Translational Regulation of Angiotensin Type 1a Receptor Expression and Signaling by Upstream AUGs in the 5’ Leader Sequence*

Received for publication, June 29, 2004, and in revised form, July 29, 2004
Published, JBC Papers in Press, August 19, 2004, DOI 10.1074/jbc.M407261200

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Rat angiotensin type 1a receptor (AT1aR) is regulated by four upstream AUGs present in the 5’ leader sequence (5’-LS). Disruption of all four upstream AUGs (QM) results in 2–3-fold higher levels of angiotensin type 1 receptor (AT1R) densities in transiently transfected rat aortic smooth muscle cells (A10) and stably transfected Chinese hamster ovary cells. Cells expressing QM have 5-fold higher levels of angiotensin II-induced inositol phosphate production than wild type (WT). Polysome analysis showed that QM mRNA is present in heavier fractions than the WT transcript, and 5.7-fold more AT1R protein is produced by in vitro translation from QM transcripts compared with WT transcripts. The AT1aR comprises 3 exons. Exon 3 (E3) encodes the entire open reading frame and 3’-untranslated region. Exons 1 and 2 (E1 and E2) and 52 nucleotides of E3 encode the 5’-LS. The AUGs in both exons contribute to the inhibitory effect on AT1R expression but not to the same degree. Disruption of the AUGs in exon 2 (DM2) relieves half of the inhibition, whereas disruption of the AUGs in exon 1 (DM1) is without effect. Disruption of the AUGs in exon 2 results in a complete lack of translation and translation that are indistinguishable from the alternative splice variant E1,3, which we previously showed was more efficiently translated than the E1,2,3 transcript. Individual mutations revealed that only the fourth AUG increased AT1R translation. In conclusion, all four AUGs present in the 5’-LS function cumulatively to suppress AT1R expression and signaling by inhibiting translation. These data also show that both AUGs in E2 contribute to the inhibitory cis element present in this alternatively spliced exon.

In the majority of eukaryotic mRNAs, the first AUG downstream from the 5’ cap site is the start of translation (1). In contrast, mRNAs that code for key regulatory proteins, such as transcription factors, protooncogenes, and key signaling molecules, commonly possess AUGs upstream of the AUG start codon. Upstream AUGs can play a critical role in the control of gene expression by causing ribosomal pausing or by forming a translation-competent ribosome that can initiate, terminate, and reinitiate. Both of these mechanisms can lead to reduced translation of the downstream open reading frame. Alternatively, an N-terminal extended protein can be synthesized from initiation at the upstream AUG, thereby competing with translation at the downstream open reading frame (2).

We have been studying the post-transcriptional regulation of the angiotensin type 1 receptor (AT1R) (3), which is a G protein-coupled receptor that plays a critical role in regulating blood pressure and fluid homeostasis. Antagonists of this receptor are widely used to control hypertension and reduce the rate of progression of cardiovascular and renal disease (4). The rat AT1aR comprises three exons (see Fig. 1). E3 harbors the entire open reading frame and the 5’-untranslated region, whereas the 5’-LS comprises exon 1 (E1), exon 2 (E2), and 52 nucleotides of E3. There are four upstream AUGs present in the 5’-LS, two in E1 and two in E2. E2 is alternatively spliced in a tissue-specific manner and contains an unidentified cis element that is inhibitory to receptor expression (5). In this study, we investigated the function of the upstream AUGs present in the 5’-LS on AT1R expression and signal transduction in transiently transfected rat aortic smooth muscle (A10) cells and in stably transfected Chinese hamster ovary (CHO) cells. We also studied the role of upstream AUGs on receptor translation by polysome analysis in transfected cells and by in vitro translation (IVT) assays.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis—The AUGs in the 5’-LS of the AT1aR cloned into the pcDNA5/FRT vector (Invitrogen) were subjected to site-directed mutagenesis using the QuickChange site-directed mutagenesis system (Stratagene).

A10 Cell Culture andTransient Transfections—A10 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 4 mM L-glutamine, 10% fetal bovine serum, and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin). When cells were 60–75% confluent, 20 μg of plasmid DNA per 100 mm dish was transiently transfected by the calcium phosphate method (Invitrogen).

CHO Cell Culture and Stable Transfections—CHO cells were cultured in Ham’s F-12K with 1.5 g/liter sodium bicarbonate, 2 mM L-glutamine, 10% fetal bovine serum, and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin). When cells were 60–75% confluent, 20 μg of plasmid DNA per 100 mm dish was transiently transfected by the calcium phosphate method (Invitrogen). Individual clones were cultivated as described previously (6). AT1R Radioligand Binding—A10 and CHO cell membranes were used in radioligand binding assays using [125I-Sar1,Ile8]Ang II and a Brandel cell harvester as described (7). Kd and Bmax values from Scatchard analysis were determined.

The abbreviations used are: AT1R, angiotensin type 1 receptor; AT1aR, angiotensin type 1a receptor; 5’-LS, 5’ leader sequence; WT, wild type; IVT, in vitro translation; E1, exon 1; E2, exon 2; E3, exon 3; CHO, Chinese hamster ovary; Ang II, angiotensin II; IP, inositol phosphate; QM, disruption of all four upstream AUGs; DM1, disruption of the AUGs in exon 1; DM2, disruption of the AUGs in exon 2.

* This research was supported by American Heart Association Beginning Grant-in-aid 0080265U, a National Kidney Foundation grant-in-aid (to H. J.), and National Institutes of Health Grant HL57502 (to K. S.). 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.
chard plots were determined using the nonlinear regression analysis program PRISM.

**Ribonuclease Protection Assay**—Total RNA was isolated, and E1,3 and E1,2,3 mRNA were measured by ribonuclease protection assay as described previously (3) using a probe based on the coding region and thus common to both transcripts (see Fig. 1). In brief, the cDNA encoding the rat AT1aR coding region in the pBluescript II vector (Stratagene) was linearized with EcoRI and transcribed in \textit{vitro} with T7 RNA polymerase to yield a 380-bp protected cRNA fragment after hybridization with 3 μg of total RNA followed by ribonuclease digestion according to the Ribonuclease Protection Assay III protocol (Ambion). The probe for β-actin was generated from pTRI-β-actin-mouse cDNA (Ambion) with T7 RNA polymerase and yielded a 245-bp cRNA fragment. Radioactive signals were detected by a phosphorimaging device after electrophoresis on a 5% acrylamide gel.

**Real Time PCR**—Total RNA was extracted using TRIzol reagent (Invitrogen). First strand cDNA was made from total RNA using iScript cDNA synthesis kit (Bio-Rad) with Moloney murine leukemia virus RNase H\textsuperscript{-} reverse transcriptase, oligo(dT), and random hexamers. Quantitations of specific mRNAs and 18 S rRNA (for control) were performed by real time PCR using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). The PCR reaction mixture consisted of RNase-free water, TaqMan Universal PCR Master Mix (Applied Biosystems), 300 nM specific primers, 10 μM probe (forward primer 5\textsuperscript{-}TGG AGA A-3\textsuperscript{\textsuperscript{\prime}}/H11032; reverse primer: 310R (E1,2,3 and E1,3), 5\textsuperscript{-}CTC TGC CAC ATT CCC TGG TC-3\textsuperscript{\textsuperscript{\prime}}; reverse primer: 119F (E1,2,3), 5\textsuperscript{-}CTC TGT CAC CCT CCC TCC TC-3\textsuperscript{\textsuperscript{\prime}}/H11032). The specificity of these primers was confirmed in CHO cells stably expressing E1,3 or E1,2,3. That is, we did not detect any amplified products using E1,3-specific primers in the E1,2,3-expressing cells and vice versa. The expression of 18 S rRNA, E1,3, and E1,2,3 mRNA in each sample was quantitated using respective primers. PCR conditions without reverse transcription were included to control for contamination by genomic DNA. Real-time standard curves for 18 S rRNA, E1,3 and E1,2,3 mRNA were made from a series of 10\textsuperscript{-n} dilutions (5, 5\textsuperscript{n}, 5\textsuperscript{2n}, 5\textsuperscript{3n}, 5\textsuperscript{4n}, 5\textsuperscript{5n} and 5\textsuperscript{6n}) for each cDNA. The cell and tissue levels of these cDNAs were calculated based on the standard curves.

**Inositol Phosphate Assay**—CHO cells stably expressing WT and QM were cultured to 70% confluence in 24-well plates before being treated for 16 h with Dulbecco’s modified Eagle’s medium containing 3 μCi/ml [\textsuperscript{3}H]inositol (Amersham Biosciences). Ang II-induced IP production was assayed as described (8).

**Polysome Analysis**—AT1R mRNA was translated in vitro with T7 RNA polymerase and yielded a 245-bp cRNA fragment. Radioactive signals were detected by a phosphorimaging device after electrophoresis on a 5% acrylamide gel.
**Fig. 2.** Comparison of WT and QM $\mathrm{AT}_{1}\mathrm{R}$ binding in transiently transfected A10 cells. A, saturation isotherms of $[\mathrm{125I}-\mathrm{Sar}^1,\mathrm{Ile}^8]\mathrm{Ang} \ II$ binding to membranes from A10 cells transiently transfected with WT and QM plasmid DNA, using a computerized nonlinear regression analysis program, PRISM. Representative data from three transfection experiments are shown; each was performed in triplicate. B, Scatchard plot of the saturation isotherm data. C, $\mathrm{AT}_{1}\mathrm{R} B_{\text{max}}$ values obtained from Scatchard analysis of the saturation curves for WT- and QM-expressing cells. D, $\beta$-galactosidase ($\beta$-Gal) activity in A10 cells co-transfected with $\beta$-galactosidase and WT or $\beta$-galactosidase and QM plasmid DNA. The data are averaged from three transfection experiments performed in triplicate. E, $\mathrm{AT}_{1}\mathrm{R}$ mRNA levels determined by ribonuclease protection assay in A10 cells transiently expressing WT and QM normalized to $\beta$-actin. The data are averaged from three experiments performed in triplicate. F, $\mathrm{AT}_{1}\mathrm{R} B_{\text{max}}$ values normalized to $\mathrm{AT}_{1}\mathrm{R}$ mRNA levels in WT- and QM-expressing cells. AU, arbitrary unit.

**Fig. 3.** Comparison of WT and QM $\mathrm{AT}_{1}\mathrm{R}$ binding in stably transfected CHO cells. A, saturation isotherms of $[\mathrm{125I}-\mathrm{Sar}^1,\mathrm{Ile}^8]\mathrm{Ang} \ II$ binding to membranes from CHO cells stably transfected with WT and QM plasmid DNA, using a computerized nonlinear regression analysis program, PRISM. Representative data from three binding experiments are shown; each was performed in triplicate. B, Scatchard plot of the saturation isotherm data. C, $\mathrm{AT}_{1}\mathrm{R} B_{\text{max}}$ values obtained from Scatchard analysis of the saturation curves for WT- and QM-expressing cells. D, $\beta$-galactosidase ($\beta$-Gal) activity in CHO cells co-transfected with $\beta$-galactosidase and WT or $\beta$-galactosidase and QM plasmid DNA. The data are averaged from three transfection experiments performed in triplicate. E, $\mathrm{AT}_{1}\mathrm{R}$ mRNA levels determined by ribonuclease protection assay in CHO cells transiently expressing WT and QM normalized to $\beta$-actin. The data are averaged from three experiments performed in triplicate. F, $\mathrm{AT}_{1}\mathrm{R} B_{\text{max}}$ values normalized to $\mathrm{AT}_{1}\mathrm{R}$ mRNA levels for all six clones normalized to their respective $\mathrm{AT}_{1}\mathrm{R}$ mRNA levels. AU, arbitrary unit.
denser and more actively translated polysome fractions (fractions 1 and 2) in the QM-expressing cells (Fig. 5A). To determine whether QM was also translated in vitro more rapidly than WT, capped WT and QM RNAs were IVT in wheat germ extracts (Fig. 5B, inset). These IVT assays showed that 5.7-fold more AT1R protein was synthesized by QM compared with WT (Fig. 5B).

Relative Contribution of Upstream AUGs in E1 and E2 on Inhibition of AT1R Densities and IVT—We used site-directed mutagenesis to create DM1, in which the two AUGs in E1 were disrupted and DM2, in which the two AUGs in E2 were disrupted as well (Fig. 1). Radioligand binding analysis of transfected A10 cells revealed that AT1R densities were 1.4-fold higher in DM2- compared with WT-transfected cells, whereas no differences in AT1R densities were observed between DM1- and WT-transfected cells (Fig. 6A). AT1R densities in DM2-transfected cells were not as high as in QM-transfected cells; transfection of A10 cells with QM resulted in 1.4-fold higher AT1R densities than transfection with DM2 and 2.0-fold higher densities than transfection with WT cDNA.

To determine how upstream AUGs contribute to inhibitory RNA cis elements within the 5'-LS, IVT assays were performed on QM, DM1, and DM2 RNA transcripts. No differences in AT1R translational efficiency were observed between the DM1 and WT transcripts, although DM2 resulted in a 2.0-fold increase in AT1R protein levels (Fig. 6B). The level of IVT in DM2-expressing cells was not as high as QM; QM resulted in 2.3-fold higher levels than DM2 and 4.5-fold higher levels than WT.

To further dissect the contribution of individual upstream AUGs in inhibiting AT1R expression, the two AUGs in E2 were individually mutated by site-directed mutagenesis to create M1 and M2 (Fig. 1). Radioligand binding studies showed an incremental increase in AT1R densities for M1 (1.1-fold) and a 1.3-fold increase for M2 (Fig. 7A). IVT assays showed a similar trend; M2 resulted in a 1.6-fold increase in IVT, whereas IVT of M1 was indistinguishable from WT (Fig. 7B). Because relief from translational repression was only observed in M2 and only the fourth AUG was in optimal Kozak consensus sequence (the +4 position is G and the −3 position is A (1)), we investigated whether initiation could occur at this fourth AUG in DM2-expressing cells. No larger protein encompassing the extra 44 amino acids, however, was not detectable by IVT (data not shown).
Comparison of the Effects of DM2 and the Splice Variant E1,3 on AT1R Expression and IVT—

The two AT1aR splice variants (E1,2,3 and E1,3) differ only in the length of their 5'UTR. Thus, both transcripts code for identical proteins. We recently showed that E2 contains an inhibitory RNA cis element, which results in reduced AT1R expression and signaling (5). As found previously, radioligand binding assays showed that AT1R densities in E1,3-expressing cells were 1.4-fold higher than WT-expressing cells, which were indistinguishable from DM2 (Fig. 6A). IVT assays showed a similar result; the E1,3 transcript was translated 2.0-fold more efficiently than WT, and no differences were observed between the levels of IVT for DM2 and E1,3 (Fig. 6B).

**DISCUSSION**

In this paper, we show that disruption of all four AUGs leads to marked increases in AT1R binding in both transiently transfected and stably transfected cells. β-galactosidase was equivalently expressed in WT and QM co-transfection experiments, and at least three independent and randomly selected stable clones of QM expressed higher levels of AT1R binding when compared with three independent and randomly selected WT
clones; these findings rule out the likelihood that differences in transfection efficiencies or different sites of integration account for these results. Scatchard analysis of radioligand binding studies indicates that the increase in AT, R binding in cells expressing QM compared with cells expressing WT arises from an increase in receptor density rather than increased receptor affinity. This is consistent with the fact that WT and QM code for identical proteins. It is unlikely that the increase in AT, R densities in QM-expressing cells is due to increased QM mRNA levels, because AT, R densities were still significantly higher after normalization to AT, R mRNA levels in both transiently and stably expressing cells.

Ang II-stimulated IP production is markedly higher in QM-compared with WT-expressing cells, which illustrates the functional significance of the 2-fold increase in AT, R densities in QM-expressing cells. These data also support studies showing a close correlation between AT, R density and signal transduction in vascular smooth muscle cells (11). In response to Ang II stimulation, QM-expressing cells produce more IP than WT-expressing cells when normalized to AT, R Bmax and this is consistent with the expected amplification of signaling that occurs in signal transduction cascades.

The observation that QM mRNA was associated with heavier polysome fractions (and thus with more actively translated mRNAs) than WT mRNA suggests that upstream AUGs inhibit translational efficiency in cells. This is further supported by IVT assays, in which QM is translated in vitro with greater efficiency than WT mRNA.

Mutagenesis studies show that disruption of both AUGs in E1 offers no liberation from the 5' -LS inhibition of AT, R expression. The data showing that AT, R densities in DM2-transfected cells were half the levels present in QM-transfected cells indicate that the upstream AUGs in E1 at least partially contribute to the RNA inhibitory cis elements within the 5' -LS. It is possible that part of the effect of QM involves a conformational change in the 5' -LS that only occurs when all four AUGs are disrupted. Thus, even though disruption of the AUGs in E1 had no effect on AT, R binding or translation, their disruption contributed to more efficient translation of QM.

Disruption of the two AUGs in E2 completely relieved the effects of the E2 inhibitory cis element on receptor expression and signaling (5). This finding suggests that the third and fourth AUGs comprise a major component of the inhibitory cis element within E2 and thus may be the RNA cis elements that contribute to control of AT, R regulation by alternative splicing. By controlling the degree of alternative splicing of E1,2,3, an additional level of control is available by which the cell can tightly regulate the expression and function of the AT, R.

We recently showed by Northern blot analysis that all three exons are expressed in rat tissue and that both splice variants (E1,3 and E1,2,3) are expressed in all rat tissues studied thus far, indicating that the splice variants are not the result of cDNA errors due to incompletely spliced introns or reverse transcription-PCR errors (5). Furthermore, we found that splicing is regulated in a tissue-specific manner and that the splice ratio of E1,3 to E1,2,3 tightly correlates with tissue specific differences in AT, R expression, suggesting that regulation of alternative splicing contributes to differential tissue-specific expression of the AT, R. Thus, in addition to regulation by other mechanisms (such as transcription, mRNA stability, RNA binding proteins, receptor desensitization, ligand-mediated receptor internalization, and receptor recycling), alternative splicing of the AT, R is one more mechanism by which AT, R expression can be controlled (3, 12–14).

DM2 and E1,3 result in the same levels of AT, R expression, but this finding does not rule out the presence of additional inhibitory cis elements within E2; it is possible that inhibitory cis elements exist which are distinct from the two AUGs but are disrupted by forming DM2. In this regard, we have recently reported that deletion of the loop in a putative hairpin in E2 markedly relieved the translational repression of E2 (5).

In most mammalian mRNAs, the small (40 S) ribosomal subunit complexes with the 5'-end of the mRNA and then begins to scan linearly along the mRNA until it reaches the first AUG. At this point, the anticodon in Met-tRNAi base pairs with the AUG codon, the large 60 S ribosomal subunit joins the complex, and translation ensues. This “first AUG rule” of translation initiation can be escaped by several mechanisms (15).

Context-dependent leaky scanning allows the ribosome to pass by the first AUG until it finds an AUG in optimal Kozak context (GCCACCAUGG; the AUG is italicized and the most crucial flanking sequences for optimal context are indicated by boldface type). The first three AUGs in the AT, R 5' -LS are not in optimal context and thus could be bypassed as initiation codons by the mechanism of leaky scanning. Studies suggest that AUGs close to the 5'-end of a mRNA are poorly recognized by ribosomes (16); Thus, it is likely that the two AUGs in exon 1 are too close to the 5'-end to sustain ribosomal initiation even if they were in optimal context. A third mechanism of escape is “reinitiation.” Reinitiation at a downstream AUG in optimal context can occur after initiation at an upstream AUG when the upstream AUG is followed in-frame by a stop codon. All of the four AUGs in the AT, R 5' -LS are followed in-frame by a termination codon (Fig. 1).

A longer AT, R was not detected by IVT when the in-frame stop codon in E2 was disrupted, which suggests that the fourth upstream AUG inhibits AT, R expression by ribosomal pausing rather than by initiation, termination, and reinitiation or by another potential escape mechanism, internal ribosomal entry (17). In this regard, the rat AT, R appears distinct from the human AT, R, in which initiation can occur at an upstream AUG in the E1,3,4 splice variant, resulting in a longer form of the receptor that is functionally distinct from the short form (18). M2 caused a significant increase in IVT, whereas M1 or the two AUGs in E1 (DM1) did not, which suggests that ribosomal pausing is greatest at the fourth AUG and is consistent with the observation that the fourth AUG (Fig. 1) is the only one in optimal Kozak consensus sequence (1). However, these studies do not rule out the possibility that initiation occurs at the fourth AUG but at a level that is undetectable under the assay conditions.

There is accumulating evidence that an unfavorable 5'-LS can serve a physiological purpose. It is well documented that upstream open reading frames inhibit translation of the major open reading frame in many key regulatory proteins such as growth factors, protooncogenes, transcription factors, and key signaling molecules (19, 20). There are also several examples of how mutations affecting mRNA translational efficiency can contribute to pathological conditions. For example, a mutation in the 5'-LS that results in an out-of-frame AUG codon leads to reduced translation of the P16 tumor suppressor; accordingly, this mutation results in a predisposition toward melanoma (21). In another example, a splicing mutation that eliminates the upstream AUGs in the 5'-LS of thrombopoietin mRNA results in efficient translation of this protein and subsequent thrombocytopenia (22). It is thus possible that aberrant splicing of the AT, R under certain pathological conditions could have profound repercussions on the renin angiotensin system and its control of blood pressure and fluid homeostasis.

In summary, these data indicate that upstream AUGs in both E1 and E2 in the 5'-LS of the AT, R act cumulatively to repress receptor expression and signaling by inhibiting trans-
lation. The upstream AUGs in E1 require the presence of the upstream AUGs in E2 to be inhibitory, whereas the upstream AUGs in E2 are inhibitory in and of themselves. However, the inhibitory effects of AUGs in E2 are amplified by the presence of the upstream AUGs in E1, suggesting that 5′-LS secondary structure is also important to the translational repression. Translational repression by these upstream AUGs is most likely due to ribosomal pausing rather than reinitiation because a longer form of the AT1R was not detected when the in-frame stop codon in E2 was disrupted. In addition, these data suggest that the third and fourth upstream AUGs are part of the inhibitory cis element present in E2 and therefore may contribute to regulation of AT1aR expression by alternative splicing.

**REFERENCES**

1. Kozak, M. (1999) *Gene (Amst.*) **234**, 187–208
2. Meijer, H. A., and Thomas, A. A. (2002) *Biochem. J.* **367**, 1–11
3. Wu, Z., Maric, C., Roesch, D. M., Zheng, W., Verbalis, J. G., and Sandberg, K. (2003) *Endocrinology* **144**, 3251–3261
4. Sandberg, K., and Ji, H. (2006) *Semin. Nephrol.* **20**, 402–410
5. Zhang, Y., Ji, H., Zheng, W., Falconetti, C., Fabucci, M. E., and Sandberg, K. (2004) *Gene (Amst.*) **341**, 93–100
6. Tian, Y., Baukal, A. J., Sandberg, K., Bernstein, K. E., Balla, T., and Catt, K. J. (1996) *Am. J. Physiol.** **270**, E831–E839
7. Ji, H., Leung, M., Zhang, Y., Catt, K. J., and Sandberg, K. (1994) *J. Biol. Chem.* **269**, 16533–16536
8. Vanderheyden, P. M., Fierens, F. L., De Backer, J. P., Fraeyman, N., and Vaquelin, G. (1999) *Br. J. Pharmacol.* **126**, 1057–1065
9. Ji, H., Krishnamurthi, K., Wu, Z., and Sandberg, K. (2000) *Methods Mol. Med.* **51**, 171–192
10. Davies, E., and Abe, S. (1995) *Methods Cell Biol.* **50**, 209–222
11. Lasségue, B., Alexander, R. W., Nickenig, G., Clark, M., Murphy, T. J., and Griendling, K. K. (1995) *Mol. Pharmacol.* **48**, 601–609
12. Thekkumkara, T. J., Thomas, W. G., Motel, T. J., and Baker, K. M. (1998) *Biochem. J.* **329**, 255–264
13. Xu, K., and Murphy, T. J. (2000) *J. Biol. Chem.* **275**, 7604–7611
14. Thomas, W. G. (1999) *Regul. Pept.* **79**, 9–23
15. Kozak, M. (2000) *Genomics* **70**, 396–406
16. Kozak, M. (1991) *Gene Expr.** **1**, 111–115
17. Martin, M. M., Garcia, J. A., McFarland, J. D., Duffy, A. A., Gregson, J. P., and Elton, T. S. (2003) *Mol. Cell. Endocrinol.* **212**, 51–61
18. Martin, M. M., Victor, X., Zhao, X., McDougall, J. K., and Elton, T. S. (2001) *Mol. Cell. Endocrinol.* **183**, 81–91
19. Brown, C. Y., Mize, G. J., Pineda, M., George, D. L., and Morris, D. R. (1999) *Oncogene* **18**, 5631–5637
20. Child, S. J., Miller, M. K., and Geballe, A. P. (1999) *J. Biol. Chem.* **274**, 24335–24341
21. Liu, L., Dilworth, D., Gao, L., Monzon, J., Summers, A., Lassam, N., and Hogg, D. (1999) *Nat. Genet.* **21**, 128–132
22. Wiestner, A., Schlemper, R. J., van der Maas, A. P., and Skoda, R. C. (1998) *Nat. Genet.* **18**, 49–52
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J. Biol. Chem. 2004, 279:45322-45328. doi: 10.1074/jbc.M407261200 originally published online August 19, 2004

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