Angiotensin II (Ang II), the major bioactive peptide of the renin–angiotensin system, was originally described as a potent vasoconstrictor. It is now recognized as a multifunctional hormone influencing many cellular processes, including cell growth, apoptosis, migration, inflammation, and fibrosis.\(^1,2\) Under pathological conditions, the vasoconstrictor, mitogenic, proinflammatory, and profibrotic actions of Ang II contribute to altered vascular tone, structural remodeling, and endothelial dysfunction.\(^3–6\) Thus, Ang II plays a key role in the pathogenesis of cardiovascular diseases.

The biological responses of Ang II are mediated by its interaction with 2 distinct high-affinity G protein–coupled receptors now designated Ang II type 1 receptor (AT\(_1\)R) and Ang II type 2 receptor.\(^7\) Most of the known physiological and pathophysiological effects of Ang II are mediated via the AT\(_1\)R.\(^1–7\) This receptor subtype is expressed in cardiovascular-relevant cell types including vascular smooth muscle cells (VSMCs), endothelial cells, cardiac myocytes, cardiac fibroblasts, and renal mesangial cells.\(^7\) The multiple actions of Ang II, mediated through the AT\(_1\)R, are a result of complex intracellular signaling pathways including stimulation of the phospholipase C/inositol 1,4,5-trisphosphate/diacylglycerol cascade, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases, tyrosine kinases, and RhoA/Rho kinase.\(^1,2,4–6,7\) In addition, AT\(_1\)Rs mediate many of their pathophysiological effects by stimulating reactive oxygen species generation via a reduced nicotinamide-adenine dinucleotide phosphate oxidase-dependent mechanism.\(^2,8\) Reactive oxygen species, in turn, influences downstream signaling molecules, including transcription factors, tyrosine kinases/phosphatases, Ca\(^{2+}\) channels, and MAPKs.\(^2,8\)

The expression level of the AT\(_1\)R defines the biological efficacy of Ang II; hence, overexpression of the AT\(_1\)R is one potential mechanism by which Ang II can contribute to cardiovascular disease. Importantly, aberrant expression of the AT\(_1\)R has been shown to have pathophysiological relevance in cell culture, animal studies, and in clinical interventional trials (reviewed in Reference 5). This review will summarize the transcriptional and posttranscriptional mechanisms by which AT\(_1\)R gene expression is regulated and discuss the pathological relevance of these mechanisms in mediating cardiovascular disease.

### AT\(_1\)R Gene Structure

To initiate the investigations into the mechanisms by which AT\(_1\)R expression is regulated, the AT\(_1\)R gene has been cloned from a variety of species. Uniquely, the rodent genome harbors 2 distinct AT\(_1\)R genes: AT\(_{\text{i,1}}\)R and AT\(_{\text{i,2}}\)R.\(^9–15\) The AT\(_{\text{i,1}}\)R gene has been localized to chromosomes 17 and 13 of the rat and mouse, respectively, whereas the AT\(_{\text{i,2}}\)R gene has been localized to chromosomes 2 and 3 of the rat and mouse, respectively.\(^14,15\) It has been suggested that both receptor subtypes are pharmacologically and functionally identical\(^16–20\); however, both of these receptor subtypes are expressed in a tissue-specific manner and seem to be differentially regulated.\(^12,16,20\) In vivo studies suggest that the AT\(_{\text{i,1}}\)R is primarily responsible for the regulation of blood pressure and cardiovascular function.\(^21\) These conclusions were based on the observation that AT\(_{\text{i,1}}\)R knockout (AT\(_{\text{i,1}}\)-R\(^{-}\)) mice were found to exhibit low blood pressure and attenuated Ang II pressor response.\(^22–24\) However, recent studies suggest that the AT\(_{\text{i,2}}\)R predominantly mediates Ang II–induced contraction in mouse abdominal aorta, femoral artery, and mesenteric resistance vessels.\(^25,26\)

The rat AT\(_{\text{i,1}}\)R (rAT\(_{\text{i,1}}\)R) gene is composed of 3 exons and spans >40 kb (Figure 1).\(^27–29\) Exons 1 and 2 constitute the 5′-untranslated region (UTR) sequence, whereas exon 3 harbors the uninterrupted open reading frame (ORF) for the rAT\(_{\text{i,1}}\)R and the entire 3′-UTR, which contains 2 polyadenylation sequences. Primer extension and S1 mapping identified a single transcription initiation site 31 bp downstream of a TATA sequence.\(^29\) The rAT\(_{\text{i,2}}\)R gene is also composed of 3 exons, with exons 1 and 2 encoding the 5′-UTR and the third exon harboring the ORF.\(^30\) Unfortunately, there is little additional information regarding the regulation of the rAT\(_{\text{i,2}}\)R gene.

In contrast to rodents, the human genome harbors only a single AT\(_1\)R gene. Curnow et al\(^{13}\) mapped the human AT\(_1\)R (hAT\(_1\)R) gene to chromosome 3q and found no evidence of related hAT\(_1\)R genes on other chromosomes. In support of this conclusion, Southern blot analyses confirmed the presence of only a single AT\(_1\)R gene\(^32\) in the human genome. The
The hAT1R gene is composed of ≥4 exons and spans >60 kb (Figure 1).32–35 Exons 1, 2, and 3 constitute the 5′-UTR sequence, whereas exon 4 harbors the entire uninterrupted ORF for the hAT1R. RNA ligase-mediated rapid amplification of cDNA ends36 and primer extension experiments33 demonstrated that there is 1 major transcription initiation start site located 25 bp downstream of a consensus TATA box element.

Figure 1. A schematic representation of the rat and human AT1R genes and their potential to generate AT1R mRNA splice variants. ATGs denote the beginning of uORFs. Although the AT1R gene structure is not known for most animals, a highly homologous 5′-UTR exon 2-like sequence has been identified in 8 additional species.37,40 All of these sequences harbor uORFs and, therefore, may represent a common AT1R translational inhibitory mechanism. Currently, it is not known whether the rAT1B gene undergoes alternative splicing. Only the human and primate genomes harbor a 5′-UTR exon 3 sequence.37

Regulation of AT1R Expression by Alternative Splicing

While, characterizing the hAT1R gene it was demonstrated that ≥4 alternatively spliced hAT1R mRNAs were transcribed in human tissues (Figure 1).33,34 The hAT1R mRNA splice variants were composed of the following exons: exons 1 and 4; exons 1, 2, and 4; exons 1, 3, and 4; and exons 1, 2, 3, and 4; therefore, alternatively spliced hAT1R mRNAs differ only in the inclusion or exclusion of exon 2 and/or 3 (reviewed in Reference 37).

Splice variants that harbor exon 2 are functionally interesting because they contain 2 upstream AUG start codons (Figure 1),34,38 which are predicted to generate upstream ORFs (uORFs) of different lengths. PCR analysis demonstrated that hAT1R mRNA splice variants, which included exon 2 (ie, exons 1, 2, and 4), were composed of ≥30% of the total hAT1R mRNA transcripts expressed in a given tissue; this suggests that this splice variant is physiologically important.34,38–40 Curnow et al34 and Warnecke et al38 demonstrated that the presence of exon 2 inhibited the translation of the hAT1R mRNAs in vitro and decreased luciferase activity in reporter gene assays. Martin et al40 extended these earlier findings and demonstrated that the inclusion of exon 2 in hAT1R mRNA transcripts dramatically reduced hAT1R protein levels and significantly attenuated Ang II responsiveness. These results also suggested that both AUGs present in exon 2 could initiate translation and, therefore, reduce the number of ribosomes that initiated translation at the downstream hAT1R ORF harbored in exon 4. However, the importance of the 2 upstream ORFs in vivo remains undefined.

Alternative splicing can also occur in 1 of the 2 identified rat AT1R genes, the rAT1AR gene (Figure 1).41–44 When the rAT1AR gene is transcribed, 2 mRNA transcripts are synthesized, 1 harboring exons 1 and 3 and another harboring exons 1, 2, and 3. It was shown that AUGs/uORFs present in exon 2 of the rAT1AR mRNA inhibited reporter gene assays.41–44 Zhang et al42 and Ji et al43 extended these studies by demonstrating that the inclusion of exon 2 in rAT1AR mRNAs inhibited AT1R expression and AT1R-mediated signaling. Importantly, the presence of exon 2 did not modulate rAT1AR mRNA stability and, like the hAT1R mRNA splice variants (ie, exons 1, 2, and 4 and exons 1 to 4), both AUGs harbored in the rat exon 2 were involved in the inhibition of receptor expression.

The hAT1R mRNA splice variants, which harbor exon 3 (ie, exons 1, 3, and 4 and exons 1 to 4 transcripts), are also of particular interest, because exon 3 contains an AUG start codon, which is in-frame with the downstream ORF located in exon 4.34,37,40 When these transcripts are translated, a novel hAT1R isoform ("long" hAT1R) is synthesized, which is composed of an additional 32 amino acids at the N-terminus. Martin et al40 demonstrated that the long receptor isoform has a 3-fold diminished affinity for Ang II. Therefore, depending on the concentration of Ang II, the percentage of the long and short (ie, synthesized from exons 1 and 4 or exons 1, 2, and 4 hAT1R mRNA transcripts) hAT1R isoforms activated will differ; thus, the potency of the Ang II response will vary. Ultimately, Ang II responsiveness can be fine-tuned by regulating the relative synthesis of the long and short hAT1R.
isoforms in a given tissue. PCR analysis has shown that this splice variant is relatively rare, with the exception of adrenal tissue, where exon 1, 3, and 4 mRNAs composed ~50% of the hAT1R alternatively spliced mRNAs.40 Interestingly, sequence comparisons between species have demonstrated that an exon 3-like sequence was only conserved in humans and primates suggesting that this fine-tuning mechanism does not occur in all species.37

Because the inclusion or exclusion of exon 2, exon 3, or both has a profound influence on the control of AT1R density and function, it is important to investigate whether alternative splicing can be regulated. It is obvious from PCR studies that AT1R mRNA splice variants are regulated in a tissue-specific manner.34,37,39,40 In addition, data from Warnecke et al18 suggested that hAT1R mRNA splicing events may be regulated by pathophysiological stimuli. These investigators demonstrated that, in normal and failing human heart tissue, the exon 1, 3, and 4 splice variants represented 93% to 98% of all hAT1R mRNAs. The ratio of hAT1R mRNA transcripts harboring exon 2 versus those lacking exon 2 was decreased in atria from failing hearts compared with control atria. Similar results were obtained when comparing endomyocardial biopsy samples from patients with normal and impaired left ventricular function and when comparing the left ventricle of explanted normal hearts with those of 14 end-stage failing explanted hearts.36 The increased expression of the exon 1, 3 splice variant, and the decreased levels of the exon 1, 2, 4 splice variant would result in enhanced hAT1R density and signaling events; hence, it could be argued that this dysregulation plays a role in mediating heart failure. Alternatively, increased hAT1R density and function may be needed to improve cardiac function in patients with heart failure.

In support of the hypothesis that physiological/pathophysiological stimuli may modulate hAT1R mRNA alternative splicing events, Martin et al40 demonstrated that transforming growth factor-β1 treatment of human lung fibroblasts upregulated exon 1,4 mRNAs 2-fold, and exon 1, 2, 4 transcripts were increased 6-fold. In contrast, the exon 1, 3, 4 hAT1R mRNA splice variants were downregulated 18-fold. Because enhanced AT1R expression can be detrimental,4 the TGF-β1 upregulation of hAT1R mRNAs, which include exon 2, may represent a mechanism to limit the pathogenicity of hAT1Rs. In addition, Cowling et al44 demonstrated that cytokine treatment of rat cardiac fibroblasts upregulated all of the rAT1aR splice variants, with exon 1, 3 mRNAs showing the greatest increase. The increased expression of the exon 1, 3 rAT1aR mRNA splice variant would lead to enhanced receptor density and signaling, thereby implicating a role in mediating cardiac fibrosis.

In conclusion, these studies demonstrate that alternative splicing can be regulated and that, ultimately, the relative abundance of each AT1R mRNA splice variant controls AT1R protein levels and, thus, consequently affects the potency of the Ang II response. Therefore, dysregulation of AT1R mRNA splicing mechanisms could play a role in mediating cardiovascular disease. Currently, very little is known regarding the regulation of these mechanisms, and clearly more studies are needed to address this important question. It appears that the primary mechanism by which alternative splicing regulates AT1R expression is through a translational mechanism that involves the uORFs contained in exon 2.

**AT1R Transcriptional Regulation**

A large number of studies have shown that a wide range of physiological and pathophysiological stimuli modulate AT1R expression in a number of cell types and tissues (reviewed in Reference 45). Although the molecular mechanisms by which the AT1R density is regulated are not completely elucidated, some of these stimuli have been shown to modulate AT1R gene transcription rates (Figure 2). Examples of such agonists are Ang II, interferon-γ, growth factors, NO, thyroid hormone, all-trans-retinoic acid, peroxisome proliferator-activated receptor (PPAR)-γ, and activator ligands (ie, 15-deoxy-Δ12,14-prostaglandin J2, troglitazone, rosiglitazone, and pioglitazone).55–57 All of these act by transcriptionally suppressing the rAT1aR gene in cultured rat VSMCs. In contrast, glucocorticoids, insulin-like growth factor, interleukin (IL)-6, and progesterone induced an upregulation of rAT1aR expression in VSMCs by a transcrip-

![Figure 2. Transcriptional regulation of AT1R gene expression. C-reactive protein, cyclosporine, glucocorticoids, growth hormone, interleukins (IL-1β and IL-6), insulin-like growth factor-1, progesterone, and TNF-α all transcriptionally activate the AT1R gene. The increased transcription rates result in enhanced AT1R steady-state mRNA levels, which, in turn, lead to increased AT1R expression. On activation by Ang II, augmented AT1R density subsequently leads to increased signaling (eg, increased reactive oxygen species release), which, in turn, may lead to vascular remodeling, endothelial dysfunction, inflammation, atherosclerosis, and hypertension.](http://hyper.ahajournals.org/)

- IFNγ: interferon-γ
- Nitric oxide
- PPARγ: peroxisome proliferator-activated receptor-γ
- Retinoic acid
- Statins
- Thyroid hormone

**Figure 2.** Transcriptional regulation of AT1R gene expression. C-reactive protein, cyclosporine, glucocorticoids, growth hormone, interleukins (IL-1β and IL-6), insulin-like growth factor-1, progesterone, and TNF-α all transcriptionally activate the AT1R gene. The increased transcription rates result in enhanced AT1R steady-state mRNA levels, which, in turn, lead to increased AT1R expression. On activation by Ang II, augmented AT1R density subsequently leads to increased signaling (eg, increased reactive oxygen species release), which, in turn, may lead to vascular remodeling, endothelial dysfunction, inflammation, atherosclerosis, and hypertension. In contrast, interferon-γ (IFNγ), NO, PPARγ, retinoic acid, statins, and thyroid hormone all decrease AT1R gene transcription rates. These stimuli lead to decreased AT1R expression and signaling and, therefore, reduce cellular events that lead to cardiovascular disease.
tional mechanism. In addition, it has been demonstrated that tumor necrosis factor (TNF)–α, IL-1β, and dexamethasone can transcriptionally enhance the rAT1R gene in cardiac fibroblasts.64–66

Several studies have been published investigating the regulation of the hAT1R gene in human cells. Avdonin et al67 and Wang et al66 demonstrated that cyclosporine and C-reactive protein could increase hAT1R density by increasing hAT1R gene transcription rates in human VSMCs. Finally, Wyse et al69 demonstrated that growth hormone increased, whereas Thomas and Thekkumkara70 demonstrated that glucose decreased hAT1R gene transcription rates in human proximal tubule cells.

Because rodents harbor 2 AT1R genes and humans only harbor 1, it has been debated whether or not these genes will be regulated by the same mechanisms. When the hAT1R promoter region was compared with the rAT1R and rAT1R promoter sequences, very little homology was found. Therefore, it was concluded that the mechanisms that govern the rodent AT1R genes cannot be assumed to regulate the hAT1R gene.71 In support of this conclusion, it was demonstrated that Sp1 and Sp3 are predominantly responsible for regulating the basal expression of the hAT1R in all of the cell types investigated, including human VSMCs.36,72–74 In contrast, MEF-2 heterodimers75 and, to a lesser degree, Sp155,76,77 are required for basal expression of the rAT1R gene.

Transcriptional Suppression of the AT1R Gene

Although a variety of physiological stimuli regulate AT1R density by modulating AT1R mRNA transcription rates, the exact mechanisms of transcriptional control are poorly understood (Figure 2). The best-characterized mechanism to date is the PPAR-γ-mediated transcriptional suppression of the rAT1R gene.55–57 PPAR-γ belongs to the nuclear receptor family of ligand-activated transcription factors, which also includes PPAR-α and PPAR-β/δ. PPARs heterodimerize with the retinoid X receptor and then act as transcription factors to modulate the functions of many target genes.78 All of the PPARs are expressed in VSMCs where they exert antiatherogenic, anti-inflammatory, and vasculoprotective actions. Activators of PPAR-α (fibrates) and PPAR-γ (thiazolidinediones or glitazones) antagonize Ang II effects in vivo and in vitro, in part by decreasing the expression level of the AT1R.55–57,79,80

Sugawara et al65 demonstrated that a GC-box–related sequence within the −58/−34 bp region of the rAT1R promoter was responsible for the PPAR-γ suppression. Sp1 overexpression stimulated rAT1R gene transcription via the GC-box–related sequence, which was inhibited by additional PPAR-γ overexpression. Furthermore, glutathione S-transferase pull-down assays demonstrated that PPAR-γ and Sp1 directly interacted with each other. It has also been shown that the MAPK pathway and coactivator CREB-binding protein, CBP/p300, may antagonize PPAR-γ regulation of the rAT1R gene.57 Interestingly, activation of the MAPK pathway and CBP/p300 has been reported recently to be atherogenic.81,82 In summary, these findings suggest that activated PPAR-γ suppresses rAT1R gene expression at the transcriptional level by inhibiting Sp1 binding activity via protein–protein interactions. In contrast, PPAR-γ phosphorylation by the MAPK pathway and CBP enhanced activity of Sp1. As a result, gene transcription rates of rAT1R are increased. Thus, PPAR-γ ligands may inhibit Ang II–induced cell growth and hypertrophy in VSMCs by inhibiting AT1R expression. Such implications could be beneficial for treatment of diabetic patients with hypertension and atherosclerosis.

In addition to their cholesterol-lowering activities, statins exert pleiotropic anti-inflammatory effects and improve endothelial function.83,84 The exact mechanisms involved in the anti-inflammatory properties of statins are unresolved; although it has now been demonstrated that PPAR-α, PPAR-β/δ, and PPAR-γ may mediate these effects.85–87 Importantly, Takeda et al84 have demonstrated that cerivastatin and fluvastatin transcriptionally repressed the rAT1R gene in VSMCs. Although rAT1R promoter analysis was not performed in this study, VSMCs are known to express all of the PPAR isoforms; therefore, rAT1R transcriptional repression by statins may result from a similar mechanism as described with PPAR-γ downregulation of rAT1R expression.

Ichiki et al81 also found that the first 61 bp of the rAT1R promoter were necessary for NO suppression of transcription. Two DNA binding proteins were observed by electrophoretic mobility-shift assays, and 1 of these binding proteins was decreased with NO stimulation. NO is known to decrease Sp1 binding to GC elements on other genes88,89; therefore, one could speculate, as described for PPAR-γ ligands, that reduced Sp1 binding to the GC-box–related sequence is responsible for the transcriptional suppression of the rAT1R gene. This inhibitory mechanism of AT1R gene expression may be partially implicated in the antiatherogenic properties of NO.

Currently, it is not known whether the GC-box described in the hAT1R promoter region36,72–74 also plays a critical role in mediating a PPAR/Sp1 regulatory system in human cells. It appears, however, that fibrates, glitazones, and statins can also transcriptionally repress the expression of the hAT1R gene in H295-R cells (T.S. Elton, unpublished observation, 2006).

In conclusion, these studies suggest that PPAR activators, statins, and the anti-inflammatory and anti-atherogenic properties of NO result, in part, by downregulating AT1R density. The decrease in AT1R expression, therefore, diminishes the potency of the Ang II response (ie, reactive oxygen species generation and subsequent oxidative damage). Recently, it was demonstrated that the AT1R blockers irbesartan and telmisartan are not only AT1R antagonists but PPAR-γ activators.90 Therefore, PPAR-γ–activating AT1R blockers may be superior to nonactivating AT1R blockers in the clinical setting, because these antagonists may also reduce AT1R expression levels. These studies also support the theory that there is a mechanistic rationale for the use of “combination therapies” in treating cardiovascular diseases.

Transcriptional Activation of the AT1R Gene

Because the overexpression of the AT1R is thought to play a role in cardiovascular disease,3 there is great interest in understanding the transcriptional mechanisms that activate
the AT1R gene (Figure 2). Recently, it was demonstrated that the proinflammatory cytokines TNF-α and IL-1β upregulated rAT1R density on rat cardiac fibroblasts.64,65 Cowling et al65 used pharmacological inhibitors and a degradation-resistant mutant inhibitor of nuclear factor κB (NF-κB)-α to demonstrate that the transcription factor, NF-κB, was necessary for cytokine-induced rAT1R upregulation. TNF-α treatment did not alter rAT1R mRNA stability. In addition, RT-PCR of heterogeneous nuclear RNA showed increased rAT1R heterogeneous nuclear RNA levels.65 These results suggested that cytokines increased rAT1R density in cardiac fibroblasts by a transcriptional mechanism. Because both TNF-α and IL-1β are known to activate NF-κB, these investigators hypothesized that NF-κB binding sites present in the rAT1R promoter region must help in mediating this response. Cowling et al44 subsequently demonstrated that 2 cis-response elements located at −365/−355 bp and −2540/−2530 bp bound NF-κB and could transactivate a minimal promoter. These investigators concluded that these proinflammatory cytokines activated NF-κB, which resulted in enhanced AT1R transcription rates. These data have been extrapolated to propose that the augmented AT1R levels would increase Ang II–induced signaling, which may lead to increased diastolic stiffness and, in turn, cause contractile dysfunction by enhancing cardiac fibrosis.91,92

The rAT1R density is increased in several animal models of cardiac myocyte hypertrophy suggesting that these receptors may play a role, in part, in mediating these pathological events.93 To investigate whether a transcriptional mechanism was involved, an rAT1R promoter/reporter construct was injected into adult rat hearts subjected to an acute pressure overload.94 Importantly, pressure overload increased expression from the rAT1R promoter. The induced expression was blocked by mutation of either a consensus-binding site for AP-1 (−395/−375 bp) or GATA (−290/−273 bp) transcription factors in the rAT1R promoter; however, these mutations had no effect on basal expression. In addition, these investigators demonstrated that a Fos–JunB–JunD complex involving the AP-1 and GATA sites was involved in mediating this augmented rAT1R expression, whereas Ang II, working through the induction of immediate early gene products, activates expression of the rAT1R gene, thus providing a potential positive feedback loop for potentiation of Ang II effects.

Gluocorticoids are involved in the regulation of blood pressure, and enhanced vascular responsiveness has been considered to be 1 of the major contributing factors.95 Glucocorticoids increase vascular sensitivity to Ang II by enhancing promoter activity of the rAT1R gene, which, in turn, leads to increased receptor levels.58–60 Three putative glucocorticoid response elements (GREs) were identified in the rAT1R promoter; however, only 1 GRE at −770/−756 bp was found to be responsive to dexamethasone. Supershift assays demonstrated that glucocorticoid receptors could specifically interact with this rAT1R promoter sequence.99 In contrast, no consensus GREs are present in the hAT1R promoter; however, there are a number of GRE half-sites (GRE 1/2s) harbored in this gene.71 This observation suggests that glucocorticoid-induced upregulation of the hAT1R gene may be mediated by multiple GRE 1/2s96,97 or a GRE 1/2 plus an additional accessory element.98

Unfortunately, very few studies have been published investigating the mechanisms by which stimuli transcriptionally augment the hAT1R gene. Wyse et al69 demonstrated that growth hormone increased the rate of transcription of the hAT1R gene through a sequence harbored at −161/−149 bp in human proximal tubule cells. These investigators also demonstrated that an 18-kDa protein specifically interacted with this site and that protein binding was increased with growth hormone activation. Finally, they determined that epidermal growth factor, platelet-derived growth factor, and insulin activated this binding protein.

Recently, Li et al89 demonstrated that, in cultured human coronary artery endothelial cells, oxidized low-density lipoprotein (LDL) upregulated hAT1R expression by a mechanism that involved NF-κB. However, these investigators did not specifically investigate whether oxidized LDL enhanced hAT1R expression by a transcriptional and/or a posttranscriptional mechanism. Computer analysis of the hAT1R promoter region demonstrated that it harbored a single consensus NF-κB site at −1240/−1228 bp. Therefore, it is plausible that oxidized LDL does regulate hAT1R expression by a transcriptional mechanism and suggests that the hAT1R gene could also be regulated by a cytokine/NF-κB–mediated mechanism described by Cowling et al.44

In conclusion, little is known regarding the mechanisms that enhance AT1R transcriptional rates. Some important cis-regulatory elements and trans-factors have been identified; however, nothing is known regarding the factors involved in mediating the insulin-like growth factor-I,61 IL-6,62 and C-reactive protein48 transcriptional activation of the AT1R gene. This is a critical deficiency in our understanding, because these factors all play a role in mediating cardiovas-

cular disease.

**Regulation of AT1R Expression by Modulating mRNA Half-Life**

It is now well established that a number of stimuli can regulate AT1R expression levels by modulating AT1R mRNA stability or half-life (Figure 3). For example, Ang II,100 cAMP stimulating agents,101 estrogen,63 growth factors,99 and thyroid hormone53 decrease rAT1R density by increasing rAT1R mRNA decay rates (ie, decrease in mRNA stability) in rat VSMCs (Figure 3). Inversely, insulin,102 LDL,103 and progesterone93 increase rAT1R density by decreasing rAT1R mRNA decay rates (ie, increase mRNA stability) in rat VSMCs (Figure 3).

Investigating the mechanisms by which Ang II leads to the destabilization of the rAT1R mRNA in VSMCs, it was demonstrated that Ang II stimulation induces proteins to bind specifically to an AUUUUA sequence located at the distal end of the rAT1R mRNA.100,104 One of these proteins was purified by multiple chromatographic steps and identified by mass spectroscopy to be calreticulin.105 Ang II stimulation
was shown to induce the phosphorylation of calreticulin, which enabled this protein to bind to the 3′-UTR of the rAT₁₃,R mRNA, and subsequently accelerated the decay of this mRNA. Interestingly, hAT₁₃,R mRNAs also harbor an identical AUUUUA sequence localized to the same region as described in the rAT₁₃,R mRNA 3′-UTR. Therefore, calreticulin may also play a role in regulating the Ang II–mediated decay of hAT₁₃,R mRNAs in human cells.

Because the studies described above were performed using transcriptional inhibitors (ie, actinomycin D or 5,6-dichloro-1β-D-ribofuranosylbenzimidazole) that have multiple nonspecific effects, Xu and Murphy used a tetracycline-suppressible promoter to express recombinant rAT₁₃,R mRNA mimics and measured decay rates without the use of these inhibitors. They demonstrated that Ang II and PDGF did not increase rAT₁₃,R mRNA decay rates and suggested that only a transcriptional mechanism was responsible for the decreased rAT₁₃,R mRNA levels. In contrast, however, it was shown that forskolin downregulated rAT₁₃,R expression by a posttranscriptional mechanism. Interestingly, these investigators demonstrated that the forskolin response was mediated by the 5′-UTR, and not the 3′-UTR, of the rAT₁₃,R mRNA. The cis-acting element(s) or trans-factor(s) responsible for this activity were not identified.

In conclusion, the mechanisms that regulate AT₁₃,R mRNA stability are still poorly understood. The importance of calreticulin in Ang II–mediated decay of AT₁₃,R mRNAs has not been confirmed and remains controversial, because it is not clear how this protein plays a role in mRNA degradation. To date, no studies have been published that investigate the mechanisms by which insulin and LDL increase mRNA stability. Clearly more studies are needed to define these mechanisms governing AT₁₃,R expression are extremely complicated and suggest the crucial need to fine tune Ang II responsiveness by modulating AT₁₃,R density. As additional studies are conducted, the complexity of AT₁₃,R gene regulation is certain to expand. In support of this hypothesis, a novel posttranscriptional hAT₁₃,R regulatory mechanism was identified recently that involved the specific interaction of micro-RNAs with hAT₁₃,R mRNAs. Clearly more studies are needed to validate and to determine the impact of micro-RNAs on AT₁₃,R gene expression. In conclusion, aberrant expression of the AT₁₃,R can result from the dysregulation of any of the transcriptional and posttranscriptional mechanisms described herein and may lead to cardiovascular disease. Therefore, it is important to continue these lines of investigation with the hope that this new knowledge may result in the development of novel therapies to treat cardiovascular disease.

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None.

**References**


42. Oliverio MI, Best CF, Smithies O, Coffman TM. Regulation of sodium balance and blood pressure by the AT(1A) receptor for angiotensin II. *Hypertension*. 2000;35:550–554.


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