Chem. 271, 33169–33172
23. Chou, M. M., and Blenis, J. (1996) Cell 85, 573–583
24. Crespo, P., Bustelo, X. R., Aaronson, D. S., Coso, O. A., López-Barahona, M., Barbacid, M., and Gutkind, J. S. (1996) Oncogene 15, 455–460
25. Perona, R., Esteve, P., Jiménez, B., Ballester, R. P., and Lacal, J. C. (1993) Oncogene 8, 1285–1292
26. Nabel, G., and Baltimore, D. (1987) Nature 326, 711–713
27. Genot, E. M., Parker, P. J., and Cantrell, D. A. (1996) J. Biol. Chem. 271, 9833–9839
28. Montaner, S., Rosas, A., Perona, R., Esteve, P., Carnero, A., and Lacal, J. C. (1995) Oncogene 10, 2213–2220
29. Lee, F. S., Hagler, J., Chen, Z. J., and Maniatis, T. (1997) Cell 88, 213–222
30. Tarakhovsky, A., Turner, M., Schaal, S., Mee, P. J., Duddy, L. P., Rajewsky, K., and Tybulewicz, V. L. J. (1995) Nature 374, 467–470
31. Zhang, R., Alt, F. W., Davidson, L., Orkin, S. H., and Swat, W. (1995) Nature 374, 470–473
32. Crespo, P., Schuebel, K. E., Ostrem, A. A., Gutkind, J. S., and Bustelo, X. R. (1997) Nature 385, 169–172
33. Hart, M. J., Evt, A., Evans, T., Aaronson, S. A., and Cerione, R. A. (1991) Nature 354, 311–314
34. Hart, M. J., Evt, A., Zangaglia, D., Aaronson, S. A., Evans, T., Cerione, R. A., and Zheng, Y. (1994) J. Biol. Chem. 269, 62–65
35. Jiménez, B., Arends, M., Esteve, P., Perona, R., Sánchez-Frieto, R., Rámón y Cajal, S., Yllolie, A., and Lacal, J. C. (1995) Oncogene 10, 811–816
36. Esteve, P., del Peso, L., and Lacal, J. C. (1995) Oncogene 11, 2657–2665
37. Qiu, R.-G., Chen, J., Dinosaur, F., and Simons, M. (1995) Nature 374, 457–459
38. Prenosil, G., Kharasvari-Far, R., Solski, P. A., Kurzawa, H., Lebowitz, P. F., and Der, C. J. (1995) Oncogene 10, 2289–2296
39. Kharasvari-Far, R., Solski, P. A., Clark, G. J., Kilch, M., and Der, C. J. (1995) Mol. Cell. Biol. 15, 6443–6453
40. Qiu, R.-G., Abo, A., McCormick, F., and Simons, M. (1997) Mol. Cell. Biol. 17, 3449–3458
41. Dever, Y., Rosset, C., DiDonato, J. A., and Karin, M. (1993) Science 261, 1432–1445
42. Finco, T. S., and Baldwin, Jr., A. S. (1993) J. Biol. Chem. 268, 17676–17679
43. Kurokawa, A. C., Chen, E. Y., Mivechi, N. F., Denk, N. C., Stambrook, P., and Giacca, A. J. (1996) Cancer Res. 54, 5273–5279
44. Meyer, C. F., Wang, X., Chang, C., Templeton, D., and Tan, T.-H. (1996) J. Biol. Chem. 271, 8971–8976
45. Hirano, M., Osada, A., Aoki, T., Hirai, S., Hosaka, M., Inoue, J., and Ohno, S. (1996) J. Biol. Chem. 271, 13234–13238