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Chapter 21

# Angiotensin-(1-7) and Mas: A Brief History

**Natalia Alenina**[*,†,‡], **Robson Augusto Souza dos Santos**[§]

*[*]Max-Delbrueck-Center for Moleculare Medicine (MDC), Berlin-Buch, Germany, [†]Federal University of Minas Gerais, Belo Horizonte, Brazil, [‡]Instituto Nacional de Ciência e Tecnologia em, NanoBiofarmacêutica (NanoBIOFAR), Belo Horizonte, Brazil, [§]Departamento de Fisiologia e Biofísica—ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*

## INTRODUCTION

Angiotensin-(1-7) is a vasoactive peptide of the renin–angiotensin system (RAS), which is generated mainly by angiotensin-converting enzyme 2 (ACE2) and exerts its actions via activation of its receptor Mas. The Ang-(1-7)/ACE2/Mas axis is nowadays considered to be a main mechanism, which counterbalances the vasoconstrictive actions of classical RAS, which includes renin, ACE, ANG II, and its receptors $AT_1$ and $AT_2$ (Figure 1). Whereas the classical RAS has been known for more than 100 years,[1] the protective arm of the RAS was relatively recently discovered. Both Mas[2] and Ang-(1-7)[3,4] were first described almost 30 years ago; however, it took an additional 15 years until the interaction of these components was revealed.[5] The third component, ACE2, was the latest to be discovered, in 2000.[6] Interestingly, besides carboxypeptidase activity, ACE2 turned out to have totally different functions and was shown to mediate the adsorption of large amino acids in the gut[7] and to be the receptor for the human severe acute respiratory syndrome virus.[8] Here, we will shortly describe the story of Mas and Ang-(1-7), which was full of errors and uncertainty at the beginning, until the interrelationship between the two was unveiled in 2003.

## DISCOVERY OF MAS: IS IT A PROTO-ONCOGENE?

The Mas gene was first described in 1986.[2] Intriguingly, the first three main discoveries about this gene turned out to be erroneous, mostly due to the fact that the quantity of collected data and the general knowledge at that time obscured the interpretation of the obtained results and led to inappropriate conclusions.

The human Mas gene was originally isolated from DNA of a human epidermoid carcinoma cell line due to its ability to transform NIH3T3 cells upon transfection[2] and therefore was called a proto-oncogene. Since computer analysis of the Mas amino acid sequence suggested that the protein belongs to the class of G protein-coupled receptors (GPCRs) with seven transmembrane domains,[2] this provided the first direct evidence for an oncogenic activity of a GPCR. Though such tumorigenicity was confirmed in independent experiments,[9,10] the oncogenic potential of Mas was challenged. In these experiments, the transfected cells or the tertiary tumor in nude mice contained amplified Mas sequences characterized by rearrangements in 5′- and 3′-noncoding regions, such as an insertion of human centromeric alpha-satellite repeat DNA.[10] However, the original tumor DNA, used in the first round of transfection, was neither rearranged nor amplified or mutated in the Mas coding sequence and therefore cannot be considered as the driving cause for tumor development. Presumably, the *Mas* 5′-noncoding region represents a hot spot of recombination, and the rearrangement of the 5′-noncoding sequence, which occurred during transfection, was responsible for the activation of the *Mas* gene in the tumorigenicity assay. Thus, *Mas* is not an oncogene, but can transform cells, when artificially overexpressed.

## FUNCTIONS OF MAS: IS IT AN ANG II RECEPTOR?

To investigate the functions of Mas, Jackson et al.[11] expressed Mas transiently in *Xenopus* oocytes and stably in a mammalian cell line. Under voltage-clamp conditions, oocytes injected with *Mas* RNA exhibited a dose-dependent induction of an inward current in response to the Ang I, II, and III, whereas in the transfected cells, stimulation of Mas with Ang II

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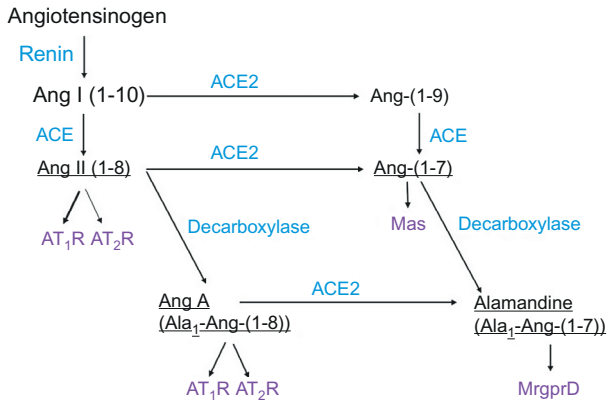


**FIGURE 1** Renin-angiotensin system (RAS). The system consists of several components. First, the protein angiotensinogen is cleaved by renin to the decapeptide, angiotensin I (Ang I, Ang (1-10)). Subsequent cleavage of Ang I by ACE leads to the formation of the octapeptide, Ang II (Ang-(1-8)), which can activate its receptors, $AT_1$ and $AT_2$. This part of the system is the most studied one and is called the "classical RAS" or ACE/Ang II/$AT_1$ axis. However, Ang II is not the only active peptide of the system. Another important component is Ang-(1-7), a heptapeptide, which is mainly produced by the enzyme ACE2 and acts on its receptor Mas. The axis is called ACE2/Ang-(1-7)/Mas and exerts cardioprotective actions. Two further vasoactive peptides of RAS were recently identified: Ang A and alamandine.[61,62] Both are produced by yet unknown aspartate-decarboxylating enzymes from Ang II and Ang-(1-7), respectively. While Ang A binds $AT_1$ and $AT_2$ receptors, alamandine was shown to interact with MrgprD, a receptor of the Mas-related gene (Mrg) family. Enzymatic pathways (blue), peptides (black), and receptors (magenta) in the RAS.

and III led to the mobilization of intracellular $Ca^{2+}$ and to the initiation of DNA synthesis. Based on these results, Mas was proposed to be a functional Ang II receptor—a molecule whose identification had been pending for years. Although a number of further studies were in agreement with this assumption,[12–15] the activation of inward currents by Ang II in *Mas* mRNA-injected oocytes was not inhibited by Ang antagonists.[11] Moreover, Ambroz et al.[16] could show that the intracellular $Ca^{2+}$ increase in *Mas*-transfected cells after Ang II treatment was only observed in cells already expressing endogenously Ang II receptors. Therefore, doubts arose as to whether the *Mas* gene product per se is an Ang II receptor. Moreover, cloning of the Ang II receptor $AT_1$ in 1991[17,18] did not favor the original hypothesis of Ang II being a ligand for Mas. The later identification of Ang-(1-7) as Mas agonist in 2003 and of the direct interaction between Mas and $AT_1$ receptors in 2005[19,20] partly explained the original observations of Jackson et al.[11] in *Xenopus* oocytes and clarified that Mas is not an Ang II receptor per se, but a modulator of $AT_1R$ signaling.

## MAS, ITS ANTISENSE RNA, AND IMPRINTING

A third intriguing story about Mas was published in 1994. Mas was reported to be maternally imprinted in mice during embryonic development and for some organs such as the tongue and heart also in adults[21] and in human breast tissue.[22] In genomic imprinting, one of the two parental alleles of an autosomal gene is silenced epigenetically by a *cis*-acting mechanism. The *Mas* gene is located in close proximity to the imprinted *Igf2r* gene in the mouse and human genomes.[23,24] Imprinting of the maternally expressed *Igf2r* gene is controlled by an intronic imprint control element that contains the promoter of the long noncoding RNA, Airn (antisense *Igf2r* RNA noncoding), which overlaps the silenced paternal *Igf2r* promoter and partially the *Mas* gene in an antisense orientation.[25,26] Our work using Mas-deficient mice and RNase protection assay clearly demonstrated that Mas is biallelically expressed.[27] Thus, due to the lack of strand selectivity in the RT-PCR assays used by Villar and Pedersen[21] and Miller et al.,[22] the maternally imprinted RNA detected by them was most probably not the coding mRNA but the antisense RNA of the *Mas* gene as part of Airn. Altogether, these data demonstrate that Airn but not the *Mas* mRNA is monoallelically expressed in mouse and human.

## MAS EXPRESSION

The distribution of *Mas* expression in different organs of rodents was extensively investigated. The highest expression was found in the brain and testis. In the rat and mouse brain, Mas transcripts are localized not only in the hippocampus and cerebral cortex, in particular in the dentate gyrus, the CA3 and CA4 areas of the hippocampus, the olfactory tubercle, the pyriform cortex, and the olfactory bulb, but also at lower levels all over the neocortex and especially in the frontal lobe.[28,29] In the rodent testis, Mas expression is not detectable in newborn animals, but starts a few weeks after birth and continuously increases during puberty.[30,31] On the cellular level, Mas is confined to Leydig and Sertoli cells, with a clear preference for

Leydig cells.[30] Mas expression was also discovered in other tissues of mice and rats such as the heart, kidney, lung, liver, spleen, tongue, and skeletal muscle.[21,31–33] This ubiquitous low-level presence of Mas mRNA may partly be due to its expression in the endothelial layer of vessels in different organs, as has been shown for the brain,[34] heart,[32] and corpus cavernosum,[35] supporting an important role of this protein in the function of the endothelium. Mas expression was also detected in cardiomyocytes[36] and more recently in cardiac fibroblasts[37] and in the sinoatrial node.[38] Moreover, Mas is present in several tissues involved in glucose and lipid metabolism including the pancreas,[39,40] liver,[41] adipose tissue,[42,43] and skeletal muscle.[44,45]

## MAS FUNCTIONS: MAS-DEFICIENT MICE

Despite this well-described expression pattern of Mas, early studies failed to identify its functions (see above). Some progress was made after mouse deficient for Mas was generated in 1998.[46] These studies showed the importance of Mas for the anxiety-related behavior and indicated Mas as the first GPCR involved in the modulation of long-term potentiation in the dentate gyrus of male mice, whereas spatial learning was not affected in these animals. Interestingly, these behavior phenotypes turned out to be gender-specific and were not detected in female *Mas*-deficient mice.[47] Elucidation of cardiovascular parameters in these mice, which were on the mixed genetic background at that time, did not reveal significant differences in heart rate or blood pressure between knockout and control mice (later, it was shown that *Mas* deficiency on the FVB/N background leads to the elevation in blood pressure, whereas *Mas*-deficient mice on the C57BL/6 background are normotensive[48,49]). Interestingly, female mice showed a reduction of heart rate variability, and knockout animals of both genders showed an increased sympathetic tone.[50] However, the molecular bases of its actions remained obscure until 2003, when finally, the ligand for Mas was identified.

## DISCOVERY OF ANG-(1-7)

Based on the lack of demonstrable pressor effect in structure–activity relationship studies, the heptapeptide Ang-(1-7) was initially considered inactive (reviewed in Ref. [51]). One decade later, this concept was reinforced by the demonstration that it also lacks one of the most classical actions of Ang II: induction of drinking behavior.[52] In keeping with this concept, enzymes capable of forming Ang-(1-7) were called angiotensinases.[53–55] In 1988, we observed that 125I-Ang-(1-7) was the principal product of the 125I-Ang I by micropunches of brain stem homogenates.[3] The formation of Ang-(1-7) was independent of ACE activity. In the same year, the first biological action of Ang-(1-7) in the brain, release of vasopressin from the rat hypothalamo-neurohypophyseal system, was reported.[4] It should be pointed out that 2 years before, Kono et al.[56] described the first biological action of Ang-(1-7) in human. However, the pressor effect described in this study with a high dose of the heptapeptide probably was due to the stimulation of $AT_1$ receptors.[56]

## CLOSING THE GAP: MAS IS AN ANG-(1-7) RECEPTOR

The fact that Ang-(1-7) was equipotent to Ang II for release of vasopressin from hypothalamo-neurohypophyseal explants,[4] contrasting with the lack of its effect on drinking behavior,[52] was the first evidence for the existence of a distinct receptor for Ang-(1-7). Moreover, Ang-(1-7) was reported to release nitric oxide, induce diuresis, and have a vasodilatory effect, favoring a lowering of blood pressure.[56,57] These vascular and baroreflex actions of Ang-(1-7), counteracting the effects of Ang II, suggested that Ang-(1-7) mediates its effects through a novel non-$AT_1$/$AT_2$ receptor subtype. Finally, the description of a selective antagonist for Ang-(1-7) in 1994[58–60] clearly indicated the existence of a receptor for this heptapeptide.

However, only in 2003, more definitive evidence for a specific binding site for Ang-(1-7) was obtained with the demonstration that Mas is a receptor for the heptapeptide.[5] In this study, specific binding of 125I-Ang-(1-7) to Mas-transfected cells was reported. Moreover, the specific binding of 125I-Ang-(1-7) to kidney sections was abolished by genetic deletion of Mas. In addition, *Mas*-deficient mice completely lack the antidiuretic action of Ang-(1-7) after an acute water load, and Mas-deficient aortas lost their Ang-(1-7)-induced relaxation response. These findings provided a clear molecular basis for the physiological actions of this biologically active peptide. At this point, an orphan receptor met an orphan peptide filling an important gap in our understanding of the RAS.

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