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Ribozymes, riboswitches and beyond: regulation of gene expression without proteins

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Abstract

Although various functions of RNA are carried out in conjunction with proteins, some catalytic RNAs, or ribozymes, which contribute to a range of cellular processes, require little or no assistance from proteins. Furthermore, the discovery of metabolite-sensing riboswitches and other types of RNA sensors has revealed RNA-based mechanisms that cells use to regulate gene expression in response to internal and external changes. Structural studies have shown how these RNAs can carry out a range of functions. In addition, the contribution of ribozymes and riboswitches to gene expression is being revealed as far more widespread than was previously appreciated. These findings have implications for understanding how cellular functions might have evolved from RNA-based origins.

More than 40 years of extensive research has revealed that RNAs participate in a range of cellular functions. Some RNAs, despite being composed of only four chemically similar nucleotides, can fold into distinct three-dimensional architectures. In many cases, these constitute simple scaffolds that provide binding sites for proteins that function together with the RNAs. However, the discovery of the first catalytic RNAs — later named RNA enzymes, or ribozymes — in the 1980s demonstrated that RNAs can also have important functional roles in their own right[1,2]. The list of naturally occuring ribozymes is short, and additions over the years have been rare, with each new discovery eliciting considerable excitement in the field.

Studies of molecular evolution suggest that RNA molecules significantly influenced the development of modern organisms by mediating the genetic flow from DNA to proteins, as well as through their own contribution to catalytic functions. The ‘RNA world’ hypothesis implies that RNA molecules appeared before DNA and proteins[3]. Nevertheless, even with a head start, RNA catalysts do not prevail in the modern world. Moreover, most ubiquitous ribozymes require proteins for efficient catalysis *in vivo*, and most true protein-devoid catalytic RNAs have been found in only a few viral-like sources, suggesting that the contribution of protein-free RNAs to the functions of modern cells has been limited.

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Competing interests statement

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The discovery of riboswitches[4–6] and other RNA-based sensors reignited interest in the roles of protein-devoid RNA-based elements. These RNA sensors can be broadly defined as mRNA regions that are capable of modulating gene expression in response to internal and external inputs, without the initial participation of proteins. Unlike ribozymes, many of these sensors direct gene expression purely through changes in RNA conformation. For instance, riboswitches alter their conformations in response to small metabolites. However, the identification of the bacterial ribozyme *glmS*, which specifically binds a metabolite and cleaves the mRNA encoding the protein that controls the metabolism of that metabolite, has established a closer link between riboswitches and ribozymes[7]. Recent findings of novel riboswitches, ribozymes and other RNA-based regulatory elements further highlight the essential contribution of such RNAs in gene expression regulation.

In this Review, we mainly focus on naturally occurring RNAs that have distinct three-dimensional structures and that can function without the help of proteins. We first present an overview of ribozymes, riboswitches and other related RNA sensors, and highlight their impact on gene regulation. We then analyse their structure–function relationships, dissecting the features that are essential for gene expression control and other cellular processes. Finally, we compare the functions of protein-free RNAs with those of proteins and RNA–protein complexes, providing a perspective on the evolution of the role of RNAs in the gene regulatory processes of modern organisms.

Ribozymes

Although naturally occurring ribozymes, excluding the ribosome, all catalyse the same reaction of RNA strand scission and ligation, they can be divided into two groups according to their main function: cleaving ribozymes, which include self-cleaving RNAs and *trans*-cleaving ribonuclease P (RNase P); and splicing ribozymes, which are large RNAs involved in the excision of introns from precursor RNAs (pre-RNAs) (TABLE 1).

Cleaving ribozymes

Self-cleaving ribozymes range from ~40–200 nucleotides in length, and have various secondary structures and three-dimensional folds (FIG. 1). For the purpose of this Review, this group can be tentatively split according to their function in either RNA replication or mRNA cleavage.

The ribozymes of the first subgroup, which include the hammerhead[8], hairpin[9], hepatitis δ virus (HDV)[10,11] and Varkud satellite (VS)[12] ribozymes (FIG. 1a–d), are predominantly found in satellite RNAs of plant origin. These RNAs are the product of a rolling-circle replication mechanism involving ribozyme processing of long multimeric RNAs into short monomers that, following cyclization, participate in the next round of replication.

The second subgroup includes the recently discovered, more diverse ribozymes that reside within eukaryotic pre-mRNAs (the *CPEB3* (REF. 13) and co-transcriptional cleavage (CoTC)[14] ribozymes) and a bacterial mRNA (the *glmS* ribozyme)[7]. The *CPEB3* ribozyme (FIG. 1e) lies within the second intron of the mammalian *CPEB3* gene, which encodes cytoplasmic polyadenylation element binding protein 3 (REF. 13). The secondary structure

and cleavage-site organization of this catalytic RNA closely resemble those of the HDV ribozyme (FIG. 1c). Although the exact function of the *CPEB3* ribozyme is unclear, it might function in the regulation of CPEB 3 biosynthesis by interfering with mRNA splicing and facilitating mRNA degradation and/or the production of truncated protein forms.

The CoTC element (FIG. 1f) has been identified downstream of the protein-coding sequences and poly(A) sites of primate β-globin genes, suggesting that CoTC has a role in transcription termination and RNA processing[14]. Indeed, CoTC integrity was found to be crucial for efficient termination of transcription of these genes by RNA polymerase II[15], and CoTC RNA can be targeted by several proteins that have been implicated in RNA processing and degradation (A. Akoulitchev, personal communication). Auto-catalytic cleavage by the CoTC ribozyme requires the presence of GTP or its derivatives. Because the rate of CoTC self-cleavage is low, the biological significance of this ribozyme remains to be validated.

The *glmS* ribozyme (FIG. 1g), which was identified in the 5′ region of the bacterial *glmS* gene, also requires a specific cofactor for self-cleavage. This gene encodes an amidotransferase[7], which generates glucosamine-6- phosphate (GlcN6P), a molecule that is used in cell-wall biosynthesis. When sufficient GlcN6P is present, the ribozyme uses GlcN6P as a coenzyme in the self-cleaving reaction that is thought to cause degradation of the *glmS* mRNA, thereby turning off the gene.

Almost all self-cleaving ribozymes break the RNA backbone through the same reversible phosphodiester-cleavage reaction (FIG. 2a). Nevertheless, they have different catalytic pockets and use different cleavage mechanisms, which are still being debated[16]. The CoTC ribozyme catalyses a different reaction[14], producing 3′-hydroxyl (3′-OH) and 5′-phosphate (5′-P) ends, and apparently carries out autolytic cleavage in the same way as RNase P.

RNase P is the only naturally occurring ribozyme identified so far that performs a multiple-turnover RNA cleavage reaction *in trans*, involving multiple substrate molecules[2]. This ubiquitous ribozyme removes extra sequences from the 5′ ends of pre-tRNAs and some other RNAs, by a different mechanism from the reaction catalysed by self-cleaving ribozymes (FIG. 2b). RNase P contains distinct protein subunits that are essential for its *in vivo* function, but protein-free RNase P from both bacteria[2] and eukaryotes[17] exhibits detectable activity *in vitro*. Despite differences in sequence, length and secondary structure, RNAse P RNAs contain five universally conserved regions that constitute the structural core of the RNA[18]. These regions include the P4 helix, the P10–P12 and the P2–P15.2 regions (FIG. 1h).

RNase P is structurally and evolutionarily related to RNase MRP, which is found only in eukaryotes. Both RNases contain similar RNA components and share several proteins[19]. Nevertheless, each RNase has its own substrate preferences. In contrast to RNase P, RNase MRP is implicated in 5.8S pre-ribosomal RNA (pre-rRNA) processing and in RNA cleavage during mitochondrial DNA synthesis, but not in pre-tRNA processing.

Splicing ribozymes

Typical nuclear mRNA splicing requires a complex machinery that involves the assembly of RNA–protein complexes. Splicing ribozymes can perform the precise excision of an intron and the covalent linkage of the boundary exons, with or without assistance from specific protein factor(s). This group encompasses two classes of heterogeneous self-splicing introns (groups I and II), which can be found in many tRNA, mRNA and rRNA precursors (TABLE 1).

Most large self-splicing introns fold into two types of multidomain secondary structure that define their division into groups I and II[20,21] (FIG. 1i,j). In each group, helical elements that form the catalytic core of the molecule are relatively conserved, whereas the peripheral regions vary, allowing classification into several subgroups. Smaller introns that have a group II-like domain VI and a streamlined domain I, but lack domains II–V, establish a separate family[21]. Self-splicing occurs *in vitro* for most group I and for several group II introns; however, a protein machinery is required for efficient splicing *in vivo*[22]. Introns can also encode RNA maturases, homing endonucleases and reverse transcriptases (with only group II introns), which help introns to splice and to spread within genomes.

In contrast to the cleaving ribozymes, the splicing ribozymes perform two consecutive reactions: cleavage and ligation of RNA. Sequence specificity and the locations of splice sites are defined by the interactions between the 5′ region of the intron (the internal guide sequence, or IGS) and two exons (domains P1 and P10) in group I introns (FIG. 1j), and by two or three pairs of interactions between intron binding sites (IBSs) and exon binding sites (EB Ss) in group II introns[22] (FIG. 1i). The first step of self-splicing requires an exogenous nucleophile, and is therefore similar to the RNase P-mediated reaction. However, instead of water, group I and II introns use external guanosine (αG) and internal adenosine as nucleophiles, respectively (FIG. 2c,e). Growing evidence suggests that some group II introns that lack the crucial adenosine undergo an alternative hydrolytic pathway[23] (FIG. 2f). Remarkably, the active sites of group II introns can accommodate a diversity of nucleophiles (2′-OH group, 3′-OH group and water) and substrates (DNA and RNA) and, in addition to splicing, can catalyse several other reactions, which are either adaptations of the basic splicing activity or distinct reactions such as terminal transferase activity[24]. Somewhat unexpectedly, the group I-like ribozyme GIR1 from the slime mould *Didymium iridis* forms a 2′,5′-lariat, similar to that of the group II introns[25] (FIG. 2d). This lariat structure apparently serves as a cap, which protects the intron-encoded endonuclease mRNA against degradation.

RNA switches

The term riboswitch is usually applied to metabolite-sensing RNA switches, which share important characteristics with other RNA sensors that are responsive to cations, temperature and regulatory RNA molecules (TABLE 1). Indeed, all these mRNA-based control systems, identified in many prokaryotes and some eukaryotes, can sense various stimuli and transduce them to the gene expression apparatus, with proteins being recruited only at a later stage. By contrast, in classical transcriptional attenuation control, ribosomes or specialized

proteins are directly involved in the initial step of regulation by helping to sense metabolite levels.

Temperature sensing

RNA thermosensors[26,27] are the simplest RNA switches, yet they control adaptation to an important physical parameter that affects multiple cellular processes. Because RNA secondary structure is highly influenced by temperature, a thermosensor can be as primitive as a stem–loop RNA structure that bears a ribosome binding site (RBS) and an initiation codon[28] (FIG. 3a). At low temperatures, this RNA-based thermometer adapts a conformation that masks the RBS and prevents ribosome binding. At elevated temperatures, secondary structure elements melt locally, thereby allowing ribosome binding to the exposed RBS and translation of the mRNA. Intrinsic thermometers are well documented in two instances: during pathogenic invasion and during heat-shock responses. When the pathogen *Listeria monocytogenes* enters an animal host, it encounters a warmer environment, and virulence-associated genes that are normally silent at low temperatures become activated. This occurs because of a thermosensor located within the 5′ untranslated region (5′-UTR) of the *prfA* mRNA[28] (FIG. 3a). A simple RNA thermometer, the repression of heat-shock gene expression (ROSE) element, which was identified in the *hspA* 5′-UTR, also upregulates a heat-shock gene at high temperatures in the bacterium *Bradyrhizobium japonicum*[29]. More complex RNA structures, including large *trans*-acting RNAs, can participate in temperature sensing, typically during heat and cold shocks, in various organisms including bacteriophages[26], bacteria[27,30] and eukaryotes[31].

RNA controls RNA

A similar strategy of either sequestering or exposing an RBS is utilized in gene expression control by some regulatory antisense short RNAs (sRNAs) in bacteria. These riboregulators are transcribed in response to various changes in the environment, and can in turn regulate genes by base pairing with target mRNAs in the regions overlapping or adjacent to the RBS. The sRNA–mRNA interactions can occur within a single region, as for the replication control of plasmid R1 by CopA RNA[32] and the control of biosynthesis of the RNA polymerase σ^s-factor by DsrA RNA[33,34], or within two regions that are distant or close to each other, as for DsrA- and OxyS-mediated control of transcription factor production[33,35,36] (FIG. 3b–c). Note that many sRNAs require pairing with the RNA chaperone protein Hfq (host factor Q) for their activity[37,38]. In addition to translation, RNAs can also direct transcription of genes by targeting transcription termination signals. The most spectacular example of such regulation involves tRNA. Non-aminoacylated $tRNA^{Tyr}$ (REF. 39), $tRNA^{Gly}$ (REF. 40) and others[41] can selectively activate transcription of the cognate aminoacyl-tRNA synthetase gene via specific interaction of the anticodon and acceptor stem with the so-called T-box region located in the 5′-UTR (FIG. 3d). This binding promotes formation of the anti-terminator stem and disruption of the terminator hairpin, thereby producing the ‘on’ state of the gene. However, aminoacylated tRNA does not bind mRNA using its aminoacylated acceptor stem; therefore, the terminator stem forms, rather than the anti-terminator, causing transcriptional abortion.

Regulation by metabolites

Metabolite-binding riboswitches, numerous examples of which are found typically embedded in the 5′-UTRs of bacterial metabolic genes, represent genetic elements that are reminiscent of T-boxes (TABLE 1). Riboswitches typically consist of evolutionarily conserved ~35–200-nucleotide sensing or aptamer domains — which specifically bind metabolites when concentrations exceed a threshold — and expression platforms that form or carry gene regulatory signals[42]. Riboswitches can adapt alternative metabolite-free and metabolite-bound conformations, which control gene expression as on–off switches. In bacteria, this is achieved by the formation of structures that form and disrupt either transcription terminators or hairpins that carry translation initiation signals. In fungi, a thiamine pyrophosphate (TPP)-specific riboswitch located within an intron was found to be involved in the regulation of splicing[43,44]. Regulation generally depends on alternative base pairing of the small region involved in the formation of helix P1, which locks the sensing domain in the metabolite-bound conformation. Although most riboswitches are integrated into negative-feedback regulation loops, some activate gene expression; for example, an adenine-specific riboswitch achieves this by disrupting a transcriptional terminator[45] (FIG. 3e).

To date, metabolite-sensing riboswitches were found to direct expression of the bacterial genes that are implicated in the biosynthesis and transport of various metabolites, including co-enzymes[4–6,46–52], amino acids[53,54] and nucleobases[45,55,56] (FIG. 4). A sugar derivative is also sensed by the *glmS* ribozyme[7]. Discovery of the magnesium sensor that controls the magnesium transporter MgtA of *Salmonella enterica*[57] suggests that riboswitches are involved in cellular processes that until now were not thought to be subject to riboregulation. The ligands of several conserved RNA elements are still to be identifed, and these RNA elements might represent novel riboswitch classes[46,58]. Remarkably, riboswitches can discriminate between highly similar compounds, such as S-adenosylhomocysteine (SAH) and S-adenosylmethionine (SAM), which differ by only a single methyl group[47,49,52] (FIG. 4c). Recent studies have identified several metabolite-like antimicrobial compounds that function by targeting riboswitches. Among them are the TPP analogue pyrithiamine pyrophosphate (PTPP), which differs from TPP by the central ring[59] (FIG. 4b), two analogues of lysine, L-aminoethylcysteine (AEC) and DL-4-oxalysine, which contain carbon substitutions in the lysine side chain[60] (FIG. 4i), and the FMN analogue roseoflavin[61] (FIG. 4f). Interestingly, high specificity for a particular ligand can be conferred by different sequences, and some metabolites such as SAM interact with several types of sensing domain[46–49,52] (FIG. 4c–e), suggesting that riboswitches have taken on similar functions by following different evolutionary paths.

Structure–function relationships

The chemical simplicity of RNA molecules raises the question of whether diverse RNA-based genetic elements utilize a limited number of architectural components and folding rules. The recently solved three-dimensional structures of several riboswitches[62–67] and the majority of ribozyme types[68–80] have shed light on this question.

Architectures that have an impact on function

The structures of ribozymes and riboswitches highlight the concept of modularity, a principle that is also used by many other RNAs to generate and maintain specific architectures[81]. All RNAs, ranging from simple thermometers (FIG. 3) to complex ribozymes (FIG. 1), can be viewed as hierarchical assemblies that are composed of helices as the major building blocks. The helices associate into bundles by end-to-end stacking and edge-to-edge docking, and are connected together by junctional regions and tertiary interactions. Many oligonucleotide elements that are present in secondary structures as non-paired sequences can form compact and often helix-like topologies. A comparison of riboswitch and small ribozyme structures reveals that the key elements defining these architectures are junctions composed of three (FIGS 1a;4a,b) or more (FIGS 1b;4c) helical segments that converge together. In large ribozymes[68,73,80], the TPP riboswitch[63,65,67] and the hairpin[77] ribozyme, these junctions comprise structural elements that are necessary for the correct orientation of the helical domains, in some cases demonstrating a remarkable convergence in terms of global architecture[82]. This feature is also observed for the SAM-I riboswitch[64] and the P4–P6 and P3–P7 domains of group I introns[68,71,72].

To reinforce junctional conformations, spatial constraints are provided by diverse and complex tertiary interactions, which most often involve loop–loop[62,66] (FIGS 1a,h;4a), loop–helix[63,65,67,76] (FIGS 1g;4b) and helix– helix interlocking connections between peripheral regions. In the absence of crucial tertiary loop–loop contacts, the hammerhead ribozyme shows a 100-fold loss of activity[83,84], and purine riboswitches do not interact with their ligands[62]. Ribozymes, riboswitches and other RNAs share several conserved three-dimensional nucleotide combinations called structural motifs. These include the T-loop, a 5-bp hairpin that is closed by a special non-canonical pair found in the TPP riboswitch[63,65,67] and RNase P[85], and the kink-turn, a sharp bend in the backbone of the RNA strand found in the SAM-I riboswitch[64] (FIG. 4c) and the *Tetrahymena thermophila* group I intron[72].

Folding of ribozymes and riboswitches

Not surprisingly, splicing reactions and larger substrates require longer ribozymes with intricate three-dimensional structures, whereas smaller self-cleaving ribozymes and riboswitches adopt simpler folds. It is therefore notable that ongoing *in vitro* studies indicate that large ribozymes self-assemble into almost-active conformations in the presence of magnesium[86], and subsequently undergo smaller substrate-induced conformational changes. By contrast, riboswitches require their ligands for efficient folding, thus demonstrating a typical induced-fit binding mechanism. The name ‘riboswitch’ implies a switch between two states in response to the presence of metabolites; however, more than half of the putative riboswitches that have been identified are predicted to function through transcriptional attenuation, when RNA polymerase must make a choice between mRNA elongation and transcription termination soon after the sensor domain of the riboswitch has been synthesized. After that event, the energy barrier for re-folding of the domain would be too great to overcome, suggesting that, instead of switching between conformations, a riboswitch makes a ‘once-in-a-lifetime’ choice between ‘on’ and ‘off ‘ conformations[87].

Because of the high speed of RNA polymerase, some riboswitches such as the FMN riboswitch[88] might not have sufficient time for metabolite–RNA interactions to reach thermodynamic equilibrium before the regulatory decision is made; therefore, they might require higher concentrations of metabolites (relative to the apparent dissociation constants) to affect transcription[4,50]. Other riboswitches might need to attain thermodynamic equilibrium with ligands to make a choice between continuation or termination of transcription. However, the adenine-sensing riboswitch utilizes kinetic or equilibrium control depending on the speed of transcription and external variables such as temperature[89]. Interestingly, co-transcriptional folding seems to dictate the formation of the tuning-fork-like architecture that is observed for several riboswitches[62,64–67]. Such a mechanism, in which two helical segments trap the metabolite between them, thereby stabilizing the transient helix P1 and preventing it from adopting an alternative base-pairing alignment, might be superior to single-site recognition, especially for bulky and extended metabolites.

An interesting situation is seen in the context of phage and bacterial mRNAs that contain self-splicing introns. In bacteria, transcription and translation are coupled, and translating ribosomes can prevent the formation of an elaborate intron structure that requires the splice sites to be in close proximity. Therefore, efficient splicing requires that RNA folding is coordinated with transcription and translation[90].

Catalysis and binding: similar trends?

A direct comparison between molecular recognition mechanisms used by ribozymes and riboswitches is difficult to undertake owing to the fact that ribozymes target a specific sugar-phosphate backbone position whereas riboswitches recognize various small molecules. An analysis of available structures also indicates that ribozymes and riboswitches exhibit great structural diversity despite superficial similarities in their global architectures. For example, a comparison of RNAs with similar scaffolds, such as the three-way helical junctions that are shared by the hammerhead ribozyme, purine and TPP riboswitches (FIGS 1a;4a,b), shows that they are used for conceptually different goals (BOX 1). In the hammerhead ribozyme[76], an open catalytic pocket formed by a three-way junction functions to precisely position a scissile phosphate. By contrast, the three-way junction of the purine riboswitches[62,66] consists of several stacked layers of base triples, and nucleobase ligands such as adenine, guanine or the guanine analogue hypoxanthine are sandwiched between layers so that ~98% of the surface area is shielded from solvent[62]. In the TPP riboswitch[63,65,67], the junction adopts a simpler fold and the ligand is recognized outside of the junctional region by two helical segments (FIG. 4b). The same principle of bridging two helical segments by the ligand is exploited by the SAM-I riboswitch[64] (FIG. 4c). Despite different ligand-binding properties, the binding event in purine, TPP and SAM-I riboswitches leads to the same result: stabilization of the overall junctional region, which in turn contributes to the stabilization of the transient helix P1, which is involved in the gene expression switch.

Box 1

The architecture of binding and catalytic pockets

The functioning of ribozymes and riboswitches depends on the precise formation of catalytic domains and binding pockets, respectively. How do these RNA molecules achieve an exceptionally high specificity for recognition and catalysis despite a limited arsenal of functional groups, and are there common principles that underlie ligand–substrate recognition?

In the hammerhead ribozyme[76], the scissile phosphate (indicated by a yellow sphere in panel **A**) is recognized in an 'open' pocket. Nucleotides C17 and C1.1, which are neighbours to the scissile phosphate, are splayed apart. C1.1 forms hydrogen bonds (indicated by black dashed lines) with G2.1, whereas C17 is intercalated into the core of the junction. The nucleophilic 2′-hydroxyl (OH) of C17 is positioned in line with the scissile phosphate and the 5′-oxygen of the leaving group (indicated by a blue dashed line coming from the phosphate group). The conserved G8 and G12 are located nearby to facilitate acid–base catalysis. Red dashed lines indicate hydrogen bonds that are potentially active for the catalysis.

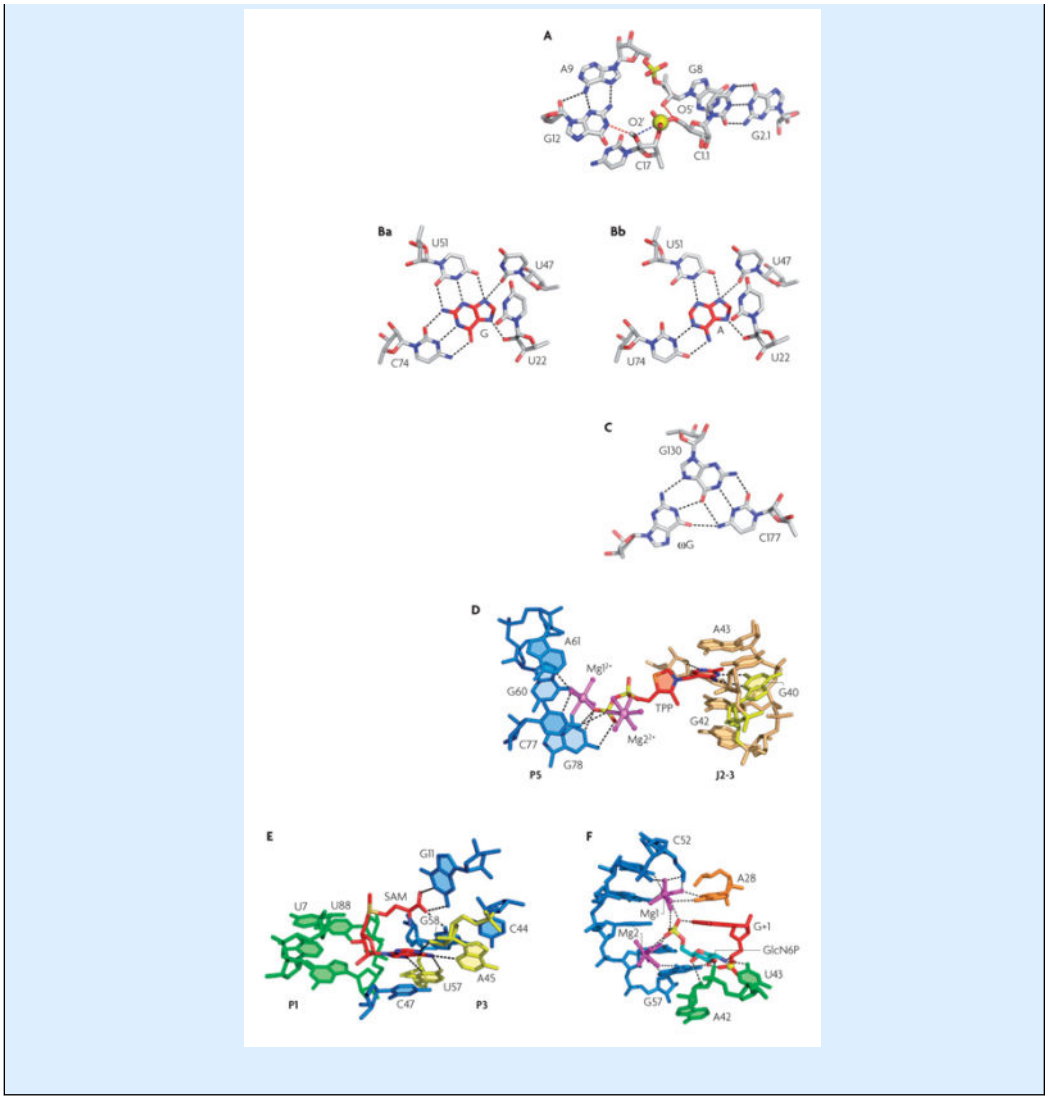
Purine riboswitches contain a 'closed' ligand-binding pocket that is located between several layers of triples that constitute the three-way junction[62,66]. The bound guanine (G) and adenine (A) are surrounded by uridines along their periphery[66] (shown in panels **Ba** and **Bb**, respectively). Specific recognition of the ligands is achieved by Watson–Crick pairing with a discriminatory nucleotide at position 74 (either cytosine or uridine in guanine and adenine riboswitches, respectively[45,55,62,66,115]).

Similarly to purine riboswitches, the guanosine-binding pocket of group I introns[68,71,72] is built by several stacked triples, and the 3′ splice site (ωG) is bound by extensive stacking and the hydrogen bond interactions of its nucleobase. However, ωG is not fully enveloped and is likely to be inserted or removed without a pronounced conformational change in the pocket. ωG interacts with a guanosine residue that is engaged in a G130–C177 base pair, forming a nucleotide triple (shown in panel **C**). Specific recognition of guanosine also has an essential role in the selection of the 5′ splice site by the ribozyme[68]. In this case, a conserved wobble pair is formed between the terminal nucleotide of the 5′ exon, U-1 and a G within the intron's internal guide sequence (IGS) (FIG. 1j). Such pairing exposes the exocyclic amine and 2′-OHs of the G for readout by a wobble receptor element, containing two non-canonical A•A pairs, in J5–4.

In the thiamine pyrophosphate (TPP) riboswitch[63,65,67] (shown in panel **D**), the ligand is bound in an extended conformation by two helical segments that lie outside of the junctional region[65]. The pyrimidine-like ring pairs with G40 (coloured yellow) and is stacked between G42 and A43, which constitute part of a T-loop motif (coloured orange). Two Mg^{2+} ions (the coordinated water molecules are shown in magenta) are bound to the pyrophosphate moiety, which interacts with the RNA through direct contact of phosphate oxygens with the base edges of C77 and G78. In addition, $Mg1^{2+}$ makes direct contacts with G60 and G78, and also makes water-mediated contacts with several other nucleotides.

The ligand also bridges two helical segments in the SAM-I riboswitch (shown in panel **E**)[64]. However, unlike TPP, the bound S-adenosylmethionine (SAM) adopts a compact conformation. The purine moiety of SAM specifically binds U57 and A45 (coloured yellow), and is sandwiched between a methionine moiety and C47. Mainchain atoms of methionine interact with the (G58–C44)•G11 base triple. SAM forms van der Waals interactions with U7 and U88, positioning P1 close to P3. These multiple interactions zip up helices P1 and P3, and stabilize the P1 helix and the overall four-way junctional fold, with the former constituting the integral part of the gene expression switch.

The preformed open glucosamine-6-phosphate (GlcN6P)-binding pocket of the *glmS* ribozyme (shown in panel **F**)[69,75] consists of nucleotides from the P2 loop (FIG. 1g) that form a double pseudoknot conformation, which is observed in the structures of the hepatitis δ virus (HDV)[70] and Diels–Alder ribozymes[116]. Like the HDV ribozyme, the double pseudoknot in the *glmS* ribozyme forms an active-site cleft, with the scissile phosphate located at the bottom. GlcN6P is locked in place by stacking with the G+1 nucleotide and multiple direct and magnesium-mediated interactions involving ring and phosphate moieties. The reaction mechanism might include deprotonation of the 2′-OH of A-1 by guanine (not shown), and protonation of the 5′-O leaving group by GlcN6P, consistent with the observation that GlcN6P is essential for catalysis. Note that Mg^{2+} does not directly participate in catalysis.



Interesting parallels are observed when riboswitch structures are compared with hairpin ribozyme and group I intron structures. Formation of the catalytic pocket in the hairpin ribozyme[77] via docking of two helical domains might be considered to be reminiscent of TPP and SAM-I riboswitches (FIG. 1b). In group I introns, the guanosine-binding pocket, formed by nucleotides at the junction of J6–J7 and P7 and occupied by ωG (the 3′ splice site)[68,71,72], is surrounded by several stacked base triples (FIG. 1j), reminiscent of the purine-binding site in purine riboswitches. Unlike other ribozymes and riboswitches, the *glmS* ribozyme contains pre-formed catalytic and ligand-binding sites and, in contrast to riboswitches, binds its ligand GlcN6P in an open, solvent-accessible pocket[69,75] (BOX 1; FIG. 1g).

Regulation of gene expression

Given that RNase P has a universal catalytic function in both prokaryotes and eukaryotes, there has been a long-standing appreciation of the important role that ribozymes have in cells. The discovery of riboswitches and novel ribozymes, however, points to a different

trend for protein-devoid RNAs; namely, a direct involvement in a range of gene-control mechanisms in both prokaryotes and eukaryotes.

The role of ribozymes in gene expression

Self-splicing introns continually modulate the genomic organization of various organisms by mediating the mobility of genetic material within the genome and by spreading between species. How important are these ribozymes for the regulation of gene expression? Self-splicing introns can interrupt genes, but the ability of introns to self-excise from mRNA potentially renders them neutral to the host[91]. Nevertheless, in the *roaA* gene of the *Euglena gracilis* chloroplast, self-splicing introns cause alternative splicing of pre-mRNAs to produce two distinct transcripts[92], highlighting one way in which these RNAs can affect gene expression. Splicing might also be an important mechanism for posttranscriptional regulation. For example, the addition of a 3′ CC A sequence, which is required for amino-acylation, to a plastid tRNA is less efficient when intron removal is blocked[93]. Importantly, in addition to homing, which occurs when introns spread into similar genes, self-splicing introns can also invade ectopic or non-homologous sites[22], and these new integration sites might not necessarily be neutral for gene expression.

Other self-cleaving ribozymes, such as the *CPEB3* and CoTC ribozymes, which seem to be involved in splicing[13] and transcription[14], respectively, might have a direct impact on gene expression. These ribozymes have been identified in only some mammalian species, and further study is needed to determine whether similar RNA elements exist, providing a more generally applicable mechanism for their function. By contrast, small self-cleaving ribozymes (hammerhead, hairpin, VS and HDV ribozymes) have specialized functions in replication, and are therefore unlikely to influence expression of the host genes specifically and directly.

A few more catalytically active self-cleaving sequences have been found in humans[13] and plants[94]. Homology searches in various genomes have also identified several hammerhead-like motifs, the activities of which have not been demonstrated[95,96]. Potentially, cleavages produced by the catalytically active elements might provide specific entry sites for distinct exonucleases, with direct implications for transcriptional termination, intron degradation and mRNA turnover. Future research should reveal whether these ribozymes are an integral part of gene expression mechanisms or are involved in specific mechanisms of gene expression regulation. So far, a regulatory function has been clearly shown for only the *glmS* ribozyme, which cleaves off the 5′ mRNA region and, probably through mRNA degradation, downregulates expression of the amidotransferase gene. Unexpectedly, the pre-rRNA-processing enzyme RNase MRP also participates in mRNA degradation by targeting specific mRNAs that are involved in cell-cycle regulation[97], suggesting that RNase MRP might be involved in gene expression control.

The complexity of riboswitch-dependent regulation in bacteria

The number of genes controlled by metabolite-sensing riboswitches (~2% in some bacteria)[55] is comparable with the number of genes regulated by metabolite-sensing proteins. Indeed, a bacterial genome can contain riboswitches that are specific to different

metabolites, as well as multiple riboswitches of the same type, each regulating a different operon. Moreover, two sensing domains or even two complete switches can lie adjacent to each other, resulting in a more complex mechanism of gene expression regulation[53,98]. For example, tandem glycine-specific aptamers bind glycine cooperatively and accomplish regulation through a single expression platform, thus providing a greater dynamic response to small changes in glycine concentration[53]. Another adjoining arrangement constitutes two complete riboswitches that independently sense different metabolites, such as SAM and adenosylcobalamin (AdoCbl), and function as a two-input Boolean NOR logic gate, wherein binding of either ligand causes repression[98]. Tandem riboswitches can also be composed of two complete riboswitches of the same type. Such composite arrangements, exhibited by some TPP riboswitches[98,99], candidate riboswitches[98] and T-box RNAs[98,100], probably enable a greater dynamic range for gene control and greater responsiveness to changes in metabolite concentration[98].

Remarkably, depending on the expression platform, riboswitches of the same type are capable of either repression or activation of gene expression, greatly increasing their regulatory potential. A similar trend is seen in regulation by bacterial sRNAs, although this involves an increased level of sophistication. The DsrA sRNA can pair with two different mRNAs, *hns* and *rpoS*, down- and upregulating their translation, respectively[33,34,101] (FIG. 3b,c). To add further complexity, *rpoS* mRNA can, in turn, be repressed by another sRNA, OxyS, possibly by titration of Hfq[37].

Riboswitches as mediators of eukaryotic gene expression

To date, virtually all riboswitch-like riboregulators have been found in bacterial species. Because of differences in transcription, translation and mRNA structure, eukaryotes rely on post-transcriptional gene-control mechanisms that differ to some extent from their prokaryotic counterparts. Nevertheless, functional TPP riboswitches have been identified in the 5′-UTR introns of fungal genes[44] and the 3′-UTRs of plant genes involved in thiamine biosynthesis[102], suggesting a regulatory role in splicing and mRNA processing, respectively. Indeed, a recent study has shown that fungal TPP riboswitches can either downregulate or upregulate gene expression using an elegant alternative splicing mechanism[43] (FIG. 3f). Computer searches have also identified TPP riboswitch-like motifs in two other eukaryotic species and in archaea, and SAM-II, preQ$_1$ and AdoCbl riboswitch-like sequences have been found in eukaryotes[103], although some of them such as preQ$_1$ and AdoCbl cannot be functional riboswitches. Furthermore, a novel candidate riboswitch that senses arginine has been recently suggested to control the arginase gene in fungi[104]. These examples highlight the plasticity of riboswitch-mediated genetic control and point out the need for experimental studies that might reveal the distinct features of eukaryotic versus prokaryotic regulatory mechanisms.

RNAs versus proteins

As they are composed of many more variable building blocks, proteins are generally considered to be more versatile than RNAs, so why did nature also implement RNA-based catalysts and regulatory elements? In fact, the versatility of RNA might have been underestimated. Studies of ribozymes and RNA sensors have shown that, despite having a

much simpler composition, RNA molecules have a number of features that are typical of protein molecules. Like their protein counterparts, ribozymes and especially riboswitches interact with small-molecule cofactors, and some of these regulatory and catalytic RNAs show allosterically controlled activity. Similarly to proteins, RNAs demonstrate high affinity and specificity towards their ligands and can recognize a wide range of molecules, from simple glycine to bulky AdoCbl. Also in common with proteins, some RNA regulators, such as tandem riboswitches, have a modular organization that seems to be crucial for cooperative and other types of complex control.

In addition to their similarities with proteins, RNAs might have functional advantages over proteins in some situations. Direct sensing of metabolites and other molecules by mRNA provides a significant advantage for cells, which can save energy and resources by eliminating the need for special regulatory proteins. Another important feature of RNA regulators that are positioned *in cis* is a synchronized response to changing surroundings. In contrast to the long-lived *trans*-acting protein factors, regulatory elements that are embedded within an mRNA exclusively control that mRNA, providing precise regulation of gene expression.

Both RNA and protein enzymes use binding interactions and energy to facilitate catalysis[105]. Remarkably, despite a limited arsenal of functional groups and a simple composition, some ribozymes turn out to be efficient catalysts. The cleavage-rate constants for the HDV[106] and hammerhead ribozymes (~1 sec^{-1} and 15 sec^{-1}, respectively)[107] approach the corresponding values for the protein enzyme ribonuclease A (160 sec^{-1} and 69 sec^{-1} for UpG and CpC substrates, respectively)[108]. Nevertheless, many ribozymes have much slower rate constants (~1 min^{-1})[109], with these values being sufficient for typical ribozyme reactions involving single cleavage and ligation *in cis*. The cleavage and ligation reactions can be carried out by specific host endoribonucleases and ligases. However, virus-like RNAs, the sources of many ribozymes, could improve their chances for survival by encoding cleavage and ligation activity within their sequences, thereby eliminating a dependence on specialized host enzymes.

Finally, cells undoubtedly benefit from RNA-dependent gene expression control under stress conditions. Relatively small sRNAs can efficiently and precisely regulate protein synthesis by base pairing with target mRNAs, thereby preventing a waste of resources for protein production.

Despite the benefits described above, some features of RNA-based regulatory systems can be limiting. For instance, because RNA typically degrades more quickly than proteins *in vivo*, RNA regulators that work *in trans*, such as sRNAs, are probably not well suited to functioning under conditions in which control is required over an extended period of time. Current research suggests that many RNA catalysts and regulators might be considered as relics of a pre-protein era, either perfectly adapted to particular functions or still subject to evolutionary pressure for replacement by proteins. Indeed, functions that are carried out by certain ribozymes and riboswitches have been taken over by protein-based systems in other species[110].

Descendants of the RNA world?

If RNAs constituted the predominant species in the early biological world as suggested by the RNA world hypothesis, are modern RNA modules direct descendants of those primordial molecules? Given the key participation of RNA in fundamental cellular processes such as protein synthesis, it is tempting to consider a positive answer, especially as it is assumed that proteins, at first, largely assisted functional RNAs. Therefore, the existing ribonucleoprotein enzymes, including the ribosome, RNase P and the spliceosome, could be considered as remnants of the RNA world, although their origins cannot be unambiguously traced owing to the many missing links in their evolutionary history. A similar problem is encountered in the evolutionary analysis of modern small ribozymes. The complexity and narrow distribution of VS, HDV and hairpin ribozymes argues against their descent from the RNA world. However, the distribution of the hammerhead ribozyme in highly divergent organisms, suggesting an ancient origin, might be misleading: this ribozyme might instead have arisen independently multiple times[111]. The evolutionary relationships between similar ribozymes in different species might be even more intricate than initially thought, given the similarity between the HDV and *CPEB3* ribozymes, which, along with the limited distribution of the HDV ribozyme, suggests a human origin for this viral ribozyme[13].

Knowledge of the origin and evolution of self-splicing introns, although uncertain, is important for understanding the origins of spliceosomal introns[112]. Indeed, terminal structures of group II self-splicing introns, which contain the parts that carry out the splicing reaction, show a remarkable similarity to the structures of the spliceosomal small nuclear RNAs (snRNAs)[22]. This prompted speculation that self-splicing introns are ancestors of spliceosomal introns. Growing evidence suggests that group II introns, which are present in many bacteria, are among the most ancient genetic entities, and probably moved into the evolving eukaryotic genome from the α-proteobacterial progenitor of mitochondria, later giving rise to the spliceosomal introns and splicing machinery[112].

Several aspects of riboswitches are suggestive of their RNA world origin. Riboswitch scaffolds have probably stayed unaltered over long periods of time, because riboswitches recognize coenzymes and nucleobases that have remained unchanged for billions of years[113]. These nucleotide-like molecules could initially have been tethered to RNAs and used as prosthetic groups for catalytic reactions. With the emergence of proteins and their take-over of catalytic functions, the ancient cofactor-dependent ribozymes could have lost their catalytic ability and instead evolved to create RNA switches[42]. In addition, the presence of some riboswitches in diverse organisms supports their ancient origin, although even this criterion is not absolute because of possible lateral gene transfer.

Perspectives and future challenges

Modern genomic and biochemical techniques provide unique opportunities to identify novel roles for RNAs that function independently of proteins, as shown by the recent discoveries of riboswitches and several ribozymes that are encoded by cellular genomes. Some of these studies, such as the discovery of riboswitches[4–6], have taken advantage of well-established biological observations, suggesting the existence of metabolite-dependent gene expression

control. Other examples, such as the metabolite-dependent *glmS* and *CPEB3* ribozymes, were discovered serendipitously during the characterization of a potential riboswitch[7] or by careful labour-intensive experimentation involving *in vitro* selection to identify self-cleaving RNAs[13]. Many of these exciting findings, such as the identification of *glmS*[7], CoTC[13] and *CPEB3*[14] RNAs, have highlighted our limited understanding of key biological processes including the termination of mRNA transcription, and mRNA processing and degradation.

A priority for future studies is to characterize the self-cleavage activities of recently identified genome-encoded ribozymes[13,94], as well as candidate ribozyme and RNA sensor sequences[46,58,95,96,103], that might reveal new aspects of genetic regulation by RNA elements. As a pivotal example, we cite the recent and long-awaited dissection of the functional role of eukaryotic riboswitches, which participate in gene expression control by alternative splicing of their pre-mRNAs[43]. This outstanding work will encourage experimentation on archaeal and other eukaryotic riboswitches, especially those that are found in the 3′ region of mRNAs and are potentially involved in novel types of regulation. These biochemical and genetic studies should be supplemented by searches for novel RNA sensors and ribozymes using new computer-based approaches that can identify candidate RNA sequences with low sequence homology[114].

Despite considerable progress in studies of ribozymes and riboswitches at the atomic level, future research must resolve many uncertainties in functional mechanisms, especially for some classical (VS ribozyme, group II introns and RNase P) and recently identified (CoTC and *CPEB3*) ribozymes, for which structures are either not yet available or are restricted to a subset of functional states. This need also applies to many riboswitches for which three-dimensional structures await determination. Comprehensive research in this area would enhance our mechanistic understanding of the biological function of these molecules and more complex RNA-containing cellular machineries such as the spliceosome, the ribosome and telomerase.

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Glossary

Satellite RNAs	Subviral agents whose multiplication in a host cell depends on coinfection with a helper virus
Rolling-circle replication	A process of replication of some circular genomes, whereby one strand is replicated first and the second strand is replicated after completion of the first one
RNA maturase	A protein enzyme, intronspecific, that acts as a cofactor to facilitate splicing
Homing endonucleases	dsDNA-specific deoxyribonucleases that recognize large asymmetrical DNA sequences, which are not stringently defined, and which mobilize these DNA elements by facilitating their

	integration into new genomic sites. Because these sites lack introns and inteins (protein introns), this form of mobility is termed ‘homing’. Homing endonucleases are encoded by intervening sequences embedded in either introns or inteins
Reverse transcriptase	An RNA-dependent DNA polymerase that transcribes ssRNA into dsDNA
Transcriptional attenuation	A regulatory mechanism whereby gene expression is controlled through the formation of alternative structures in the mRNA sequence that inhibit or facilitate the progression of transcription
Aptamer	Derived from the Latin aptus, meaning ‘to fit’, aptamers are oligonucleotide or peptide molecules that bind specific target molecules. The term is usually applied to nucleic acid molecules that are created following selection from a large random sequence pool, as well as to natural metabolitesensing domains in riboswitches, which possess similar recognition properties to artificially generated aptamers
Boolean NOR logic gate	Boolean logic, named after George Boole, is a complete system for logical operations. A logic gate performs a logical operation on one or more logical inputs and produces a single logical output. The logical NOR, or joint denial, is a logical operator, meaning that the output is true if none of the inputs are true. Consequently, if one or both inputs are true, then the output is not true
Prosthetic group	A non-protein component of a conjugated protein that is usually essential for the protein’s function. Prosthetic groups are also called coenzymes and cofactors
Lateral transfer	A process of transferring genetic material from one organism to another without reproduction. Also called horizontal gene transfer
Telomerase	An RNA-containing reverse transcriptase that adds DNA sequence repeats (telomeric repeats) to the 3′ end of DNA strands in eukaryotic chromosomes using its RNA component as a synthesis template

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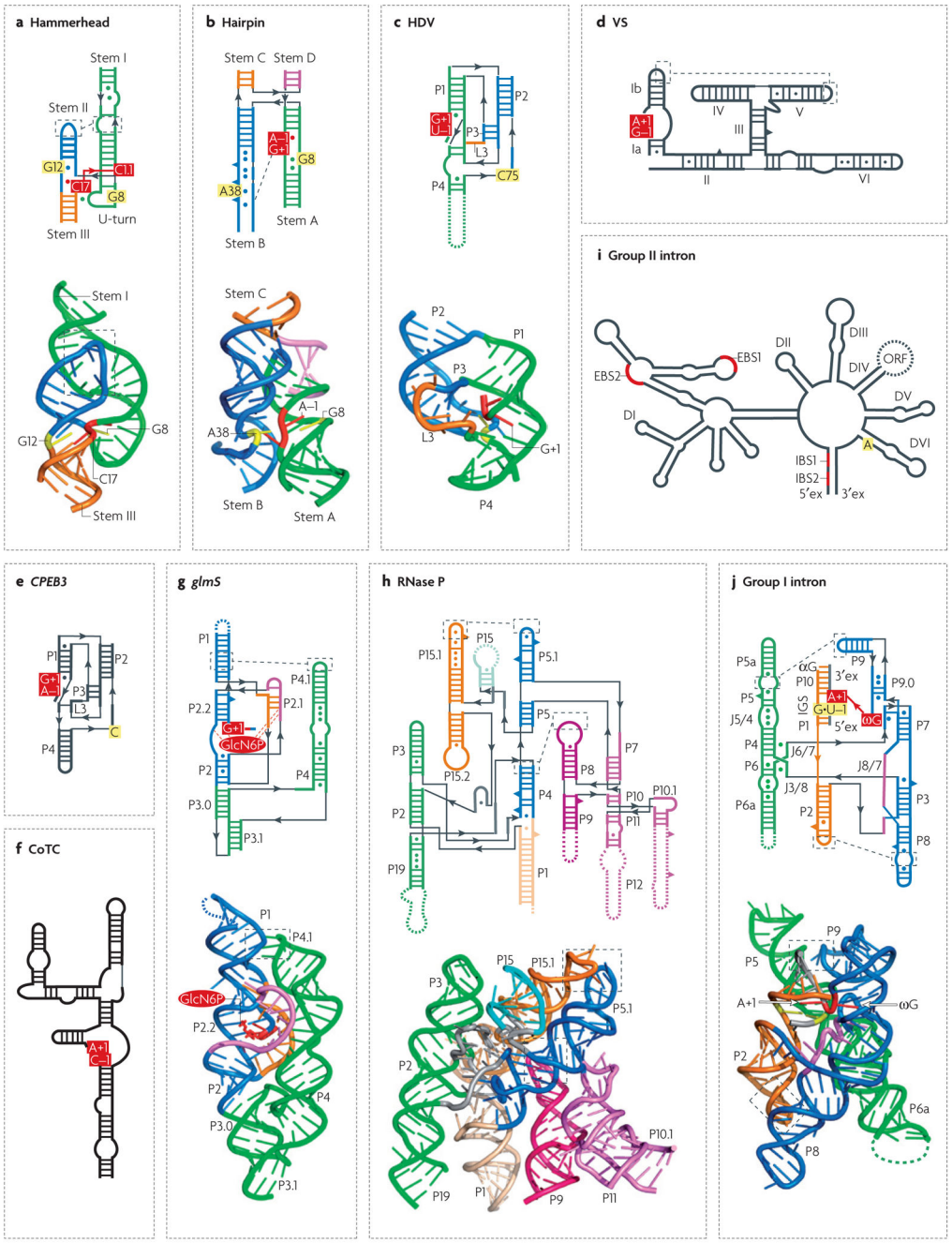

Figure 1. Domain organization and secondary and three-dimensional structures of ribozymes

Secondary structures are depicted in thick lines and are connected by thin black lines with arrows. Watson–Crick and non-canonical base pairs are shown as solid lines and circles, respectively. Bulged-out nucleotides are represented as triangles. Ribozymes with known three-dimensional structures are coloured according to secondary structure elements and domains. Three-dimensional structures, if available, are shown in a ribbon-and-stick representation below the secondary structures. The two nucleotides that lie adjacent to the scissile phosphate are indicated by red boxes. Nucleotides that are implicated in catalysis

(panels **a**–**f** and **i**) or that are essential for molecular recognition (panel **j**) are indicated by yellow boxes. Black dashed squares and lines highlight important tertiary interactions. Coloured dashed lines indicate elements that are missing in the structure or that are substituted by non-natural sequences. **a** | Hammerhead ribozyme[76]. **b** | Hairpin ribozyme[78]. **c** | Hepatitis δ virus ribozyme[74]. **d** | Varkud satellite (VS) ribozyme[12]. **e** | *CPEB3* ribozyme[13]. **f** | Human CoTC ribozyme[14]. **g** | *Bacillus antracis glmS* ribozyme; glucosamine-6-phosphate (GlcN6P) is represented as a red oval, with its interactions with RNA indicated by dashed lines[69]. **h** | *Bacillus subtilis* RNase P, B-type[73]. **i** | Domain organization of the group II intron, with IBS and EBS designating intron and exon binding sequences, respectively[21,22]. The yellow-coloured A designates a conserved unpaired adenosine that participates in splicing. **j** | *Asoarcus* spp. *BH72* group I intron in the state that precedes the second step of splicing[79]. The internal guide sequence (IGS) aligns the 5′ and 3′ exons (ex), which are shown in grey. ωG and αG designate the 3′-terminal guanosine nucleotide of the intron and the external guanosine that is linked to the intron after the first step of splicing, respectively. Secondary structures in panels **a**, **c**, **d**, **e**, **f**, **g**, **h** and **j** are modified with permission from REF. 76 © (2006) Cell Press, REF. 117 © (2007) Cell Press, REF. 118 © (1995) National Academy of Sciences (USA), REF. 13 © (2006) American Association for the Advancement of Science, *Nature* REF. 14 © (2004) Macmillan Publishers Ltd, REF. 75 © (2006) American Association for the Advancement of Science and REF. 69 © (2007) Current Biology Ltd, REF. 119 © (2006) Elsevier Sciences and REF. 120 © (2005) Elsevier Sciences, respectively.

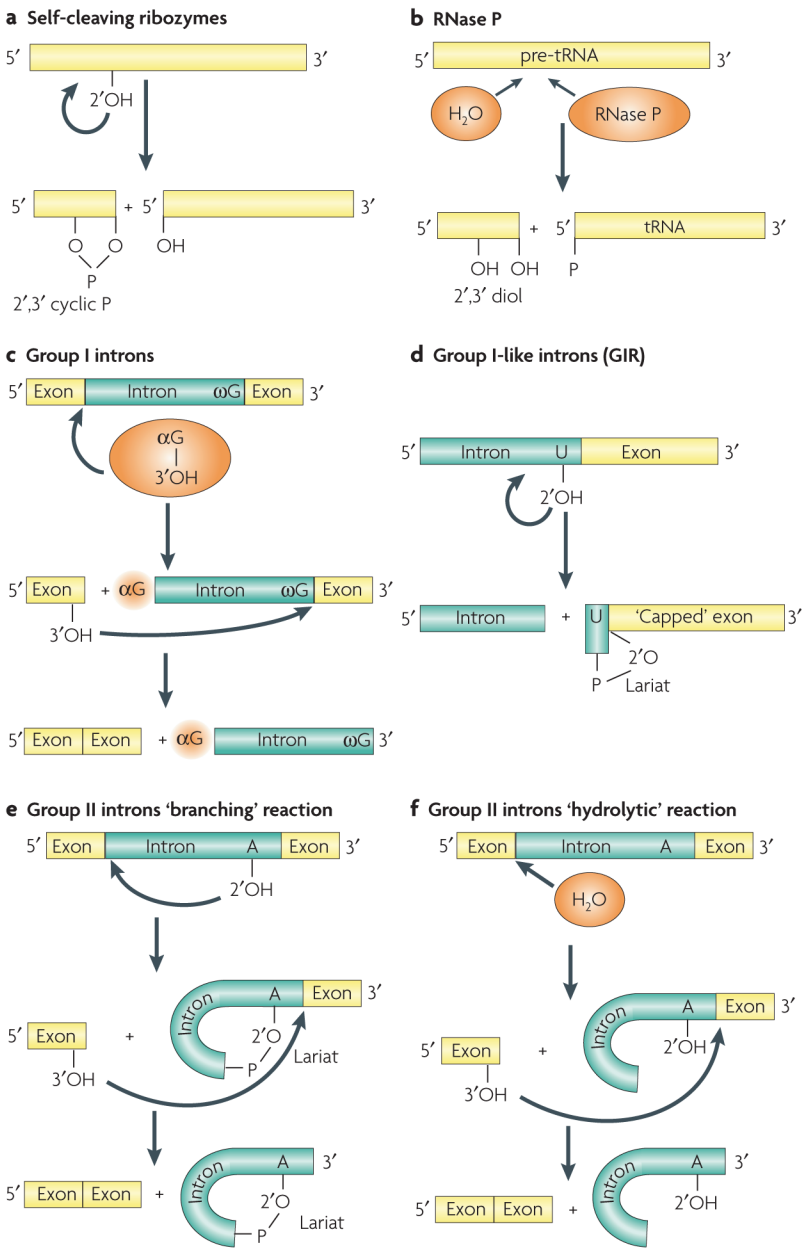

Figure 2. Reactions catalysed by ribozymes

a | The typical reaction of self-cleaving ribozymes, initiated by 2′-hydroxyl (OH) attack, and yielding 2′,3′-cyclic phosphate (P) and 5′-OH termini. **b** | The catalytic cleavage of pre-tRNA by RNase P (REF. 2). A water molecule serves as a nucleophile, and the reaction yields 2′,3′-diol and 5′-P termini. **c** | Self-splicing by group I introns[1]. The reaction is initiated by nucleophilic attack by the 3′-OH of external guanosine (αG) at the 5′ splice site. This results in covalent linkage of αG to the 5′ end of the intron and release of the 3′-OH of the 5′ exon. In the second step, the 3′-OH attacks the 3′ splice site located immediately after the conserved guanosine (ωG), resulting in excision of the intron with αG at the 5′ end and release of the ligated exons. **d** | The 'capping' reaction of the *Didium iridis* GIR1 ribozyme

is similar to the first step of the 'branching' reaction of group II introns[25]. The reaction joins nucleotides by a 2′,5′-phosphodiester linkage, thereby forming a 3-nucleotide 'lariat' that might be a protective 5′ cap of the mRNA. **e** | The self-splicing of group II introns by a branching reaction[22]. In the first step, the 5′ splice site is attacked by the 2′-OH of a conserved unpaired adenosine located in domain VI, resulting in formation of a 2′,5′-phosphodiester linkage. In the next step, the free 3′-OH group of the 5′ exon attacks the 3′ splice site, liberating the circular intron lariat and ligated exons. **f** | Alternative 'hydrolytic' self-splicing of group II introns[22]. The reaction involves a water molecule as a nucleophile and produces a linear intron.

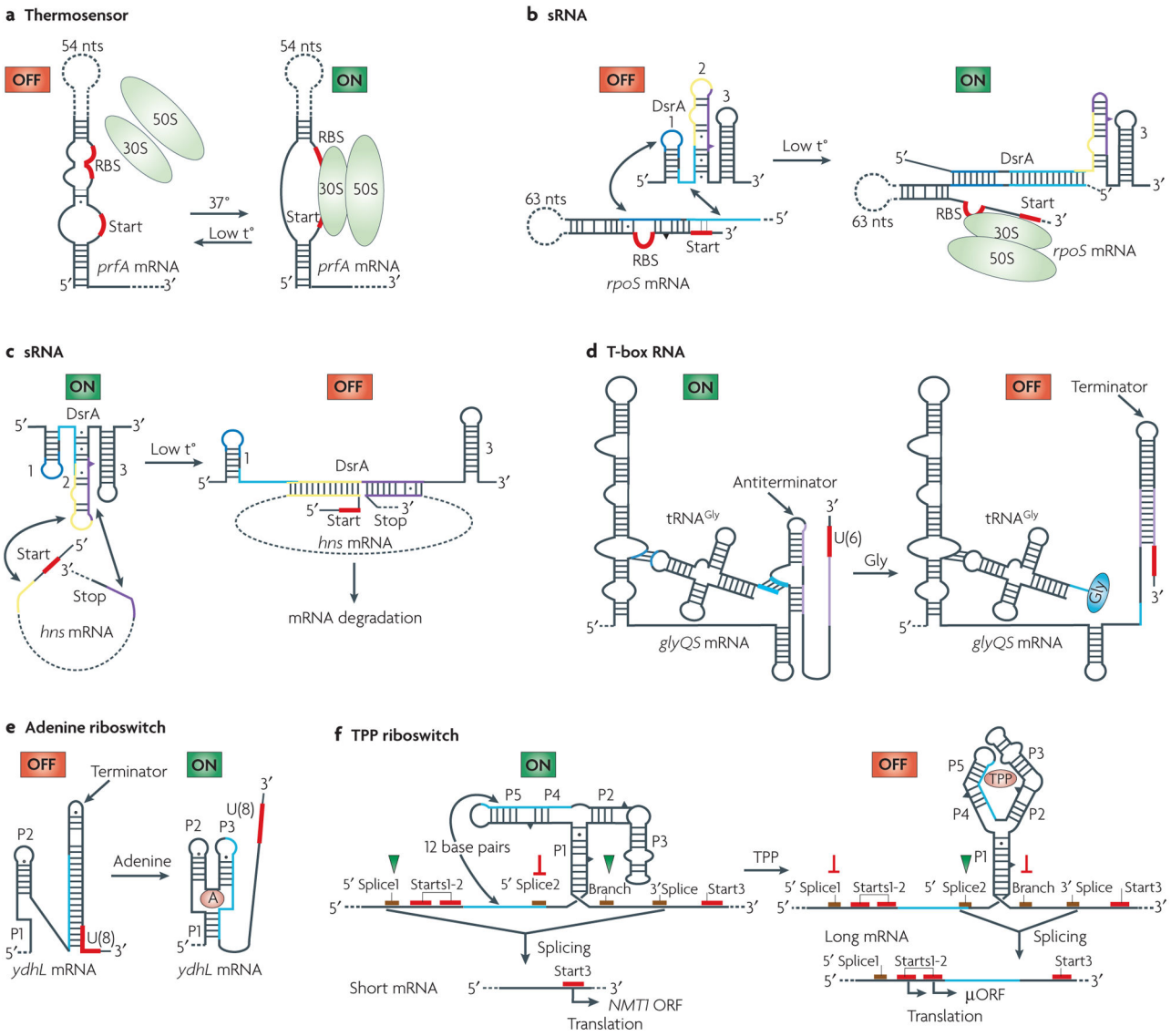

Figure 3. Gene regulation by RNA switches

RNA regions that are involved in gene expression switching are shown in the same colour. **a** | Translation activation of virulence genes in the pathogen *Listeria monocytogenes*[28]. An increase in temperature melts the secondary structure around the ribosome binding site (RBS) and start codon, allowing ribosome binding and translation initiation. **b** | Upregulation of *Escherichia coli* σ^S-factor gene by the DsrA antisense short RNA (sRNA). DsrA RNA[121] pairs with the translational operator of the *rpoS* gene[122] using two sequences (coloured blue and light blue) located within helices 1 and 2 (REFs 33,34,121). This base pairing exposes translation initiation signals for ribosome binding and increases mRNA stability[101]. **c** | Downregulation of transcription regulator HNS by DsrA sRNA. DsrA RNA, transcribed in response to low temperature, pairs with 5′ and 3′ regions of *hns* mRNA and causes faster turnover of the mRNA[101], possibly by RNase E degradation[123]. **d** | Transcription termination of the *Bacillus subtilis* glycyl-tRNA synthetase gene by aminoacylated $tRNA^{Gly}$ (REF. 124). Non-aminoacylated $tRNA^{Gly}$ interacts with the T-box

region of mRNA using an anticodon and an acceptor helix[125], and promotes the formation of the anti-terminator stem–loop structure. The aminoacylated tRNA cannot contact mRNA using the acceptor stem, thus allowing the formation of the transcription terminator. **e** | Transcription activation of the purine efflux pump by the adenine riboswitch[45]. In the absence of adenine, transcription of *B. subtilis ydhL* mRNA is aborted as a result of formation of a transcription terminator. Adenine binding stabilizes the metabolite-sensing domain and prevents the formation of the terminator. **f** | Thiamine pyrophosphate (TPP)-riboswitch-mediated alternative splicing of mRNA in *Neurospora crassa*[43]. In the absence of TPP, the mRNA adopts a structure that occludes the 5′ splice site by base pairing with the P4–P5 region of the riboswitch. Pre-mRNA splicing from 5′ splice site 1 leads to production of a short mRNA and expression of the *NMT1* gene. TPP binding causes a structural change in the RNA, opening the 5′ splice site 2 and occluding the branch site. Therefore, splicing is inhibited (not shown) and, were it to proceed, would result in the formation of a long mRNA. The alternatively spliced and non-spliced mRNAs both carry a short ORF (μORF) that begins from initiation codons 1–2 and competes with translation of the main ORF, thereby repressing *NMT1* expression. Key splicing determinants are activated (indicated by green arrows) and inhibited (indicated by red lines) during different occupancy states of the TPP-sensing domain. Panels **a**, **b** and **c**, **d** and **f** are modified with permission from REF. 28 © (2002) Cell Press, REF. 126 © (2000) National Academy of Sciences (USA), REF. 124 © (2005) Academic Press and *Nature* REF. 43 © (2007) Macmillan Publishers Ltd.

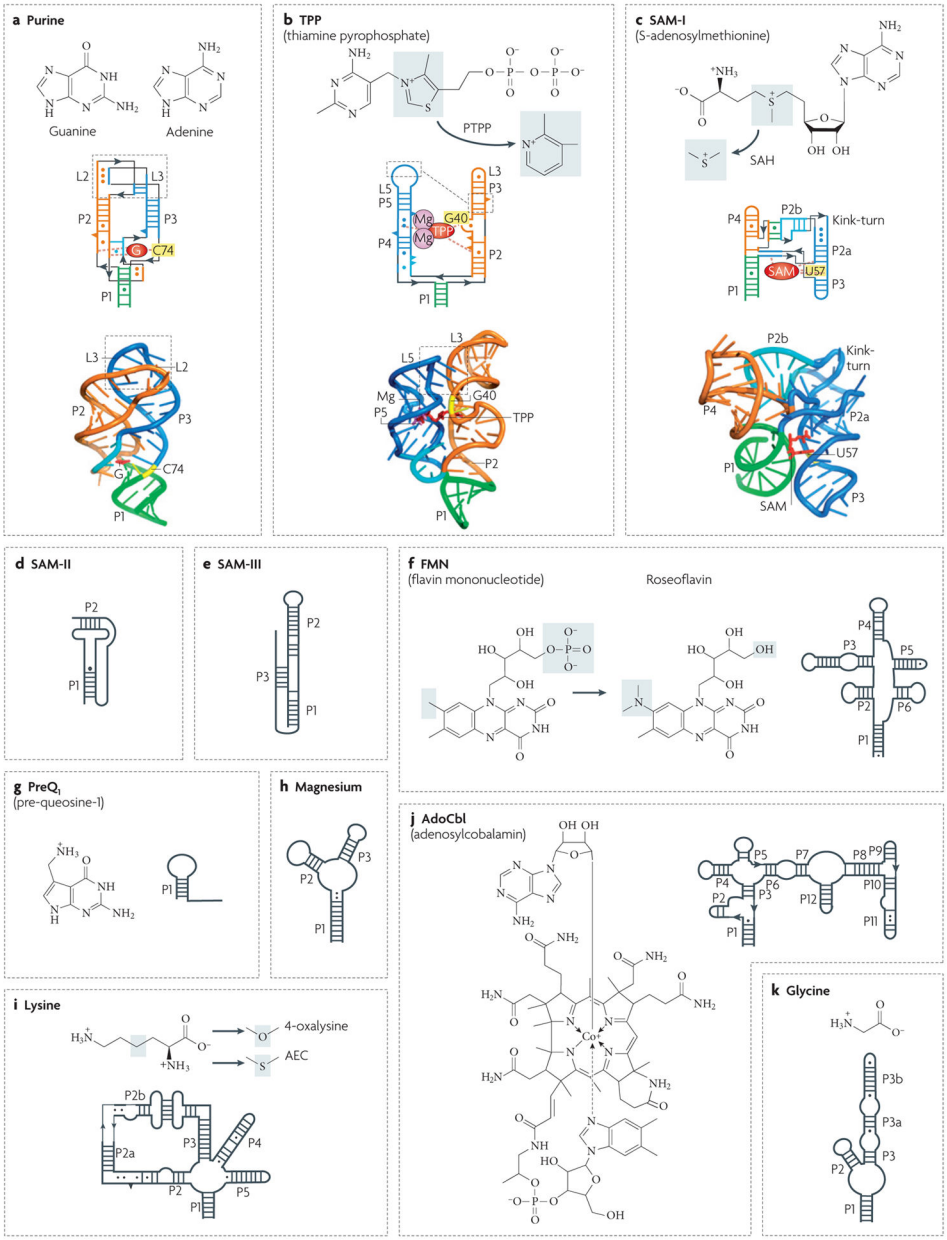

Figure 4. Secondary and tertiary structures of riboswitches

Secondary structures are depicted by thick lines and are connected by black lines with arrows. Watson–Crick and non-canonical base pairs are shown as solid lines and circles, respectively. Natural riboswitch ligands and riboswitch-binding antibiotics are shown next to the secondary structures. Grey shadings indicate areas in which the ligands undergo changes. **a** | The *Bacillus subtilis xpt* gene guanine riboswitch bound to guanine (represented as a red G)[66]. The discriminatory nucleotide C74 is coloured yellow. **b** | The TPP riboswitch from the *Escherichia coli thiM* gene bound with TPP (in red)[65]. A pair of hydrated Mg^{2+} cations is shown in magenta. G40 interacting with the pyrimidine moiety of TPP is shown in yellow. The antibiotic pyrithiamine pyrophosphate (PTPP) differs from TPP by the central ring. **c** | The class I SAM *Thermoanaerobacter tengcongensis* riboswitch in complex with

SAM (in red)[64]. U57 interacting with the purine moiety of SAM is shown in yellow. The grey area highlights a methyl group that is missing in S-adenosylhomocysteine (SAH). **d** | A class II SAM riboswitch from the *Agrobacterium tumefaciens metA* gene[46]. **e** | An S_{MK} (class III SAM) riboswitch from the *Streptococcus gordonii metK* gene[48]. Helix P3 is formed by Shine–Dalgarno and anti-Shine–Dalgarno sequences. **f** | The FMN riboswitch from the *B. subtilis ribD* gene[50]. **g** | The $preQ_1$ riboswitch from the *B. subtilis queC* gene[56]. **h** | The magnesium riboswitch from the *Salmonella enterica mgtA* gene[57]. **i** | The lysine riboswitch from the *B. subtilis lysC* gene[54]. **j** | The AdoCbl riboswitch from the *E. coli btuB* gene[5]. **k** | The glycine type II riboswitch from *Vibrio cholerae gcvT* gene[53]. Secondary structures in panels **c**, **d**, **e**, **f**, **g**, **h**, **i**, **j** and **k** modified with permission from *Nature* REF. 64 © (2006) Macmillan Publishers Ltd, REF. 46 © (2005) BioMed Central Ltd, *Nature Structural & Molecular Biology* REF. 48 © (2006) Macmillan Publishers Ltd, REF. 50 © (2002) National Academy of Sciences (USA), *Nature Structural & Molecular Biology* REF. 56 © (2007) Macmillan Publishers Ltd, REF. 57 © (2006) Cell Press, *Nature Biotechnology* REF. 61 © (2006) Macmillan Publishers Ltd, REF. 5 © (2002) Current Biology Ltd and REF. 53 © (2004) American Association for the Advancement of Science, respectively.

Table 1

Major classes and distribution of ribozymes and RNA switches

Class	Group	Members	Main functions	Natural ligand	Size (nucleotides)	Distribution
Cleaving ribozymes						
Self-cleaving	Satellite RNAs	Hammerhead	Gene control (?); processing of multimeric transcripts during rolling-circle replication		65	Viroids, plant viral satellite RNA, eukaryotes (plants, crickets, amphibians, schistosomes)
		Hairpin			75	Plant viral satellite RNA
		VS			155	Satellite RNA of *Neurospora* spp. mitochondria
		HDV			85	Human satellite virus
	mRNAs	CoTC	Transcription termination (?)	GTP	190	Primates
		CPEB3	Splicing regulation (?)		70	Mammals
		glmS	Gene control	GlcN6P	170	Gram+ bacteria
Trans-cleaving		RNase P	tRNA processing		140–500	Prokaryotes, eukaryotes
Splicing ribozymes						
Group I			Self-splicing	Guanosine	200–1500	Organelles (fungi, plants, protists), bacteria, bacteriophages, mitochondria (animals)
Group II			Self-splicing		300–3000	Organelles (fungi, plants, protists), bacteria, archaea
RNA switches						
Thermosensors			Gene control		Variable	Phages, bacteria, eukaryotes
sRNAs			Gene control	Hfq	>85	Bacteria
T-boxes			Gene control	tRNA	190	Mostly Gram+ bacteria
Metabolites	Coenzymes	TPP	Gene control	TPP	100	Bacteria, archaea, eukaryotes (fungi, plants)
		FMN	Gene control	FMN	120	Bacteria
		AdoCbl	Gene control	AdoCbl	200	Bacteria
		SAM-I	Gene control	SAM	105	Mostly Gram+ bacteria
		SAM-II	Gene control	SAM	60	α- and β-proteobacteria
		SAM-III (S_{MK})	Gene control	SAM	80	Gram– bacteria
	Amino acids	Lysine	Gene control	Lysine	175	γ-proteobacteria, *Thermotogales*, *Firmicutes*
		Glycine (I+II)	Gene control	Glycine	110	Bacteria
	Nucleobases	Guanine	Gene control	Guanine, hypoxanthine	70	Gram+ bacteria

Class	Group	Members	Main functions	Natural ligand	Size (nucleotides)	Distribution
		Adenine	Gene control	Adenine	70	Bacteria
		$preQ_1$	Gene control	$preQ_1$	35	Bacteria
Magnesium		*mgtA*	Gene control	Mg^{2+}	70	Gram– bacteria

AdoCbl, adenosylcobalamin; CoTC, co-transcriptional cleavage; FMN, flavin mononucleotide; GlcN6P, glucosamine-6-phosphate; Gram−, Gram-negative; Gram+, Gram-positive; HDV, hepatitus δ virus; Hfq, host factor Q, interacting with some sRNAs; preQ1, pre-quenosine-1; SAM, S-adenosylmethionine; sRNA, small RNA; TPP, thiamine pyrophosphate; VS,Varkud satellite. ? indicates an unconfirmed function.