The estrogen receptors (ERs) are ligand-inducible transcription factors that play key roles in the control of growth and differentiation in reproductive tissues. We showed that the novel Db1 family proto-oncoprotein Brx enhances ligand-dependent activity of ERα via a Cdc42-dependent pathway. Brx also significantly enhances ligand-dependent activity of ERβ. This enhancement is not affected by inhibition of p44/42 mitogen-activated protein kinase (MAPK) activation by PD98059. However, addition of the p38 MAPK inhibitor SB202190 abrogates the enhancement of ERβ activity by Brx, showing that p38 MAPK activity is required for the enhancement of ERβ function by Brx. In COS-7 cells, transfection of Brx leads to activation of endogenous p38 MAPK activity. Co-expression of the β2 isoform of human p38 MAPK and a constitutively active form of the p38 MAPK kinase MKK6 (MKK6-EE) synergistically augments ligand-dependent activity of ERβ. Our findings suggest that p38 MAPKs may be important regulators of ERβ activity.

The steroid hormone estrogen plays a critical role in the regulation of growth and development of reproductive tissues. The effects of estrogen in these tissues are due to changes in gene expression modulated by the estrogen receptors (ERs) that are members of the nuclear hormone receptor superfamily of transcription factors. After being activated by binding estrogen, the ER activates transcription of hormone-responsive genes. Prior to 1996 estrogen-dependent effects were thought to be mediated by a single estrogen receptor molecule, now designated ERα. Another estrogen receptor, ERβ, was cloned from human testis (1, 2). The ERs are modular transcription factors containing two transcription activation functions, AF-1 located in the N-terminal A/B domain, and AF-2 located within the C-terminal ligand-binding domain. Although AF-2 function is dependent upon ligand binding, AF-1 functions independently of ligand binding (reviewed in Ref. 3) but synergizes with AF-2 in the promotion of ligand-dependent transcription activation by the receptor (4).

The ER can also be activated by ligand-independent pathways involving signals originating from growth factor receptors. This pathway involves signaling from cell surface receptors and results in phosphorylation of the estrogen receptor (reviewed in Refs. 5, 6). Activation of ERα by epidermal growth factor was shown to involve phosphorylation of serine 118 in the AF-1 region of ERα through a Ras-Raf-MAPK signaling pathway (7, 8). Both ERs and ERβ are activated in transient transfection systems by co-expression of the oncogenic V12 mutant of Ras (RasV12). In both cases the activation depends on phosphorylation of target serine residues located within the receptors’ AF-1 domains by p44/42 MAPK (ERK1/2) (9). Ras-dependent phosphorylation of the AF-1 domain of ERβ is thought to enhance ligand-independent transcriptional activation by enhancing recruitment of transcription co-activators such as SRC-1 (10).

We previously described the cloning and characterization of a cDNA encoding a protein Brx (breast cancer nuclear hormone receptor auxiliary factor) that interacts with ERα, is preferentially expressed in reproductive and immune tissues, and augments ligand-dependent transcriptional activation by ERα (11). Sequence analysis showed that Brx belongs to the Db1 family of proto-oncogenes (reviewed in Ref. 12) that function as guanine nucleotide exchange factors (GEFs) for Rho-GTPases, small Ras-related cytoplasmic proteins involved in signal transduction pathways governing growth and morphogenic transformation. Proteins acting as GEFs shift the equilibria of small GTPases toward an active GTP-bound state. We found that activation of ERα by Brx in transient transfection assays involves the Rho-related GTPase Cdc42. Our findings demonstrated for the first time that ERα function might be modulated by pathways dependent on small GTPases other than Ras.

Brx mRNA is highly expressed in the same human tissues that express ERβ mRNA. These include, specifically, ovary, uterus, testis, spleen, thymus, peripheral blood leukocytes, and specific areas of the brain, including the amygdala and hypothalamus, which are thought to be regulated by estrogen. The present study was performed to investigate the effect of Brx on ERβ. We show that Brx enhances ligand-dependent activity of ERβ. Furthermore, we demonstrate that Brx sends signals to ERβ through a pathway involving p38 MAPKs.

EXPERIMENTAL PROCEDURES

Cell Culture and Transient Transfection Studies—Ishikawa human endometrial adenocarcinoma cells were cultured in phenol red-free...
Dulbecco’s modified Eagle’s medium/F-12 (Life Technologies, Inc.) supplemented with 5% charcoal-stripped fetal bovine serum (HyClone). For experiments examining the activation of the c-fos SRE, cells were serum-starved overnight prior to transfection. COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. For experiments examining the activation of endogenous p38 MAPK activity, COS-7 cells were plated onto 100-mm dishes and transfected with 15 μg of expression vector for Brx, pBK-RSV, or MKK6-EE using FuGENE-6 (Roche Molecular Biochemicals) as described by the manufacturer. Twenty-four hours after transfection, cells were subjected to overnight serum starvation prior to lysis and immunoblotting. For transient transfection studies Ishikawa cells were plated onto 12-well plates and 100 ng of pRSV-ERβ, 1 μg of pRSV-Brx, 500 ng of GAL4-Elk-1, 500 ng of GAL4-ERβ or empty vector, and 1.5 μg of pBK-CMV, 1.0 μg of (ERE)-tk-luciferase, 1.0 μg of SRE-tk-luciferase or 1.0 μg of G4E1b-luciferase were added to cells with FuGENE-6 as per the manufacturer’s instructions. Cells were harvested 20 h after transfection, and luciferase assays were performed and normalized as described previously (11). In all transfection experiments, total amounts of DNA were made constant by the addition of empty vectors. 17β-Estradiol (10 nM), 4-hydroxy tamoxifen (40 nM), PD98059 (10 μM), Calbiochem), SB202190 (2 μM, Calbiochem), SB203580 (2 μM, Calbiochem) were added as described.

Plasmids—Expression vectors for Brx, (ERE)-tk-luciferase, SRE-tk-luciferase, and MEKE, were described previously (11, 13–15). A full-length cDNA-encoding human ERβ (2) was amplified from a human prostate Marathon-Ready cDNA library (CLONTECH). The cDNA was subcloned into pBK-RSV (Stratagene) and sequenced to make RSV-ERβ. The ERβ cDNA beginning at the second codon was subcloned into the pIM expression vector (CLONTECH) containing the GAL4 DNA binding domain to make GAL4-ERβ. A full-length cDNA encoding Elk-1 was amplified from a human peripheral blood leukocyte cDNA library (CLONTECH), subcloned into pIM, and sequenced to make GAL4-Elk-1. Expression plasmids for MEKE and wild type MKK6 were kindly provided by Dr. Silvio Gutkind. MKK6-EE containing Ser to Glu and Thr to Glu mutations at codons 207 and 211, respectively, were derived from wild-type MKK6 by overlap amplification with primers containing site-specific mutations. The resulting cDNA was subcloned into pBK-RSV, and individual clones were sequenced to verify the presence of the mutations. Full-length cDNA encoding human p38β2 was amplified from a brain cDNA library (CLONTECH), subcloned into pBK-RSV, and sequenced.

Immunoblotting—Transfected COS-7 cells were harvested at 4 °C and lysed in SDS sample buffer by sonication. Following centrifugation, proteins were resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose (Schleicher and Schuell) by semi-dry electroblotting. Membranes were blocked in Tris-buffered saline containing 0.05% Tween-20 and 5% nonfat dry milk. Phospho-p38 MAPK primary antibody and HRP-conjugated anti-rabbit secondary antibody (both from New England BioLabs) were used and detected per the manufacturer’s instructions. Epitope-tagged Brx and GAL4-ERβ were detected with HRP-conjugated anti-FLAG M2 monoclonal antibody (Sigma Chemical Co.) and HRP-conjugated GAL4 (DBD) monoclonal antibody (Santa Cruz Biotechnology, Inc.) per the manufacturers’ instructions.

Protein Phosphorylation—Proteins were phosphorylated with active recombinant human p38β2 (Upstate Biotech Inc.) per the manufacturer’s instructions. Twenty milligrams of protein substrates were incubated with 100 ng of active p38β2 in the presence of [γ-32P]ATP (1 mCi/ml) at 30 °C for 15 min with rotation in a final reaction volume of 40 ml. Reactions were stopped by addition of SDS sample buffer and heating to 95 °C for 5 min. Labeled proteins were resolved on 10% SDS-polyacrylamide gels and detected by autoradiography.

RESULTS

Bṛx Enhances Ligand-dependent Transcriptional Activation by Estrogen Receptor β—Given our previous observation that Bṛx enhances ligand-dependent transcriptional activation by ERβ in transiently transfected Ishikawa endometrial cells, we wanted to test whether Bṛx expression could also affect the ability of ERβ to activate a reporter. Transfection of ERβ resulted in severalfold ligand-dependent activation of the G4E1b-luciferase reporter (Fig. 1A). Co-transfection of an expression vector for full-length Bṛx enhanced ligand-dependent transcriptional activation by ERβ 10-fold.

Although tamoxifen acts as an estrogen antagonist in the breast, in the uterus it acts as an estrogen agonist, and long-term treatment of breast cancer with tamoxifen results in an increased risk of endometrial cancer (16, 17). We found that in Ishikawa cells 40 nm 4-hydroxytamoxifen blocked ligand-dependent activity of ERβ (Fig. 1A). Addition of 40 nm 4-hydroxytamoxifen completely eliminated ligand-dependent activation of the reporter by ERβ in the absence of Bṛx and greatly reduced ligand-dependent activity of the receptor in the presence of Bṛx. These results suggest that the full level of activity...
of ERβ in the presence of Brx was dependent on activation of the receptor by ligand binding. In our previous study we reported an antagonistic effect of tamoxifen on the ligand-dependent activity of ERα in Ishikawa cells (11).

We tested the possibility that Brx augments receptor activity through an effect on receptor expression levels. COS-7 cells were transfected with expression vectors for Brx and GAL4-ERβ. Western analyses showed that co-expression of Brx did not affect the level of GAL4-ERβ expression (Fig. 1B), suggesting that the observed augmentation of ligand-dependent activity of ERβ by Brx is not simply due to an increase in the amount of receptor protein expressed. We also tested the specificity of Brx action by assessing the ability of Brx to activate a chimeric GAL4-VP16 transcription factor. Under conditions in which Brx enhances the ligand-dependent activity of ERβ, Brx failed to enhance the activity of GAL4-VP16 (Fig. 1C). Brx expression also did not affect the activity of the G4E1b-luciferase reporter.

Brx Signaling to ERβ Does Not Involve p44/42 MAPK (ERK1/ERK2)—ERβ has been shown to be activated independently of ligand binding by a mechanism involving phosphorylation of Ser residues in AF-1 through the Ras-Raf-p44/42 MAPK (ERK1/ERK2) signaling pathway (9). It has also been shown that Cdc42 can activate ERK2 in cooperation with Raf (18). We therefore tested whether activation of ERβ by Brx involves p44/42 MAPK. Dual specificity mitogen-activated protein kinase kinases (MEK) activate p44/42 MAPK by phosphorylating specific Thr and Tyr residues. The inhibitor PD98059 is thought to block activation of p44/42 MAPK (19) by binding to inactive MEK1 and blocking activation by upstream kinases (20). In these experiments Brx only modestly affected activity of the receptor. Addition of 10 μM PD98059 did not affect the enhancement of ligand-dependent activity of ERβ by Brx (Fig. 2A). Addition of the inhibitor also did not affect ligand-dependent activity of ERβ in the absence of Brx. In control experiments the same concentration of PD98059 effectively inhibited activation of a SRE-tk-Luc reporter by mutationally activated MEK1 (21) (Fig. 2B).

Brx Activates ERβ by a p38 MAPK-dependent Pathway—We previously showed that Brx enhances ligand-dependent activity of ERα by a Cdc42-dependent pathway. Because activation of ERα by Brx did not appear to involve p44/42 MAPK pathways, and because Cdc42 has been shown to activate p38 MAPKs (22), we tested whether the enhancement of ERα activity by Brx could involve a p38 MAPK pathway. The pyridinyl imidazole compound SB202190 inhibits the activity of p38 MAPKs by a mechanism involving direct binding to the isoforms of p38 MAPKs by a mechanism involving direct binding to the kinases (23). In our transient transfection system using ERα expressed as a GAL4 DBD fusion protein (GAL4-ERα) and the G4E1b-luciferase reporter, transfection of GAL4-ERα activated luciferase expression severalfold in the presence of ligand compared with control transfections lacking ligand. Co-transfection of Brx enhanced ligand-dependent activity of GAL4-ERα–10-fold (Fig. 3A). Addition of 2 μM SB202190 inhibited activation of ERα by Brx by ~70%, suggesting that p38α or p38β activity is required for enhancement of ligand-dependent activity of ERα by Brx. SB202190 did not inhibit the activity of a chimeric GAL4-VP16 transcription factor (Fig. 3B).

Because our results suggested that p38 MAPK activity is required for augmentation of receptor activity by Brx, we tested whether p38 activation affects activity of ERβ in Ishikawa cells. The p38 MAPKs can be activated by several dual-specificity MAPK kinases, including MKK3, MKK6, MKK4, and MKK7 (24–27). MKK4 and MKK7 also efficiently activate JNK, and MKK3 activates only p38α, γ, and δ. Each of the p38 MAPKs (α, β, γ, and δ) has been shown to be phosphorylated and activated by MKK6 (28). A constitutively active mutant form of MKK6 (MKK6-EE), contains Ser to Glu and Thr to Glu mutations at amino acid positions 207 and 211, respectively, and activates p38 MAPKs (29).

Transfection of Ishikawa cells with an expression vector for the β2 isoform of p38 MAPK or MKK6-EE alone did not activate GAL4-ERβ, but co-transfection of both synergistically enhanced ligand-dependent activity of GAL4-ERβ (Fig. 4A). The transcription factor Elk-1 is thought to be a physiological substrate of p38 MAPK (29). We therefore performed control experiments to test for activation of Elk-1 by MKK6-EE and p38β2 in Ishikawa cells. As we observed for ERβ, transfection of either p38β2 or MKK6-EE was not sufficient to activate a control GAL4-Erk-1 construct. Co-transfection of Ishikawa cells with both p38β2 and MKK6-EE synergistically activated...
The estrogen receptors (ERs) are modular ligand-inducible transcription factors that play a key role in the regulation of growth and differentiation of reproductive tissues. An important issue in the study of ER signal transduction pathways is that of ligand-independent activation (5). Studies have documented effects upon ER-mediated gene activation by epidermal growth factor and other growth factors acting as ligand-independent receptor activators. Both ERα and ERβ can be activated independently of ligand binding by a Ras-Raf-p44/42 MAPK signaling pathway involving the phosphorylation of specific Ser residues in the receptors’ AF-1 domains (9). In the case of ERβ, modification of the receptor was proposed to affect transcription by enhancing the recruitment of steroid receptor co-activator-1 (SRC-1) (10). The demonstration of signaling pathways linking Ras to regulation of ER activity was an important observation, because Ras has a critical role in cellular proliferation and oncogenesis.

Members of the Ras-related Rho family of small GTPases, including Rho, Rac, and Cdc42, regulate a variety of crucial cellular processes, including cytoskeletal organization, gene expression, cell cycle progression, cell adhesion, and intracellular membrane trafficking (30, 31). These proteins are regulated by GTP binding and are activated by guanine nucleotide exchange factors (Rho-GEFs) that catalyze the exchange of GDP for GTP. Rho-GEFs constitute a group of oncoproteins containing Dbl homology and pleckstrin homology domains that are required for the nucleotide exchange function (12). Different Rho-GEFs possess a variety of structural motifs presumably regulating function and specific interactions with other proteins. Although the Rho-GEFs are thought to be important targets of upstream activators of Rho GTPases, relatively little is known about the regulation of function of most of the GEFs. We recently described the cloning of brx, a novel Dbl family proto-oncogene, encoding Brx, a protein that enhances ligand-dependent activity of ERα by a Cdc42-dependent pathway (11). Our report was the first to implicate Rho GTPases in the regulation of ER function. Recently, Su et al. (32) demonstrated enhancement of ERα activity by the guanine nucleotide dissociation inhibitor GD1α, a finding that provided further support for a role for Rho GTPases in the regulation of ER activity. In this report we demonstrate that Brx enhances ligand-dependent activity of ERβ. The mechanism appears not to involve the p44/42 MAPK pathway, but instead involves activation of a p38 MAPK pathway (Fig. 5). This report represents the first report implicating p38 MAPK activation in the regulation of ERβ function.

Brx mRNA is highly expressed in human tissues that express ERβ mRNA, specifically including ovary, uterus, testis, spleen, thymus, peripheral blood leukocytes, and specific areas of the brain, including the amygdala and hypothalamus, which are thought to be regulated by estrogen (11).2 Immunohistochemical staining of human ovarian tissues showed high levels of Brx protein expression in granulosa cells of follicles (33). These data suggest that Brx is co-expressed in tissues expressing ERβ. We also found that Brx interacts with ERβ in binding assays in vitro.2 Given these findings, we wanted to know whether Brx affects ERβ function. In transient transfection assays using Ishikawa human endometrial adenocarcinoma cells, ligand-dependent activity of ERβ was markedly enhanced by co-transfection of a Brx expression vector. The full level of reporter activity required Brx, ERβ, the ERE, and estrogen. Addition of tamoxifen totally eliminated ligand-dependent activity of ERβ. In control experiments we demonstrated that activation of Brx is selective, because Brx did not affect the activity of the chimeric transcription factor GAL4-VP16. Additionally, analysis of protein expression levels in transfected COS-7 cells showed that Brx did not affect the level of GAL4-ERβ protein expression, a finding that suggests Brx does not augment ligand-dependent receptor activity simply by increasing receptor expression. Taken together, these results suggest that Brx affects the ligand-dependent activity of ERβ itself.
We previously demonstrated that Brx augments ligand-dependent activity of ERα by a Cdc42-dependent pathway (11). Mutationally activated Cdc42 also strongly activates JNK and p38 MAPK (14, 22). One possibility, therefore, is that Brx activates a JNK or p38 MAPK that in turn augments ligand-dependent function of ERβ either directly or indirectly. We therefore tested for an effect of the p38 MAPK inhibitor SB202190 on ERβ activation by Brx and found that 2 μM SB202190 inhibited enhancement of ERβ activity by Brx by ~70%, suggesting that p38 MAPK activity is required for activation of ERβ by Brx. We next tested directly whether a specific p38 MAPK could activate ERβ. Although transient transfection of either p38β2 or MKK6-EE was insufficient to activate the receptor, transfection of both synergistically activated the receptor. Similarly, for the GAL4-Elk-1 transfection control experiments, we found that the combination of both p38β2 and MKK6-EE cooperatively activated the luciferase reporter. We do not know why transfection of MKK6-EE was insufficient to activate ERβ. It is possible that MKK6-EE was unable to activate cellular p38 MAPK in Ishikawa cells, although it was able to do so in COS-7 cells in our control experiment. Unfortunately, our transfection efficiency for the Ishikawa cells has been too low to permit a similar analysis. Selectivity of p38 MAPK isomorph activation by MKK6 has been described by Alonso (34). This group showed that, at relatively low concentrations both in vitro and in vivo, wild-type MKK6 activated p38α but failed to activate p38γ. At present, we do not know which p38 MAPK isoforms are expressed in Ishikawa cells and we have not yet determined whether the enhancement of ERβ activity by Brx exhibits selectivity for p38 MAPK isoforms. It is also possible that MKK6 is not involved in activation of ERβ by Brx in the Ishikawa cells. Our findings do suggest, however, that endogenous p38 MAPK activity in Ishikawa is required for activation of ERβ by Brx and that activation of p38β2 MAPK by MKK6-EE effectively enhances ligand-dependent activity of the receptor. We showed that Brx expression results in the activation of endogenous p38 MAPK in COS-7 cells.

p38 MAPKs have well established roles in the regulation and function of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1; they are also activated in cultured cells in response to environmental stresses such as exposure to UV radiation and hyperosmotic conditions (reviewed in Ref. 35). Recent experiments suggest that activation of p38 MAPK pathways may also play important roles in cellular differenti-
ERβ Regulation by p38 MAPK

Publication in vitro of several cell lines has been shown to depend on p38 MAPK activity (reviewed in Ref. 36). It was recently reported that activation of p38 MAPKs is deficient in rhabdomyosarcoma cells (37). Activation of p38 MAPKs in these cells by ectopic expression of MKK6-EE resulted in growth arrest and terminal differentiation. Our findings suggest that p38 MAPKs may also play a role in the regulation of estrogen receptors, which are in turn key regulators of growth and differentiation in reproductive tissues.

During the preparation of this manuscript, Lee et al. (38) published a report showing activation of ERs by constitutively active MEK1 in Ishikawa cells. These authors proposed that MEK1 activates ERα through activation of JNK and p38 MAPK. They also demonstrated phosphorylation of ERα by p38 MAPK in vitro. These findings, considered together with our own results, suggest that p38 MAPKs may be important regulators of both forms of ER.

We do not know the mechanism for enhancement of ERβ activity by p38 MAPK and whether direct phosphorylation of the receptor by p38 MAPK is involved. However, we observed that p38 MAPK efficiently phosphorylated ERβ in vitro. By analogy with p44/42 MAPK-dependent activation of ERβ, direct phosphorylation of the receptor could affect the ability of liganded receptor to interact with or recruit a transcriptional co-activator such as SRC-1. Alternatively, p38 MAPK may indirectly affect the activity of ERβ by phosphorylating other factors required for ERβ function such as SRC-1 or p300.

Acknowledgments—We are grateful to Dr. Silvio Gutkind for the kind gifts of COS-7 cells and expression vectors for MKK6 and MEKE. We thank Drs. William Haffner and Prabir Chakraborty for their support during this project.

REFERENCES

1. Kuiper, G. G. J. M., Enmark, E., Pelto-Huikko, M., Nilsson, S., and Gustafsson, J.-A. (1996) J. Biol. Chem. 271, 5295–5300
2. Masselman, S., Polman, J., and Dijkema, R. (1996) FEBS Lett. 382, 49–53
3. Tsai, M., and O'Malley, B. W. (1994) Annu. Rev. Biochem. 63, 451–486
4. Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E., and Chambon, P. (1989) Cell 59, 477–487
5. O'Malley, B. W., Schrader, W. T., Mani, S., Smith, C., Weigel, N. L., Conney, O. M., and Clark, J. H. (1995) Recent Prog. Horm. Res. 50, 333–347
6. Kato, S., Masuhirou, Y., Watanabe, M., Kobayashi, Y., Takeda, K., Endoh, H., and Yanagimachi, J. (2000) Genes Cells 5, 593–601
7. Kato, S., Endoh, H., Masuhirou, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masuhirou, S., Gotoh, Y., Nishida, K., Kawashima, H., Metzger, D., and Chambon, P. (1995) Science 270, 1491–1494
8. Bunone, G., Briand, P. A., Miksicek, R. J., and Picard, D. (1996) EMBO J. 15, 2174–2183
9. Tremblay, G. B., Tremblay, A., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Lefebvre, F., and Giguere, V. (1999) Mol. Cell 3, 513–519
10. Rubino, D., Driggers, P., Arbit, D., Kemp, L., Miller, B., Coce, O., Guagliani, K., Gray, K., Gutkind, J., and Segars, J. (1996) EMBO J. 15, 2513–2520
11. Ceroni, R. A., and Zheng, Y. (1996) Curr. Opin. Cell Biol. 8, 216–222
12. Nunez, S., Medin, J., Brauniant, O., Kemp, L., Wahl, W., Ozato, K., and Segars, J. (1997) Mol. Cell. Endocrinol. 127, 27–40
13. Coce, O., Chiariello, M., Yu, J., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. (1995) Cell 81, 1137–1146
14. Fromm, C., Coce, O., Montazer, S., Xu, N., and Gutkind, J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10988–10103
15. Rutqvist, L. E., Johansson, H., Signomklao, T., Johansson, U., Forsander, T., and Wilking, N. (1995) J. Natl. Cancer Inst. 87, 645–651
16. Fisher, B., Costantine, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., Vogel, K., Robidou, A., Dimioit, N., Atkins, J., Daly, M., Weand, S., Tan-Chiu, E., Ford, L., and Wolmark, N. (1998) J. Natl. Cancer Inst. 90, 1371–1388
17. Frost, J. A., Steen, H., Shapiro, P., Lewis, T., Ahn, N., Shaw, P. E., and Cobb, M. (1997) EMBO J. 16, 6426–6438
18. Pang, L., Sawada, T., Decker, S. J., and Saltiel, A. (1995) J. Biol. Chem. 270, 13285–13298
19. Aleski, D. R., Cuenca, A., Cohen, P., Dudley, D. T., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 27484–27494
20. Cowley, S., Paterson, H., Kemp, P., and Marshall, C. J. (1994) Cell 77, 841–852
21. Minden, A., Lin, A., Clarlet, F. X., Abo, A., and Karin, M. (1995) Cell 81, 1147–1157
22. O'Malley, B. W., Laydon, J. T., McDowell, F. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., and Landvatter, S. W. (1994) Nature 372, 739–746
23. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kerr, K., and Chambon, P. (1989) Science 243, 486–489
24. Cowley, S., Paterson, H., Kemp, P., and Marshall, C. J. (1994) Cell 77, 841–852
25. Lin, A., Minden, A., Martinetto, H., Clarlet, F. X., Lange-Carter, M., Mercuri, F., Johnson, G. L., and Karin, M. (1995) Science 268, 286–290
26. David, B., Raisingh, J., Barrett, T. S., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) Science 267, 682–685
27. Hu, M. C.-T., Wang, Y.-F., Mikhail, A., Qiu, W. R., and Tan, T.-H. (1997) J. Biol. Chem. 272, 7095–7102
28. Keeler, G. A., Bray, J., Hunt, J., Johnson, D. A., Gleason, T., Yao, Z., Wang, S.-W., Parker, C., Yamane, H., Cole, C., and Lichtenstein, H. S. (1998) Protein Expr. Purif. 14, 221–228
29. Raisingh, J., Whilmarch, A., Barrett, T., Derijard, B., and Davis, R. J. (1996) Mol. Cell. Biol. 16, 1247–1255
30. Van Aelst, L., and D'Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
31. kjeller, L., and Hall, A. (1996) Exp. Cell. Res. 233, 166–179
32. Su, I. F., Knoblauch, R., and Garabedian, M. J. (2001) J. Biol. Chem. 276, 3231–3237
33. Miller, B., Rubino, D., Driggers, P., Haddad, B., Cisar, M., Gray, K., and Segars, J. (2000) Am. J. Obstet. Gynecol. 182, 295–298
34. Alonso, G., Ambrosino, C., Jones, M., and Nebreda, A. R. (2000) J. Biol. Chem. 275, 4041–4048
35. Paul, A., Wilson, S., Belham, C. M., Robinson, C. J. M., Scott, P. H., Gould, G. W., and Plevin, R. (1997) Cell. Signal. 9, 403–410
36. Nebreda, A., and Porras, A. (2000) Trends Biochem. Sci. 25, 257–260
37. Puri, P. L., Wu, Z., Zhang, P., Wood, L. D., Bhakta, K. S., Han, J., Feramisco, J. R., Karin, M., and Wang, J. Y. (2000) Genes Dev. 14, 574–584
38. Lee, H., Jiang, F., Wang, Q., Nicosia, S. V., Yang, J., Su, B., and Bai, W. (2000) Mol. Endocrinol. 14, 1882–1896
The Proto-oncoprotein Brx Activates Estrogen Receptor β by a p38 Mitogen-activated Protein Kinase Pathway
Paul H. Driggers, James H. Segars and Domenica M. Rubino

J. Biol. Chem. 2001, 276:46792-46797.
doi: 10.1074/jbc.M106927200 originally published online September 28, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106927200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 38 references, 13 of which can be accessed free at http://www.jbc.org/content/276/50/46792.full.html#ref-list-1