International Journal of Medical Microbiology 304 (2014) 79–92

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Mini review

The promise of riboswitches as potential antibacterial drug targets

Christina E. Lünse, Anna Schüller, Günter Mayer*

Life & Medical Sciences Institute, University of Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany

ARTICLE INFO

Keywords:
Riboswitch
Ribozyme
Metabolite
FMN
TPP
Glucosamin-6-phosphate
Lysine
Adenine
Guanine

ABSTRACT

Riboswitches represent promising novel RNA structures for developing compounds that artificially regulate gene expression and, thus, bacterial growth. The past years have seen increasing efforts to identify metabolite-analogues which act on riboswitches and which reveal antibacterial activity. Here, we summarize the current inventory of riboswitch-targeting compounds, their characteristics and antibacterial potential.

Introduction

In order to ensure economical use of nutrients and available resources and to quickly adapt to environmental changes, all organisms have to regulate their gene expression tightly. The concerted regulation of genes occurs on different cellular levels and includes the alteration of transcriptional or translational processes (Jacob and Monod, 1961; Browning and Busby, 2004; Mandal and Breaker, 2004a,b). Research of the past decade has elucidated a novel way of gene regulation on the mRNA level that is mediated by so-called riboswitches.

Riboswitches are RNA elements that are mainly embedded in the 5′-untranslated region (UTR) of mRNA molecules and they efficiently regulate gene expression in many fundamental metabolic pathways. These RNA elements usually consist of a highly conserved aptamer domain and a so-called expression platform (Fig. 1). The aptamer domain recognizes a cognate ligand with high affinity and remarkable specificity. Binding of the ligand to the aptamer domain induces a switch between two mutually exclusive RNA conformations in the expression domain, that lead to an alteration of gene expression by induction of transcription attenuation or inhibition of translation initiation.

At present more than 20 riboswitch classes are known (Table 1), some of which represent orphan riboswitches, whose natural ligands remain unidentified to date (Breaker, 2011). Among the known riboswitch classes, seven ligands respond to enzymatic cofactors, like adenosyl-cobalamine (Nahvi et al., 2004), thiamine pyrophosphate (Winkler et al., 2002a,b), flavin mononucleotide (Winkler et al., 2002a,b), S-adenosylhomocysteine (Wang and Breaker, 2008; Wang et al., 2008), molybdenum cofactor (Regulski et al., 2008), S-adenosylmethionine (Grundy and Henkin, 1998; Winkler et al., 2003; Corbino et al., 2005; Lu et al., 2008; Poiata et al., 2009) and tetrahydrofolate (Ames et al., 2010; Trausch et al., 2011). Three riboswitches were described that sense the amino acids glycine, lysine and glutamine (Sudarsan et al., 2003a,b; Mandal et al., 2004; Winkler, 2005; Ames and Breaker, 2011; Trausch et al., 2011) and one recognizes an amino sugar, glucosamine-6-phosphate (Winkler et al., 2004). Moreover, several of these RNA elements specifically interact with purines such as guanine and adenine (Batey et al., 2004; Mandal and Breaker, 2004a,b) or other nucleobase ligands like 2′-deoxyguanosine, prequeuosine (Kim et al., 2007; Roth et al., 2007), cyclic di-GMP (Sudarsan et al., 2008; Lee et al., 2010) or putatively adenosine triphosphate (Watson and Fedor, 2012). Additionally, there have been riboswitches identified, which bind inorganic ligands such as magnesium or fluoride (Ramesh and Winkler, 2010; Baker et al., 2012).

Primary sequences of aptamer domains range in length from 30nt for the preQ$_1$ riboswitch (Roth et al., 2007; Rieder et al., 2010) to more than 150nt, e.g. for the TPP riboswitch (Dambach and Winkler, 2009). To recognize their structurally different ligands, aptamer domains employ diverse secondary structures. However, distinct aptamer folds exist for sensing the same ligand, the most prominent example being the SAM-responsive element, which is categorized into six riboswitch subtypes based on secondary structure constraints (Winkler et al., 2003; Corbino et al., 2005; Fuchs et al., 2006; Weinberg et al., 2008; Poiata et al., 2009; Weinberg et al., 2010).

Sequence and structure conservation within the aptamer domain is used to estimate the phylogenetic distribution and conservation of these RNA elements (Barrick and Breaker, 2007). So

* Corresponding author. Tel.: +49 228 73 4808; fax: +49 228 73 4809.
E-mail address: gmayer@uni-bonn.de (G. Mayer).

1438-4221/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ijmm.2013.09.002

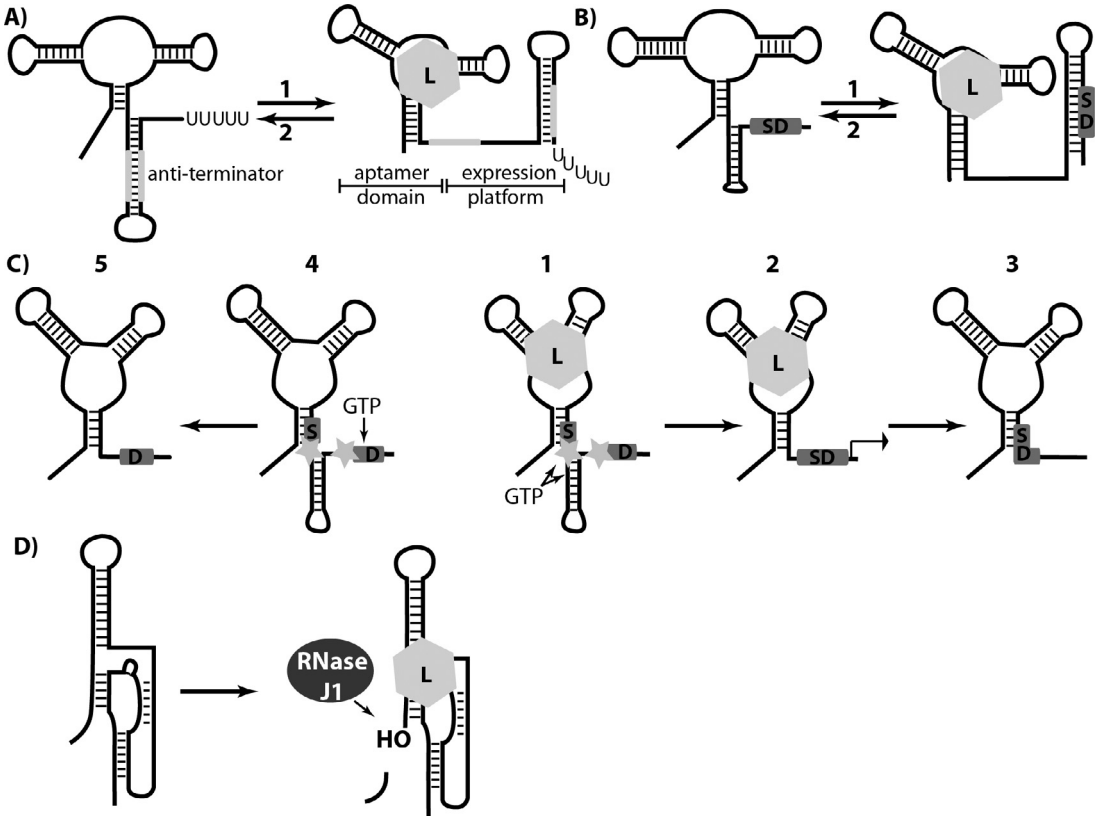

Fig. 1. Common regulatory mechanisms employed by riboswitches. (A) Transcription termination or anti-termination. Riboswitch-mediated gene regulation on the transcriptional level includes switches where cognate metabolite binding to the aptamer domain induces secondary structure changes that foster the formation of a terminator hairpin followed by a stretch of uridines ("off"-switch, 1). "on"-switches, where ligand binding leads to the release of the terminator by pairing with an anti-terminator sequence are also known (2). (B) Regulation of translation initiation. In accordance to "off" and "on"-switches described under (A), there are riboswitches which inhibit (1) or allow (2) translation initiation by sequestering the Shine-Dalgarno (SD) sequence upon metabolite recognition. (C) Intron splicing. Some riboswitches are involved in ligand mediated intron splicing. Here, the example of a class II c-di-GMP riboswitch is illustrated. It creates a functional SD-sequence in the presence of c-di-GMP and GTP by group I intron splicing at splice sites indicated by stars (1–2). The resulting structure is still responsive to the ligand, in this case decreasing concentrations of c-di-GMP lead to a sequestration of the SD-sequence (3). In the absence of c-di-GMP, the ribozyme favors GTP attack shortly before the start codon (4), excising nucleotides of the ribosome binding site (Lee et al., 2010) leading to translation inhibition (5). (D) Ribozyme cleavage and mRNA degradation. A unique regulatory mechanism is employed by the *glmS* ribozyme, which cleaves itself upon binding glucosamine-6-phosphate. Cleaved RNA fragments are prone to degradation by RNase J1 leading to a downregulation of *glmS* mRNA levels.

far riboswitches have been identified in many bacterial species, as well as in archeae (Weinberg et al., 2010), fungi (Cheah et al., 2007), phages (Sudarsan et al., 2008), plants (Wachter et al., 2007) and also in several metagenomic sequences (Kazanov et al., 2007).

Depending on the ligand they sense, riboswitches represent an elegant mechanism for bacteria to quickly react to changes in their environment. As riboswitches are often placed upstream of those genes that are involved in the metabolism of the ligand by which they are triggered, they most often provide an efficient means for feedback regulation in many metabolic pathways (Winkler et al., 2002a,b; Winkler et al., 2002a,b; Winkler et al., 2003, 2004). Other riboswitch classes induce virulence or resistance genes depending on the physiological function of the ligand (Sudarsan et al., 2008; Jia et al., 2013). For example, riboswitches that recognize the second messenger cyclic-di-GMP induce global changes in bacterial metabolism leading to the expression of virulence genes (Tamayo et al., 2007) or the induction of biofilm formation (Hengge, 2009).

Riboswitches mediate their gene regulatory functions via a variety of different mechanisms. One of the most common mechanisms of riboswitch control involves the manipulation of transcription termination (Fig. 1A). Here, ligand binding induces a conformational change in the expression platform that creates a terminator stem followed by a stretch of five to nine uridines. Upon encountering this structure the RNA polymerase pauses and eventually dissociates from its template, thus prematurely ending transcription. In the absence of ligand, a competing second structure or anti-terminator is formed that allows efficient mRNA production (Breaker, 2012; Serganov and Nudler, 2013).

Another commonly used mechanism of gene expression involves riboswitch regulation at the translation initiation level (Fig. 1B). Here, two different RNA folds control ribosome access to the ribosome-binding site, also termed Shine-Dalgarno (SD) sequence. In the presence of the ligand, a complementary-SD-sequence sequesters the ribosome binding site, which prevents association of the small ribosomal subunit and hence translation start (Breaker, 2012; Serganov and Nudler, 2013).

In addition to these widespread mechanisms, there are riboswitches that regulate splicing (Fig. 1C) (Cheah et al., 2007; Croft et al., 2007; Wachter et al., 2007), or gene expression via transcription interference or antisense action (Andre et al., 2008; Breaker, 2012), dual transcription and translation control (Rodionov et al., 2004; Andre et al., 2008) as well as RNA cleavage (Fig. 1D) (Winkler et al., 2004; Collins et al., 2007).

Riboswitch targeting compounds

In order to enable precise gene regulation, riboswitches have to specifically recognize their cognate metabolite and reliably