

## Gas-sensing riboceptors

Savani Anbalagan 

Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

### ABSTRACT

Understanding how cells sense gases or gaseous solutes is a fundamental question in biology and is pivotal for the evolution of molecular and organismal life. In numerous organisms, gases can diffuse into cells, be transported, generated, and sensed. Controlling gases in the cellular environment is essential to prevent cellular and molecular damage due to interactions with gas-dependent free radicals. Consequently, the mechanisms governing acute gas sensing are evolutionarily conserved and have been experimentally elucidated in various organisms. However, the scientific literature on direct gas sensing is largely based on hemoprotein-based gasoreceptors (or sensors). As RNA-based G-quadruplex (G4) structures can also bind to heme, I propose that some ribozymes can act as gas-sensing riboceptors (**ribonucleic acid receptors**). Additionally, I present a few other ideas for non-heme metal ion- or metal cluster-based gas-sensing riboceptors. Studying riboceptors can help understand the evolutionary origins of cellular and gasocrine signaling.

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### If there are protein-based gasoreceptors, are there nucleic acid-based gasoreceptors?



Gases are among the evolutionarily oldest molecules, likely preceding the RNA world and potentially contributing to the formation of complex polymeric structures [1–6]. As RNA-based organisms evolved, gaseous solute (gas) sensing must have been one of the earliest mechanisms to develop, playing a pivotal role not only in organism evolution but also in the evolution of molecular machinery. Table 1 I have recently proposed gasocrine signaling to unify all the gasoreceptor-based cellular signaling events and to relax the gasotransmitter criteria to include oxygen (O<sub>2</sub>) as an essential gasotransmitter [7,8]. Currently, several protein-based acute gas-sensing sensors or gasoreceptors with diverse signaling domains and activity have been identified across various genera (Table 1). However, the identity of O<sub>2</sub>-sensing gasoreceptors in vertebrates and plants is largely unknown.

Similar to proteins, RNA (ribozymes) can also sense and trigger a cellular signaling event. For instance, bacterial *nhaA-I* and *nhaA-II* RNA motif riboswitches can sense Li<sup>+</sup>, while the *glmS* ribozyme/catalytic riboswitch can sense molecules such as GlcN6P (glucosamine-6-phosphate) and glucose-6-phosphate [9–12]. However, whether gas-sensing riboswitches exist is unknown [13]. Recently, the lncRNA *HIPLS* (hypoxia-induced lncRNA for polo-like kinase 1 stabilization) was identified as essential for O<sub>2</sub> sensing, acting downstream of HIF1 $\alpha$ , one of the hypoxia-inducible factor subunits [14]. To the best of my knowledge, there are no scientific literature on RNA-based structures (ribozyme, riboswitch, RNA-based gas sensor, or receptor, etc.) that can directly sense gases. Does this mean that during evolution, the only ‘sensory’ functions of ribozymes or riboswitches

were for amino acids, metal ions, metabolites and temperature but not gases? [15]. Or are such gas-sensing ribozymes yet to be experimentally demonstrated, or have they not been described in the literature with a unifying term similar to the parable of the blind men and the elephant? [16,17]

### Heme-based protein gasoreceptors

Even before we could debate about the existence of gas-sensing ribozymes or catalytic riboswitches, a question arises: how common are heme (iron protoporphyrin IX)-based gasoreceptors or sensors other than the well-known NO (nitric oxide)-sensing soluble guanylate cyclase? [18] Theoretical arguments for the presence of heme-based gas-sensing proteins have been proposed by others, even for O<sub>2</sub> [19,20]. My arguments for mammalian protein-based gasoreceptors are based on the evolutionary conservation of gas-sensing hemoproteins and gas-binding metalloproteins [21–23]. Heme-based gasoreceptors include DosP (Direct sensor of O<sub>2</sub>), FixL, CooA in bacteria, E75/Eip75 (ecdysone-induced protein 75) in flies, soluble adenylate cyclase in *Leishmania*, and soluble guanylate cyclase in worms, mice and human (Table 1) [7,24–30]. Circadian regulators CLOCK (Clock Circadian Regulator), NPAS2 (Neuronal PAS domain protein 2), and nuclear hormone receptor family protein REV-ERB/NR1D1 are also heme-based gasoreceptors [31–34]. The activity of all these proteins has been experimentally demonstrated to be affected by the binding state of either O<sub>2</sub>, CO, NO, or CO<sub>2</sub>. The heme in the pentacoordinate (and even in hexacoordinate in the case of CooA) geometry allows binding of these gases, which is

**CONTACT** Savani Anbalagan  [savani.anbalagan@amu.edu.pl](mailto:savani.anbalagan@amu.edu.pl)  Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, ul. Uniwersytetu Poznańskiego 6, Poznań 61-614, Poland

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**Table 1.** List of gasoreceptors in diverse organisms.

Gas/solute sensed	Organism	Gasoreceptor protein	Gasoreceptor activity	Riboceptor
O <sub>2</sub>	<i>Escherichia coli</i>	DosP	Phosphodiesterase	?
	<i>Rhizobium meliloti</i>	FixL*	Histidine kinase	?
	<i>Leishmania major</i>	HemAc-Lm	Soluble adenylate cyclase	?
	<i>Caenorhabditis elegans</i>	GCY-35	Soluble guanylate cyclase	?
CO <sub>2</sub>	<i>Caenorhabditis elegans</i>	GCY-9		
CO	<i>Rhodospirillum rubrum</i>	CooA	Transcription factor	?
	<i>Burkholderia xenovorans</i>	RcoM		
	<i>Mus musculus</i>	NPAS2		
CO, NO	<i>Drosophila melanogaster</i>	E75		
	<i>Homo sapiens</i>	REV-ERBβ		
C <sub>2</sub> H <sub>4</sub>	<i>Synechocystis sp. PCC 6803</i>	Light-sensing SynEtr1	Histidine kinase	?
	<i>Arabidopsis thaliana</i>	ETR1,	Histidine kinase	?
		ETR2, ERS1, ERS2, EIN4	Serine/Threonine kinase	
NO	<i>Mus musculus,</i>	GUCY1A3	Soluble guanylate cyclase	?
	<i>Homo sapiens</i>	GUCY1B3		
?	<i>Danio rerio</i>	NO gasoreceptor candidate		
?	<i>Mus musculus, Homo sapiens</i>	Gucy1a		
?		NO-, CO-binding gasoreceptor candidate	RNase III enzyme	?
?		DGCR8/DROSHA		
?		O <sub>2</sub> -binding gasoreceptor candidate	Protease	?
H <sub>2</sub> S	?	ADGB		
CH <sub>4</sub>				
NH <sub>3</sub>				
N <sub>2</sub>				
?				

usually further stabilized by additional interactions such as hydrogen bond formation with the distal histidine [30]. Overall, heme-based gasoreceptors or sensors exist at the protein level with diverse signaling domains and activity that include phosphodiesterase, hexokinase, adenylate cyclase, guanylate cyclase, DNA-binding transcription factor activity (Table 1). However, to the best of my knowledge, the identity of O<sub>2</sub>-, H<sub>2</sub>S-, C<sub>2</sub>H<sub>4</sub>-, CH<sub>4</sub>-, NH<sub>3</sub>-, and N<sub>2</sub>-, sensing gasoreceptors in vertebrates is largely unknown [35–39]. Moreover, it is not clear if heme is the sole co-factor in gasoreceptors, as proteins can bind gases such as O<sub>2</sub>, CO, H<sub>2</sub>S, and N<sub>2</sub> either via metal ions or metal clusters [22,40–42]. Finally, it is worth noting that not all the hemoproteins may have a gas-sensing role (at least without additional binding partners). For example, O<sub>2</sub>-binding hemoprotein cytoglobin exhibits nitrite reductase activity and has been proposed as a redox sensor [43,44].

### Can heme-binding G4-based ribozymes act as RNA-based gasoreceptors?

RNA G4 (G-quadruplex) structures have been described not only in mRNA but also in small and long ncRNAs (non-coding RNAs), TERRA (telomeric repeat-containing RNA), and even in mitochondria RNA [45–48]. RNA G4 structures are widespread and enriched in the 5' UTR of ribosomal protein-coding mRNAs, regulating ribosomal protein translation [49,50]. RNA G4 has been shown to exhibit ribozyme activity, catalysing oxygen transfer reactions and acting as peroxidases and/or peroxygenases both *in vitro* and *in vivo* [51–54]. Such RNA G4-based ribozymes have also been demonstrated in the C9orf72 (chromosome 9 open reading

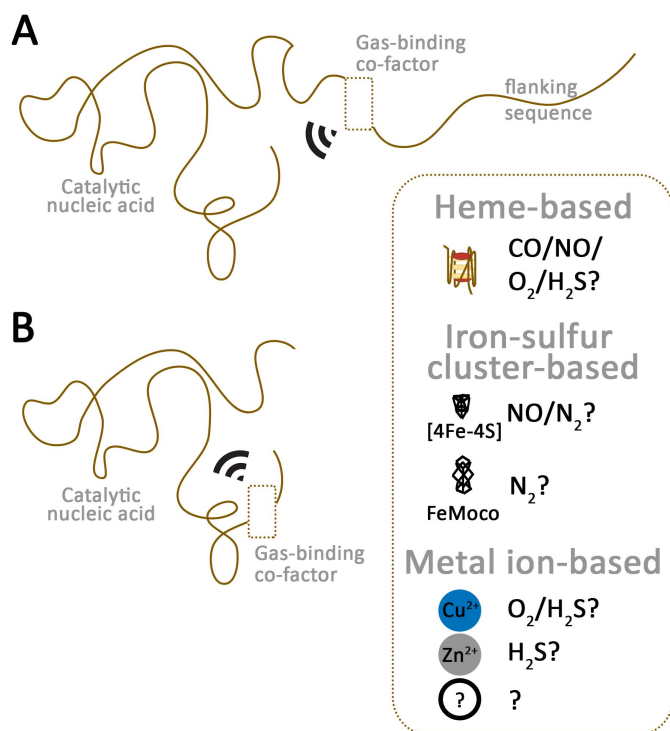
frame 72) gene, implicated in neurodegenerative diseases such as ALS (Amyotrophic lateral sclerosis) [55–58].

RNA-based G4 structures, characterized by stacked G-tetrads, have the capability to scavenge heme, which in its freeform is cytotoxic [51,52,59–62]. Even human rRNA and ribosomes have been shown to bind heme *in vivo* [63]. Functional RNA G4 heme-based ribozymes (e.g. rPS2.M/heme holoenzyme) has been reported to catalyze chlorination of organic substrates [64]. However, the question arises whether all or some RNA G4 associated with heme (heme B) can also bind gas *in vivo* under physiological conditions. In my opinion, if soluble guanylate cyclase can be activated by NO binding to its heme and this binding affects its structure and enzymatic activity, then why not consider that at least some RNA G4 structures with bound heme could similarly respond to gas binding? [65] If gas binding to the heme of RNA G4 triggers a change in the activity of G4-containing or G4-associated ribozymes, then these ribozymes could be considered RNA-based gas-sensing receptors (Figure 1).

Overall, heme-binding RNA G4 structures found within or associated with RNA-based signaling components (such as ribozymes, riboswitches, RNA-based transcription factors), besides their role as heme scavenging, may also function as gas-sensing riboceptors (**ribonucleic acid receptors**) [63,66]. I propose 'riboceptors' as a unifying term to encompass all classes of RNA molecules capable of sensing not only for gas, ions, metal ions, and metabolites, but also temperature and water.

### How to identify heme-based gas-sensing riboceptors?

To identify putative gas-sensing riboceptors, we first need to systemically identify all RNA structures with catalytic (or



**Figure 1.** Model of putative gas-sensing riboreceptors. (A) a riboreceptor can consist of a ribozyme or deoxyribozyme associated with sequences capable of binding diverse gas-binding cofactors. (B) a riboreceptor can consist of a ribozyme or deoxyribozyme containing sequences capable of binding diverse gas-binding cofactors. The gas-binding must modulate the ribozyme activity.

transcription factor) activity that contain or are associated with heme-binding G4-forming sequences and structures. Next, we should determine which heme-based ribozymes can bind to gases such as CO, CO<sub>2</sub>, NO, or O<sub>2</sub> and exhibit a change in ribozyme activity upon binding. The binding of the gases may be stabilized through additional interactions with the RNA, such as hydrogen bonding via guanine or cytosine, similar to distal histidine-based hydrogen bonding seen in O<sub>2</sub>-binding hemoproteins. If binding of the gas does not alter ribozyme activity, then heme-binding could serve a catalytic, scavenging, or transport role for the gas. However, if binding of the gas alters ribozyme structure and activity *in vivo*, then these ribozymes function as heme-based gas-sensing riboreceptors. Mutational studies disrupting heme-binding (or additional interactions with the gas) should confirm loss of gas-binding activity and modulation of ribozyme activity.

### Binding partner, co-riboceptor, or riboreceptor activity-modifying RNA?

Formation of protein-protein or protein-lipid-based functional homodimers, heterodimers, and heteromultimers/oligomers can alter or enhance the protein activity [67–71]. For example, the Wnt/ $\beta$ -catenin pathway requires interaction of the Wnt ligand with the receptor Frizzled, as well as the co-receptor LRP5/6 (lipoprotein receptor-related protein 5 or 6) [72]. Similarly, ligand-selectivity can be regulated depending on the identity of RAMPs (receptor activity-modifying

proteins) and their interaction with receptors [73,74]. In another extreme example, gas-sensing bacterial protein CooA, CO-binding is important for CooA homodimerization and its transcriptional activity [75]. Finally, the RNA-binding protein DGCR8 (DiGeorge Critical Region 8), which interacts with DROSHA to form a pri-miRNA (primary miRNA) processing complex, is also a gas-binding hemoprotein [76–80]. We should consider whether DGCR8 or the pri-miRNA processing factor can be classified as gasoreceptor, given that CO and NO has been shown to inhibit pri-miRNA activity. This suggests that CO and NO act as antagonists of the pri-miRNA processing factor. Debating about it may allow us to consider riboreceptors based on dimerization of ribozymes with other gas-sensing modules (Figure 1). Overall, drawing parallels with protein-based signaling and gas-sensing mechanisms, it remains unknown whether RNA-based gas sensing also requires dimerization of ribozymes or other RNA structures, co-riboceptors, or riboreceptor activity-modifying RNAs (RAMRs).

### Non-heme-based gas-sensing riboreceptors

In proteins, gas-binding occurs not only via heme, but also via metal ions and even metal clusters. For instance, arthropod hemocyanin bind O<sub>2</sub> and CO via Cu<sup>2+</sup> ions, while hemoglobin in vestimentiferan tubeworms can bind H<sub>2</sub>S via Zn<sup>2+</sup> ions [41,42,81]. Ethylene-sensing gasoreceptors in Arabidopsis and cyanobacteria require Cu<sup>2+</sup> ions [82,83]. Metal cluster-based gas binding occurs via FeS (Iron-sulphur), FeMoco (Iron molybdenum cofactor), etc [40,84]. In bacteria, FeS cluster-containing transcription factors FNR and NsrR has been proposed as O<sub>2</sub>- and NO-sensing proteins, respectively [84–87]. However, apart from proteins, nucleic acids can also interact with metal ions [88–92]. Whether metal ion-binding ribozymes or other RNA structures act as gas-sensing riboreceptors is currently unknown (Figure 1). Theoretically, FeS clusters can interact with RNA via coordination bonds, but their stability and role in gas sensing require experimental verification.

Additionally, besides RNA, G-rich DNA can also form G4 structures that bind heme, and DNA (deoxyribozymes or DNzyme) can exhibit catalytic activity [93–96]. Therefore, it is theoretically plausible that heme- or non-heme-based gas-sensing deoxyriboceptors may exist. Overall, until all putative heme- and non-heme-based nucleic acids have been systemically characterized for their ability to bind and sense gases, it remains challenging to dismiss the possibility of gas-sensing riboreceptors. If gas-sensing riboreceptors don't exist, it suggests that the origin of gasocrine signaling is only via protein-based gasoreceptors, which I think is very unlikely if the RNA world theory is true, since gases preceded the RNA world. Finally, as I recently proposed that some of the heme-based gasoreceptors may also function as water-sensing aquareceptors, it remains to be determined whether gas-sensing riboreceptors may also have a dual role as water-sensing riboreceptors [97].

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## Author contributions

Savani Anbalagan: conceptualization, writing of the original draft, and review and editing.

## ORCID

Savani Anbalagan  <http://orcid.org/0000-0003-4839-9439>

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