Riboswitches as Therapeutics Targets

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Regulation of gene expression includes a wide range of mechanisms that are used by cells to control the production of specific gene products. It has long been recognized that organisms make extensive use of protein based control systems to regulate gene expression. In eukaryotes, the networks of protein signaling and gene control factors are very complex, where numerous factors typically work together to influence transcription, translation, mRNA processing/degradation and other mechanisms that control the levels of gene products in a cell. However, research over the past several years have found that RNA, which was considered only as a cellular messenger carrying out genetic information also plays a more intimate role in the control of gene expression. A variety of microRNAs (miRNAs) and related short-interfering RNAs (siRNAs) are functioned by a series of protein-mediated processing events that regulates gene expression by forming base paired structures with target mRNA resulting in inactivation of the targeted gene by subsequent nuclease processing or by other non-nucleolytic mechanisms.

One of the most striking recent examples of how RNA regulates gene expression was revealed by the discovery of riboswitches, a common means of genetic regulation at the mRNA level in the bacterial kingdom. Riboswitches are complex folded RNA domains that serve as receptors for specific metabolites. These domains are found in the 5′-untranslated region (UTR) of mRNAs that exert their regulatory control over the transcript in a cis-fashion, by harnessing allosteric structural changes that are brought about by metabolite binding without the obligate involvement of a protein factor. They are mostly located upstream regions of bacterial mRNAs associated with metabolism and transport of their cognate metabolites.

The term 'riboswitch' was first coined by Breaker and co-workers confirming the existence of a 5′-UTR sequence in *E.coli btuB* mRNA which selectively binds to the coenzyme B_{12}. A number of different riboswitches that bind and sense different cellular metabolites like amino acids and their derivatives, carbohydrates, and nucleobases and their derivatives have been discovered. Some of the recently discovered riboswitches include those that bind to c-AMP-GMP, 5-Amino-4-imidazolecarboxamide riboside 5′-monophosphate (ZMP), cations Mn^{2+} and Ni^{2+}/Co^{2+} binding motifs and anions such as fluoride.

Riboswitches typically consists of two parts: an aptamer domain and an expression platform. The aptamer domain which functions as a molecular sensor adopts a compact three-dimensional fold to scaffold the ligand binding pocket. It has high selectivity and specificity to discriminate and bind to chemically similar metabolites. The expression platform is typically located immediately downstream from the aptamer domain, and in many instances the two domains overlap to some extent. The role of the expression platform is to transduce metabolite-binding events into gene-control consequences by allosteric modulation of the structure of the 5′-UTR. Switching sequence located between these two domains plays a pivotal role, whose pairing directs folding of the RNA into one of the two mutually exclusive structures in the expression platform that represent the on and off states of the mRNA.

Many mechanisms are known for the regulation of genes by riboswitches at various levels of transcription and translation. One of the most general mechanisms involves the formation of ligand-dependent intrinsic terminator stem, which is a GC rich stem and typically trailed by a run of six or more U residues. This causes RNA polymerase to abort transcription before the coding portion of the mRNA has been made. When the aptamer domain is un-complexed with ligand, it permits formation of an anti-terminator stem, which precludes formation of the intrinsic terminator stem and thereby permits transcription of the complete mRNA. In the second mechanism, similar structural changes in full-length mRNA control ribosome access to the ribosome binding site or start codon sequences thereby leading to blockage of translation initiation. Furthermore certain ligand associations with riboswitches bring about self-cleavage of mRNA while certain other riboswitches lead to the production of antisense RNA. Additionally there are combined forms of riboswitch dependent gene regulations such as tandem riboswitches.

Emergence of antibiotic resistance is a serious public health issue all over the world. Riboswitches, as metabolite sensing domains of bacterial mRNAs, represent a promising novel solution to multiple drug resistance (MDR), since they can be considered as antimicrobial targets when agonistic ligands are employed to knock down the expression of associated gene or genes. Examples of such riboswitches used as antimicrobial targets include: thymine pyrophosphate (TPP) riboswitch, glycine riboswitch, lysine riboswitch, FMN riboswitch, glmS riboswitch and guanine riboswitch.

TPP, which is a target of one of the most widespread riboswitch classes, is commonly involved as a coenzyme for decarboxylase enzymes. This riboswitch negatively regulates the expression of proteins involved in the biosynthesis and transport of thymine in bacteria. It binds to its ligand with a

dissociation constant of 100 nM and discriminates by a factor of 100 fold against thiamine phosphate (TP) which only differs from TPP by one phosphate. Pyrithiamine which is an isosteric pyrimidine analog of thiamine is phosphorylated to pyrithiamine pyrophosphate and then binds to the TPP riboswitch.

Lysine riboswitches are involved in the control of biosynthesis and transport of lysine. L-aminoethylcysteine (AEC) and DL-4-oxalysine are lysine analogs that inhibit the growth of some Gram-positive bacteria. They bind to the *lysC* riboswitch of *B. subtilis* and repress expression of a lysine riboswitch regulated reporter gene in *B. subtilis*. Roseoflavin, an analog of riboflavin and FMN, is a pigment from *Streptomyces davawensis* with antimicrobial activity. Roseoflavin inhibits the growth of several Gram-positive bacteria, and roseoflavin resistant mutants overproduce riboflavin. In Gram-positive bacteria, all genes involved in riboflavin synthesis are under the control of a single FMN riboswitch. It was found that roseoflavin binds FMN riboswitch *in vitro* and down regulates the expression of a *lacZ* reporter gene under the control of FMN riboswitch. Mutation in all the above mentioned riboswitches cause disruption of antimicrobial activity and inhibit their activity.

glmS riboswitch has the unique capacity of mRNA self-cleavage through binding to GlcN6P. A 5′-OH terminus RNA product of cleavage is recognized by RNase J1 which degrades *glmS* mRNA. The cognate ligand, GlcN6P, is the precursor of peptidoglycan biosynthesis making it an essential metabolite needs for bacterial cell wall synthesis. In addition, most of the *glmS* riboswitches are present in Gram-positive organisms and it has been shown that *glmS* riboswitch-ribozyme cleavage-inhibition leads to inability of sporulation or forming biofilm. According to different analog studies, three functional groups of GlcN6P including the anomeric hydroxyl, the amine and the phosphate are important to interact with *glmS* riboswitch. As a result, some compounds such as glucosamine (GlcN), L-serine, serinol, tris and ethanolamine which contain vicinal amine and hydroxyl groups are weak activators of *glmS* riboswitch.

Targeting the riboswitches provides many advantages over other molecular targets. Compared to the rRNA, which is one of the most common targets of antibiotics, riboswitches bind small molecules more selectively and specifically. This is because riboswitches are RNA receptors. Most of the riboswitches exist mainly in bacteria and not in eukaryotes. Therefore this will reduce the cross reactivity of bacterial riboswitch sensing ligands. Since most of the riboswitches are associated with genes that are important for survival or/and resistance, targeting them will lead to the death of the organism or weakening of the organism.

Riboswitches offer new therapeutic approaches to address human diseases due to their structural sophistication, specificity, and their function as genetic regulators of essential bacterial genes. Structure-guided rational design, high-throughput screening methods, and riboswitch- specific assays have been applied to the discovery of novel riboswitch-targeted drugs. These efforts have produced compounds with *in vivo* antibacterial activity that appear to be functioned by targeting riboswitches. Previously reported antibiotics and newly identified compounds that function through riboswitches emphasize the progress made in the field and provide a foundation for future discovery of new riboswitch-targeting compounds.

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