Peroxisome Proliferator-activated Receptor α (PPARα) Turnover by the Ubiquitin-Proteasome System Controls the Ligand-induced Expression Level of Its Target Genes*

Received for publication, December 5, 2002, and in revised form, May 14, 2002
Published, JBC Papers in Press, July 12, 2002, DOI 10.1074/jbc.M110598200

Christophe Bianquart‡, Olivier Barbier§, Jean-Charles Fruchart, Bart Staels, and Corine Glineur†

From the INSERM U954, Département d’Athérosclérose, Institut Pasteur de Lille, 1 rue du Pr. Calmette 59019 Lille, France and the Faculté de Pharmacie, Université de Lille II, 59000 Lille, France

Peroxisome proliferator-activated receptor α (PPARα) is a ligand-activated transcription factor belonging to the nuclear receptor family. PPARα is implicated in the regulation of lipid and glucose metabolism and in the control of inflammatory response. Recently, it has been demonstrated that a number of nuclear receptors are degraded by the ubiquitin-proteasome pathway. Since PPARα exhibits a circadian expression rhythm and since PPARα is rapidly regulated under certain pathophysiological conditions such as the acute phase inflammatory response, we hypothesized that PPARα protein levels must be under tight control. Here, we studied the mechanisms controlling PPARα protein levels and their consequences on the transcriptional control of PPARα target genes. Using pulse-chase experiments, it is shown that PPARα is a short-lived protein and that addition of its ligands stabilizes this nuclear receptor. By transient cotransfection experiments using expression vectors for PPARα and hemagglutinin-tagged ubiquitin, it is demonstrated that PPARα protein is ubiquitinated and that its ligands decrease the ubiquitination of this nuclear receptor, thus providing a mechanism for the ligand-dependent stabilization observed in pulse-chase experiments. In addition, treatment with MG132, a selective proteasome inhibitor, increases the level of ubiquitinated PPARα and inhibits its degradation in transfected cells. Furthermore, MG132 treatment enhances the level of endogenous PPARα in HepG2 cells. Finally, transient transfection and quantitative reverse transcription-PCR show that inhibition of PPARα degradation increases its transcriptional activation and expression of target genes such as apoA-II and fatty acid transport protein (FATP). Taken together, these data demonstrate that PPARα is degraded by the ubiquitin-proteasome system in a ligand-dependent manner. Regulation of its degradation provides a novel regulatory mechanism of transcriptional activity of this nuclear receptor.

The peroxisome proliferator-activated receptors (PPARs)1 are members of the nuclear receptor superfamily that act as ligand-dependent transcription factors. PPARα is highly expressed in liver, skeletal and cardiac muscle, and proximal tubular epithelium of kidney. A significant expression of PPARα has also been shown in endothelial cells, smooth muscle cells, and cells involved in the inflammatory process (1). The ligands of PPARα are natural fatty acids and derivatives such as leukotriene B4 and 8-S-hydroxyeicosatetraenoic acid or oxidized phospholipids from oxidized low density lipoprotein. The fatty hypolipidemic drugs are synthetic PPARα ligands (1). PPARα plays a role in intracellular fatty acid metabolism and in triglyceride metabolism by regulating genes involved in the transport and degradation of fatty acids in mitochondria and peroxisomes (2). PPARα is also implicated in the metabolism of lipids and lipoproteins. As a result, PPARα activation decreases the hepatic very low density lipoprotein secretion and plasma triglyceride levels (3). Furthermore, PPARα agonists increase plasma concentration of high density lipoprotein protein particles by regulating the transcription of the major high density lipoprotein apolipoproteins (apo) in liver including apoA-II (4). PPARα regulates gene expression by binding, as a heterodimer with the retinoic acid receptor, to specific DNA sequences, called PPAR response elements (PPRE), resulting in the transcriptional activation of target genes (5). More recently, PPARα has also been shown to play a negative role in the inflammatory response by interfering negatively with the AP-1 and NF-κB signaling pathway (6).

Physiological responses to nuclear receptor ligands not only depend on the potency of the ligand but also on the expression levels of the nuclear receptors in a given tissue. Regulation of nuclear receptor expression occurs at both protein and mRNA levels. For instance, expression of certain nuclear receptors is repressed in the acute phase inflammatory response (7). This effect is very rapid, which suggests a tight control not only of nuclear receptor mRNA but also of protein levels, likely via a control of the degradation and stability of the nuclear receptors. Indeed, several nuclear receptors, such as the retinoid X receptor α, the retinoic acid receptor γ (8, 9), the retinoic acid receptor α (9), the thyroid hormone receptor (10), and PPARγ (11), have been shown to be degraded by the ubiquitin-proteasome system. This degradation pathway is implicated in the regulation of many short-lived proteins involved in essential functions of the cells, including cell cycle control, transcription regulation, and signal transduction (12). The proteins degraded by this pathway are covalently modified by fixation of an 8-kDa apolipoprotein; FATP, fatty acid transport protein; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; RIPA, radioimmuno-precipitation assay; TK, thymidine kinase; Wy 14,643, 4-chloro-6-(2,3-xylidino)-2-pyrimidinyl-thio)acetic acid.

* 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 1734 solely to indicate this fact.
‡ Supported by a fellowship from the Région Nord-Pas-de-Calais and the Institut Pasteur de Lille.
§ Supported by a fellowship from la Ligue contre le Cancer.
¶ To whom correspondence should be addressed. Tel.: 33-3-20-87-77-75; Fax: 33-3-20-87-71-98; E-mail: Corine.Glineur@pasteur-lille.fr.
1 The abbreviations used are: PPAR, peroxisome proliferator activated-receptor; PPRE, PPAR response elements; HA, hemagglutinin; apo,
polypeptide, called ubiquitin, on lysine residues in a three-step process. In the first step, ubiquitin is activated by a ubiquitin-activating enzyme (E1). Then, the activated ubiquitin is transferred to a ubiquitin carrier protein (E2). Finally, ubiquitin-protein isopeptide ligase (E3) catalyzes the covalent bond of ubiquitin to the target protein. Following this process, multiubiquitinated proteins are rapidly degraded by the 26 S proteasome (13).

We demonstrated previously that PPARα mRNA and protein levels follow a circadian rhythm (14). More recently, it was reported that PPARα mRNA is rapidly down-regulated in the acute phase inflammatory response (7). Since these responses imply a rapid regulation also at the levels of PPARα protein, the present study was designed to test whether PPARα protein levels are controlled by the ubiquitin-proteasome degradation pathway. Our results demonstrate that PPARα is an unstable protein that is rapidly degraded and that ligand activation stabilizes this nuclear receptor. Moreover, we show that the degradation of PPARα involves the ubiquitin-proteasome pathway and that its stabilization observed in the presence of the ligand is due to a decrease of PPARα ubiquitination. Inhibition of the proteasome increases the amount of PPARα protein and consequently the transcriptional activation of PPARα-dependent promoters. These results indicate that the proteasome plays an important role in the regulation of PPARα protein level, a mechanism contributing to the magnitude of ligand response.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium (DMEM) without methionine was purchased from ICN (Oray, France), and DMEM and fetal calf serum (FCS) were purchased from Invitrogen. [35S]methionine was manufactured by PerkinElmer Life Sciences. Plasmids containing the expression vector (pSG5hPPARα) (16) or the control TK-pGL3 and 50 ng of pSVβ-galactosidase control vectors were used. ExGen 500 and cytochalasin D were obtained from PerkinElmer Life Sciences (Darmstadt, Germany). Methyl-[-U-35S]-methionine, [35S]-labeled PPARα, and [35S]-[125I]-labeled PPARα were obtained from PerkinElmer Life Sciences. These compounds were used for determining the radioactivity contained in the pellet by autoradiography. The radioactivity was determined using the BCA kit. Twenty μg of protein extracts were analyzed by Western blot using a monoclonal antibody against the HA epitope. The blot was revealed with the ECL reagent (Amersham Biosciences) reagent according to the manufacturer’s protocol.

Preparation of Nuclear Extracts and Western Blot Analysis—HepG2 cells were treated with 40 μM MG132 for 2 or 4 h in the absence of serum and subsequently trypsinized and washed. Then, cells were resuspended in DMEM medium containing 10% FCS and 1% MeSO, frozen in liquid nitrogen, and conserved at −80 °C. To prepare cytoplasmic extracts, cells were centrifuged for 5 min at 800 × g and resuspended in 5 ml of HB buffer (15 mM Tris-HCl (pH 8), 15 mM NaCl, 60 mM KCl, 0.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride) centrifuged at 800 × g for 5 min, resuspended in 200 μl of HB buffer supplemented with 0.05% Triton-X-100 (Sigma), and centrifuged for 10 min at 1,000 × g, and the supernatant was collected. The pellet containing the nuclei was washed with 5 ml of HB buffer containing 0.05% Triton-X-100 and 5 ml of HB buffer. Nuclei were incubated at 4 °C for 30 min in 50 μl of HB buffer containing 360 mM KCl and centrifuged for 5 min at 10,000 × g, and the supernatant corresponding to the nuclear extract was collected. The concentration of protein in the extracts was determined using the BCA kit. Twenty μg of nuclear extracts were analyzed by Western blot using the anti-PPARα antibody. The blot was revealed with the ECL reagent.

Transfection Experiments—HepG2 cells were cultured in 24-well plates. Cells were transfected with 10 ng of PPRE-containing reporter plasmid J6-TK-pGL3 (4) or the control TK-pGL3 and 50 ng of pSVβ-galactosidase control vectors using ExGen 500. After 24 h, cells were treated with 40 μM MG132 for 2 or 4 h prior to adding 50 μM Wy 14,643 or 50 μM Wy 14,643 or Me2SO for 2 or 4 h. RNA extraction was performed using TRIzol (Invitrogen) reagent according to the manufacturer’s protocol. One μg of total RNA was reverse-transcribed with random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Invitrogen). RNA levels were measured by quantitative PCR using the LightCycler-FastStar DNA SYBR Green I kit (Roche Diagnostics). 

RESULTS

PPARα Is a Short-lived Protein That Is Stabilized by Its Ligand, Wy 14,643—To study the stability of PPARα protein, pulse-chase experiments were performed in COS 7 cells transfected with the pSG5hPPARα expression vector. PPARα protein was immunoprecipitated after a chase of 0, 2, 5, 10, and 24 h. In the absence of ligand, PPARα protein is rapidly degraded in cells (Fig. 1A). After 2 h of chase, the quantity of [35S]-labeled PPARα obtained was drastically decreased, indicating that PPARα is a short-lived protein. Since ligand activation has been shown to influence the stability of nuclear
receptor proteins (20–24), the effect of treatment with Wy 14,643, a PPARα ligand, was tested next. After 2 and 5 h of chase, a significantly higher amount of [35S]-labeled PPARα protein was observed in the presence of Wy 14,643 than in the presence of Me2SO (Fig. 1A), indicating that Wy 14,643 induces a stabilization of PPARα protein. Next, a more detailed time course was performed to determine PPARα protein stability in the presence and absence of ligand (Fig. 1B). This experiment showed that PPARα presents a half-life of approximately 1 h in the absence of ligand and of approximately 2 h in the presence of Wy 14,643. Again, when compared with the vehicle-treated cells, slower degradation of PPARα protein was observed in the presence of Wy 14,643 (Fig. 1B). Interestingly, this protective effect of the ligand was observed only during the first 3 h of activation (Fig. 1B). These data indicate that PPARα protein is rapidly degraded in cells and that ligand activation stabilizes this protein in a transitory manner.

**PPARα Protein Is Degraded by the Proteasome.**—To demonstrate the implication of the proteasome in the degradation of PPARα, a pulse-chase experiment was performed in COS 7 cells transfected with the pSG5hPPARα expression vector and treated or not with MG132. PPARα protein was immunoprecipitated after 0, 2, and 4 h. In line with the data above, the results obtained show that PPARα protein is rapidly degraded in the cells. After 2 h of chase, a very low amount of [35S]-labeled PPARα proteins was observed, which became undetectable after 4 h (Fig. 1C). In the presence of MG132, a stabilization of the protein was observed. After 2 h of chase, the quantity of PPARα protein was not reduced (Fig. 1D). Since HepG2 cells express significant amounts of endogenous PPARα protein (25), the influence of MG132 on endogenous PPARα level was analyzed. Western blot analysis using a specific PPARα antisera demonstrated that treatment of HepG2 cells with MG132 (Fig. 2, lanes 2 and 3) resulted in a significant increase of endogenous PPARα proteins levels (Fig. 2, lane 1). These results indicate that both endogenously and exogenously expressed PPARα proteins are degraded by the ubiquitin-proteasome pathway.

**PPARα Protein Is Ubiquitinated in a Ligand-dependent Manner.**—A number of nuclear receptors are degraded by the ubiquitin-proteasome pathway (8–11). To determine whether PPARα is also degraded by this system, COS 7 cells were cotransfected with expression vectors for PPARα and an HA epitope-tagged ubiquitin. After treatment with Wy 14,643 or vehicle for 5 h, PPARα proteins were immunoprecipitated, and the ubiquitinated PPARα proteins were revealed with an HA antiserum (Fig. 3, lane 3). The high molecular weight of the ubiquitinated PPARα proteins suggests the presence of numerous ubiquitination sites. Addition of Wy 14,643 decreased the amount of ubiquitinated PPARα protein (Fig. 3, lane 4), data that are in line with the stabilization effect of the ligand obtained in the pulse-chase experiment (Fig. 1). To determine whether the ubiquitinated PPARα protein is degraded by the proteasome, COS 7 cells transfected with an expression vector for PPARα were treated with the proteasome...
inhibitor, MG132, which inhibits the degradation of ubiquitin-conjugated proteins by the 26 S proteasome complex. In the presence of this inhibitor, an increase of ubiquitinated PPARα protein was observed in cells treated or not with Wy 14,643 (Fig. 3, lanes 5 and 6). These results show that ubiquitinated PPARα protein is degraded by the proteasome. To confirm that the effect of Wy 14,643 on PPARα ubiquitination is a ligand-specific effect, the influence of other PPARα ligands, including clinically used fibrates and the highly specific PPARα agonist GW7647, were analyzed next. In addition, the influence of cerivastatin, which is not a PPARα ligand but which is known to activate this nuclear receptor by modulating its phosphorylation status (25), was tested. The results obtained show that the decrease in PPARα ubiquitination was observed only with the PPARα ligands but not with cerivastatin (Fig. 4). These data demonstrate that PPARα is ubiquitinated and that ligand activation decreases PPARα protein ubiquitination.

**Inhibition of PPARα Degradation by the Proteasome Increases Its Transcriptional Activity—**To determine the consequence of inhibition of PPARα degradation on its transcriptional activity, HepG2 cells were transfected with a reporter vector containing the J site PRE of the apoA-II gene promoter (J6-TK-pGL3) or with the control reporter vector TK-pGL3 and treated or not with MG132 for 2 and 4 h prior to activation with the Wy 14,643 compound. In cells transfected with the TK-pGL3, no modification of the reporter activity was induced by the treatment with Wy 14,643 and MG132 (Fig. 5). In the untreated cells transfected with the J6-TK-pGL3, a 2-fold activation of reporter activity was observed in the presence of Wy 14,643 as compared with vehicle (Fig. 5). Pretreatment of HepG2 cells with MG132 increased both the basal and ligand-stimulated transcriptional activation of the reporter gene. This effect was already observed when the cells were pretreated for 2 h with MG132 and was even more pronounced after 4 h (Fig. 5).

To determine whether this stabilization of PPARα protein by MG132 resulted in changes in PPARα target gene expression, HepG2 cells were treated or not with MG132 for 2 h prior to activation with Wy 14,643, and the expression level of two established PPARα target genes, apoA-II (4) and FATP (26), was analyzed by quantitative PCR. Pretreatment with MG132 increased Wy 14,643-induced expression of both the apoA-II (Fig. 6A) and FATP (Fig. 6B) genes. These data show that MG132 stabilization of PPARα protein levels results in an enhanced ligand-induced expression of PPARα target genes in HepG2 cells.
PPARα Degradation by the Proteasome

**DISCUSSION**

Eukaryotic cells exhibit rigorous control over gene expression by tightly regulating the expression and activity of transcription factor proteins. The concentrations of these transcriptional regulators are controlled, at least in part, through proteasome-mediated protein degradation. The first step in this process, the ubiquitination of proteins, which are subsequently degraded by the 26 S proteasome complex, is a highly regulated process leading to the modulation of transcription factor activity (13). In this report, it is shown that the ubiquitin-proteasome pathway controls the degradation of PPARα protein and as such modulates the concentration of PPARα in hepatocytes. Results from pulse-chase experiments demonstrate that PPARα protein has a short half-life, which is extended by liganding of the receptor, results that confirm and extend data that appeared when this work was in progress (27).

In addition, we show that PPARα protein stabilization by its ligand is associated with a reduction in ubiquitination of PPARα protein. Furthermore, a highly specific inhibitor of the proteasome, MG132, blocks PPARα protein degradation, and the resulting enhanced expression of PPARα leads to a higher transcriptional activation of a reporter gene driven by a PPARα-responsive element. In addition, treatment of HepG2 cells with MG132 results in an enhanced ligand-induced expression of endogenous PPARα-responsive genes. Recently, it was demonstrated that elevating expression of PPARα in HepG2 cells by overexpressing exogenous PPARα results in an increased expression of endogenous PPARα target genes (28), whereas genetic ablation of PPARα expression results in decreased basal and/or ligand-induced expression of PPARα target genes (29), indicating that PPARα protein levels are a determinant of the response to its ligands. The results from this study, demonstrating that PPARα protein expression and activity are regulated at the level of degradation, thus provide a novel mechanism of control of PPARα activity.

Although only a limited number of studies have addressed PPARα protein regulation, the concentration of PPARα has been shown to be critical under a number of physiological situations. PPARα expression has been shown to oscillate with a circadian rhythm in liver (14). The diurnal variations of PPARα mRNA is closely followed by a parallel cycling of PPARα protein. This rapid diurnal cycling of PPARα protein levels implied that the half-life of the protein should be short enough to allow its levels to significantly decrease over a period of 12 h. Our results from pulse-chase experiments demonstrate that PPARα protein half-life is approximately 1 h due to its rapid degradation by the proteasome, which provides a molecular mechanism potentially contributing to the rapid circadian cycling of this protein. Furthermore, our data indicate that regulation of PPARα protein level by controlling its degradation modulates the expression of different PPARα target genes in response to its ligand. For example, the expression of the apoA-II and FATP genes, two well-characterized PPARα target genes (4, 26), by MG132 treatment is increased in a ligand-dependent manner. Thus, in addition to PPARα control by the level of its gene transcription as well as by the potency of the ligand, the magnitude of the physiological response to PPARα activation is also regulated at the level of its stability. It will be of interest to identify physiological factors that influence PPARα degradation and as such affect the PPARα signaling pathway.

Interestingly, our study shows that ligand activation protects PPARα from ubiquitination and degradation in a rapid but transient manner. Previous studies demonstrated that the estrogen and vitamin D3 receptors are also degraded by the proteasome and that this is accelerated after ligand exposure (20, 21). Similarly, progesterone receptor protein levels are downregulated after progesterone treatment (22, 23). Other results clearly indicate a hormone-mediated destabilization of the glucocorticoid receptor (24). Under basal conditions, glucocorticoid receptor has a fairly long half-life of 18 h, whereas dexamethasone-treatment decreases its half-life to 8–9 h. In contrast to the previous receptors, PPARα has a very short half-life, and ligand activation prolongs its half-life. We therefore propose a model in which the ubiquitin-proteasome pathway may contribute to the regulation of duration and magnitude of the response to PPARα activators. The interaction with its ligand reduces the ubiquitination of the PPARα protein and consequently its degradation. This protective effect appears transitory, which may be due either to a rapid metabolization of the ligand in liver cells and/or to a ligand-dependent recruitment of coactivators that could induce the degradation of the PPARα protein. Indeed, it has been shown previously that the AF-2 domain of certain nuclear receptors binds a component of the proteasome in a hormone-dependent manner (30), which may result in the arrest of the transcriptional activation by the liganded receptor in a temporally defined manner. Further investigation is necessary to identify the mechanisms explaining the transitory stabilization of PPARα protein by its ligand.
Acknowledgments—we thank Dr. Bohman for providing the HA-tagged ubiquitin expression vector. We acknowledge the technical contribution of O. Vidal.

REFERENCES

1. Chinetti, G., Fruchart, J. C., and Staels, B. (2000) Inflamm. Res. 49, 1–9
2. Schoonjans, K., Staels, B., and Auwerx, J. (1996) J. Lipid Res. 37, 907–925
3. Fruchart, J. C., Duriez, P., and Staels, B. (1999) Curr. Opin. Lipidol. 10, 245–257
4. Vu-Dac, N., Schoonjans, K., Kosyk, V., Dallongeville, J., Fruchart, J. C., Staels, B., and Auwerx, J. (1995) J. Clin. Invest. 96, 741–750
5. Fruchart, J. C., Duriez, P., and Staels, B. (1999) Curr. Opin. Lipidol. 10, 245–257
6. Delerive, P., Fruchart, J. C., and Staels, B. (2001) J. Endocrinol. 191, 453–459
7. Beigneux, A. P., Moser, A. H., Shigenaga, J. K., Grunfeld, C., and Feingold, K. R. (2000) J. Biol. Chem. 275, 16390–16399
8. Boudjelal, M., Wang, Z., Voorhees, J. J., and Fisher, G. J. (2000) Cancer Res. 60, 2247–2252
9. Kopf, E., Plassat, J. L., Vivat, V., de The, H., Chambon, P., and Rochette-Egly, C. (2000) J. Biol. Chem. 275, 33280–33288
10. Daee, A., Zhao, L., Park, K. S., Furuno, T., Takamura, N., Nakanishi, M., West, B. L., Hanover, J. A., and Cheng, S. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 8985–8990
11. Hauser, S., Adelmant, G., Sarraf, P., Wright, H. M., Mueller, E., and Spiegelman, B. M. (2000) J. Biol. Chem. 275, 18527–18533
12. Mimnaugh, E. G., Bonvini, P., and Neckers, L. (1999) Electrophoresis 20, 418–428
13. Hodges, M., Tissot, C., and Freemont, P. S. (1998) Curr. Biol. 8, R749–R752
14. Lemberger, T., Saladin, R., Vazquez, M., Assimacopoulos, F., Staels, B., Desvergne, B., Wahli, W., and Auwerx, J. (1996) J. Biol. Chem. 271, 1764–1769
15. Brown, P. J., Stuart, L. W., Hurley, K. P., Lewis, M. C., Winegar, D. A., Wilson, J. G., Wilkinson, W. O., Ittoop, O. R., and Willson, T. M. (2001) Bioorg. Med. Chem. Lett. 11, 1225–1227
16. Staels, B., Koenig, W., Habib, A., Merval, R., Lehret, M., Pineda Torra, I., Delerive, P., Fadel, A., Chinetti, G., Fruchart, J. C., Najib, J., Maclouf, J., and Tedgui, A. (1998) Nature 393, 790–793
17. Treier, M., Staszewski, L. M., and Bohmann, D. (1994) Cell 78, 787–798
18. Heid, C. A., Stevens, J., Livak, K. J., and Williams, P. M. (1996) Genome Res. 6, 986–994
19. Gibson, U. E., Heid, C. A., and Williams, P. M. (1996) Genome Res. 6, 995–1001
20. Nawaz, Z., Lonard, D. M., Dennis, A. P., Smith, C. L., and O'Malley, B. W. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1858–1862
21. Masuyama, H., and MacDonald, P. N. (1998) J. Cell. Biochem. 71, 429–440
22. Torreg, J. L., and Waring, D. W. (2000) Endocrinology 141, 3432–3439
23. Syvala, H., Vienonen, A., Zhuang, Y. H., Kivineva, M., Ylikomi, T., and Tushimaka, P. (1998) Life Sci. 63, 1505–1512
24. Webster, J. C., Jewell, C. M., Bodwell, J. E., Munck, A., Sar, M., and Cidlowski, J. A. (1997) J. Biol. Chem. 272, 9287–9293
25. Martin, G., Duez, H., Blanchard, C., Berezowski, V., Poulain, P., Fruchart, J. C., Najib-Fruchart, J., Gineur, C., and Staels, B. (2001) J. Clin. Invest. 107, 1425–1432
26. Frohner, B. I., Hui, T. Y., and Bernlohr, D. A. (1999) J. Biol. Chem. 274, 3970–3977
27. Hirotsu, M., Tsukamoto, T., Bourdeaux, J., Sadano, H., and Osumi, T. (2001) Biochem. Biophys. Res. Commun. 288, 106–110
28. Hui, T. Y., Griffin, K. J., and Johnson, E. F. (2001) J. Biol. Chem. 276, 27950–27958
29. Lee, S. S., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salgueiro, P. M., Westphal, H., and Gonzalez, F. J. (1995) Mol. Cell. Biol. 15, 3012–3022
30. Vom Baur, E., Zechel, C., Heery, D., Heine, M. J., Garnier, J. M., Vivat, V., Le Douarin, B., Grumeneyer, H., Chambon, P., and Losson, R. (1996) EMBO J. 15, 110–124