Regulatory RNA

Introductory article

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Beyond their coding capacity, RNA molecules serve structural, catalytic and regulatory functions. The latter are elicited by RNA–RNA interactions (antisense-target), frequently resulting in complex three-dimensional structures, and by RNA–protein interactions. The numerous examples known to date probably present only a first glimpse at this expanding field.

Article Contents

Introduction

Ribonucleic acid (RNA) has long been regarded as a molecule whose principal function is the transfer of genetic information from genes to gene products (proteins), either as messenger or as part of the translational machinery (transfer RNA, ribosomal RNA). The importance of RNA has been underscored by the discovery of many additional biological functions in which RNAs play key roles. In particular, the finding that RNAs can act catalytically (ribozymes) – like enzymes – emphasizes the versatility of these molecules.

Today we know of a great number of RNAs that play important roles in splicing (small nuclear RNAs), ribosomal RNA modification (small nucleolar RNAs), editing (guide RNAs, gRNAs) and protein export (7S RNA in signal recognition particle). Some RNAs are subunits of enzymes (telomerase, RNAaseP), or segments of larger RNAs involved in RNA self-cleavage/ligation reactions (ribozymes). 5′ and 3′ segments of messenger RNAs (mRNAs) are frequently required for regulatory processes. For example, pattern formation in the early *Drosophila* embryo depends on localized *bcd* (Bicoid) mRNA. The 3′ untranslated region of *bcd* mRNA presents a complex structure that acts as a localization signal. The Staufen protein binds to this motif and promotes localization of *bcd* mRNA to the anterior pole of the embryo. In retroviruses, RNAs play important roles as primers for replication, modulators of viral gene expression or elements important for RNA trafficking (e.g. transfer RNAs, Tar, RRE). Although all these cases have regulatory aspects, we do not regard the primary role of these RNAs as regulation. Hence, these RNAs are described elsewhere. This chapter focuses on the diverse roles and properties of RNAs whose main function is in regulation of gene expression. This can result from direct interaction with a target RNA or may be mediated by effects on the activity of a protein. Most regulatory RNAs have been identified in bacteria, often encoded by their accessory genetic elements, and in viruses, but examples are found in all kingdoms of life.

Most regulatory RNAs are small and untranslated. In general, secondary and tertiary structural elements are of great importance for their function. One particular subclass of regulatory RNAs, the antisense RNAs, are easily distinguished by well-defined properties. Antisense RNAs are completely or partially complementary to target RNAs, and regulation (mostly inhibition) is dependent on base pairing. Other regulatory RNAs do not rely on base-pairing interactions, but accomplish regulation by sequestration of a regulatory protein or by modulating the activity of a protein.

Antisense Control in Plasmids

Natural antisense RNAs were first identified as regulators of plasmid copy number (ColE1, R1). Even though many antisense RNAs have been described in bacteria, archaea and eukaryotes, bacterial plasmids appear to be the genetic entities in which most known antisense RNA control systems reside.

Control of plasmid replication

Bacterial plasmids do not replicate in synchrony with the host chromosome. Their replication frequency (and, therefore, their copy number) is independently controlled by plasmid-encoded functions. This control is homeostatic: copy number fluctuations are 'counted' by the plasmid, and the replication frequency adjusted accordingly. Many unrelated groups of plasmids employ antisense RNAs as the regulatory key components (**Table 1**). These RNAs are small (60–150 nucleotides), untranslated and most often unstable inhibitors of replication. The common theme in the different mechanisms used is that the antisense RNA binds to a target RNA (a pre-primer RNA or an mRNA encoding a replication initiator protein) to inhibit replication. Since antisense RNAs (almost without exception) are constitutively transcribed and metabolically unstable, it follows that replication rate is inversely

Table 1 Regulatory RNAs, occurrence, mechanisms and properties

Biological functions	System/gene(s)	Mechanisms of action	Special features
Prokaryotic antisense RNAs			
Chromosomal			
Cell division	*dicF*/*ftsZ*	Inhibition of translation (?)	
Stress responses	*oxyS*/*fhlA*	Inhibition of translation	Second mechanism (not antisense)
Stress responses	*dsrA*/*rpoS*	Activation of *rpoS* translation	Requires Hfq; also inhibitor (below)
Stress responses	*dsrA*/*hns*	Inhibition of *hns* translation	Also activator (above)
Stress responses	*micF*/*ompF*	Partial antisense/target duplex blocks *ompF* translation	Protein cofactor likely
Virulence	*agr*	Activation of translation	
Unknown	*hok/sok* homologues	Unknown (likely inhibition of translation)	
Accessory genetic elements			
Replication control	Plasmids		
	Relatives of ColE1	Inhibition of primer maturation	Rom protein stabilizes antisense/target RNA kissing complex
	of R1, ColE2 etc.	Inhibition of translation	
	of pT181, pIP501 etc	Induced termination of transcription	Unusually stable antisense RNA (pIP501)
	of pMU720, Col1b-P9	Inhibition of activator pseudoknot	
Conjugation	F, R1 and relatives	Inhibition of *traJ* translation	FinP antisense RNA stabilized by FinO protein
Killer genes	*hok /sok* gene family (R1)	Inhibition of translation	Unusual target RNA stability, processing required for killer protein synthesis
Multimer resolution/plasmid stability	*cer* site of ColE1	Rcd RNA (encoded within *cer*) accumulates in plasmid multimer-containing cells, delays cell division (target unknown)	
Transposition	Transposons/IS elements		
	IS*10*	RNA-OUT blocks initiation of *tnp* mRNA translation	Additional levels of control
	IS*30*	RNA-C may block progress of ribosomes during translation of *tnp* mRNA	

continued

Table 1 – *continued*

Biological functions	System/gene(s)	Mechanisms of action	Special features
Lysis/lysogeny switches	Bacteriophages		
	P1, P7, P4	Antisense RNA inhibits *ant* gene expression: block of *icd* translation/premature mRNA termination	Cotranscribed with target RNA/generated by processing
	P22	Sar RNA inhibits *ant* expression	
	Phage λ	OOP RNA/*cII* mRNA duplex destabilized (RNAase III)	Second, less well-defined antisense system present in λ
Phage exclusion	P22	Sas RNA causes translational switch by blocking *sieB* translation without affecting *esc*	
Prokaryotic regulatory RNAs (others)			
Glycogen synthesis	*csrB*	Inhibition of CsrA by binding	
Stress responses	*oxyS/rpoS*	OxyS binding to Hfq decreases *rpoS* translation	Second mechanism (antisense)
Eukaryotic antisense RNAs			
Developmental control (vegetative/prespore)	*Dictyostelium*	mRNA destabilization	
Developmental control (larval stages)	*Caenorhabditis elegans*	*lin-4* RNA binds (multiple targets in) the 3′ UTR of *lin-14* and *lin-28* mRNA and downregulates translation	Likely protein involvement
Unclear	*bFGF* gene in vertebrates	Downregulation of bFGF by antisense RNA suggested. Possibly scrambling of coding information due to ADAR activity as second consequence	
Retrotransposition	*Drosophila* micropia element	Antisense RNA inhibits translation of reverse transcriptase/RNAaseH (?)	Mechanism uncertain
Viral regulatory RNAs			
Counteracting host defence	EBER RNAs	Inhibition of protein kinase R (PKR)	
Counteracting host defence	VA RNAs	Inhibition of PKR and ADAR	

Regulatory RNAs listed here are discussed in the text. This table is not intended to give a complete list of regulatory RNAs known. For some additional information, see Further Reading.

proportional to the concentration of the plasmid. The details of the inhibition pathways differ (**Figure 1**). The ColE1 relatives use an antisense RNA (RNAI) that, upon binding, induces a change in the folding of the nascent pre-primer RNA so that its conversion to an active primer is prevented. In plasmids with a genetic organization similar to that of R1, the antisense RNA (CopA) binds to an mRNA that encodes an initiator Rep protein to block its translation. Plasmids like ColIb-P9 or pMU720 use a similar inhibition pathway, but it is complicated by the involvement of a peculiar RNA structure within the mRNA (a so-called pseudoknot). Certain plasmids in Gram-positive bacteria are regulated by 'transcriptional attenuation' (e.g. pT181, pIP501). The *rep* mRNA encoding the Rep protein can follow either of two mutually exclusive folding pathways. Binding of antisense RNA forces the nascent target RNA into one of these, resulting in the formation of a Rho-independent transcription terminator. Thus, when antisense RNA binds, elongation of the mRNA is aborted before it reaches the region encoding Rep. Interestingly, the antisense RNA of pIP501 (RNAIII) is unusually stable, in striking contrast to the other copy number control RNAs. This is surprising, since proper copy number control was expected to require an unstable inhibitor. However, it appears that in addition to RNAIII, this plasmid uses a second function, the CopR protein, to achieve proper copy number control.

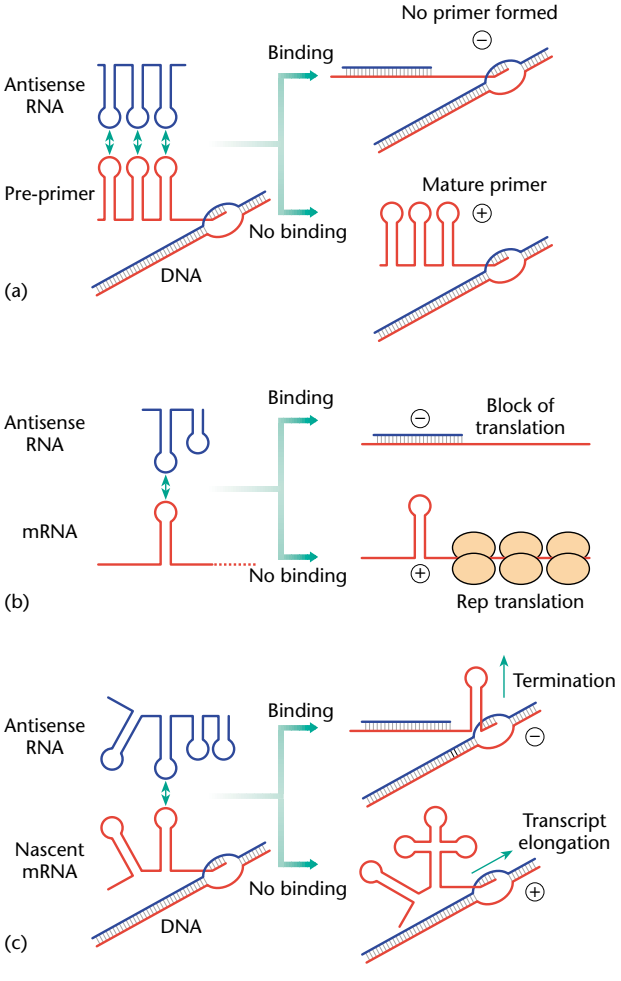

Figure 1 Examples of antisense RNA inhibition mechanisms in plasmids. (a) Mechanisms used by plasmids related to ColE1. The antisense RNA binds to its target (pre-primer) during transcription to induce a folding change that is incompatible with primer maturation. (b) A mechanism used by plasmids similar to R1, ColIbP9, and many others. Antisense RNA binds to a region within a *rep* mRNA and blocks translation. (c) The transcriptional attenuation mechanism of, for example, pIP501 and pT181. Antisense RNA binds to nascent *rep* mRNA to induce the formation of a terminator stem–loop.

In a few plasmids, proteins play modulating roles: the Rom protein of ColE1 strongly stabilizes kissing complexes formed between antisense and pre-primer RNA stem–loops. However, it has been shown that inhibition is primarily determined by the binding rate constant, and not the binding affinity of loop–loop complexes; hence the effect of Rom on the copy number is small (2–5-fold). The activities of cellular RNAases also affect antisense RNA control, since changes in RNA degradation rates must change the intracellular concentrations of the inhibitors. This then entails changes in the replication frequency and, hence, copy number. Recently, the addition of A-tails at the 3′ end of RNAs (poly-adenylation), has been shown to be an important determinant for antisense RNA decay rates.

Plasmid maintenance functions

Other plasmid-associated functions are also controlled by antisense RNAs. The conjugation of F and R1 requires the FinP antisense RNA. FinP is intrinsically unstable and requires the FinO protein to increase its functional half-life. FinP blocks translation of the *traJ* mRNA and hence downregulates the frequency of conjugal transfer. Possibly, the FinO protein also aids antisense-target RNA binding.

Many plasmids encode killer loci. The phenomenon of postsegregational killing implies that cells that fail to obtain at least one plasmid copy at cell division are killed. Thus, only plasmid-containing cells survive, and the stable maintenance of the plasmid in the population is ensured. The prototype of the family of killer systems is the *hok*/*sok* locus. Hok is a toxic protein, and Sok the antidote (an antisense RNA). The *hok* mRNA is extremely stable and accumulates as a highly structured RNA species which is both inactive in translation and antisense RNA binding. When RNAases cleave off a 3′ segment, the mRNA is activated with respect to both activities. In plasmid-containing cells, rapid Sok binding inactivates this truncated mRNA, and cells survive. In plasmid-free cells, by contrast, the unstable Sok RNA is rapidly lost (since it

cannot be synthesized *de novo* in the absence of plasmid) and, therefore, the activated mRNA is translated and the cell killed. Interestingly, several *hok*/*sok* homologues have been identified in bacterial genomes. Their functions are as yet undefined.

Plasmid multimer formation creates a problem for stable inheritance. A dimer, for instance, contains two 'copies' of a plasmid, but in a single molecule. Thus, fewer plasmid molecules than normal are available at cell division, resulting in an increased probability of plasmid loss. Site-specific recombination systems (the enzymes are host-encoded) ensure that plasmid multimers are reconverted to monomers, thus maximizing the number of units that can individually segregate to daughter cells. The *cer* sequence of ColE1 is the plasmid resolution site. In addition to this multimer resolution system, ColE1 encodes a helper function, Rcd RNA; this transcript is encoded within *cer*. Interestingly, Rcd accumulates only in cells that carry plasmid multimers. Under these conditions, cell division is delayed, thus increasing the time available for efficient resolution of multimers. The site of action of RcD is still elusive.

Binding modes and RNA structure

In all these known cases, mutational analyses have emphasized the role of antisense and target RNA structure in determining both rate and specificity of interaction and, hence, regulation. The antisense RNAs, as well as the targets, carry stem–loop motifs and single-stranded stretches important for binding (**Figure 2**). Most often, loops (6–7 nucleotides) are the preferred sites for initial recognition between the complementary RNAs ('kissing complex'); loop mutations change binding rates and specificity. The presentation of the loop as a defined structure is clearly important. The ubiquitous 'U-turn' structure motif within loops may be a preferred element for rapid binding. Subsequently, more stable complexes are formed, and hybridization can ultimately result in the formation of complete RNA duplexes. For example, in the antisense RNAs of plasmids R1, ColIb-P9 and pMU720, as well as the target structure in *hok* mRNA and the antisense RNA-OUT, it has been demonstrated that bulged-out nucleotides play an important role in permitting the transition from initial kissing to more stable complexes. The *hok*/*sok* and IS*10* systems differ from the plasmid copy number control systems in that the initial recognition step involves a single-stranded tail of one of the RNAs binding to a loop presented in the other. Interestingly, recent data indicate that full duplexes are rarely required for inhibition. The binding pathways are complex and have been elucidated in a few cases (**Figure 3**). Most antisense RNAs bind target RNAs with characteristic binding rate constants of $\approx 10^6\,M^{-1}\,s^{-1}$.

Antisense Control of DNA Transposition

Transposons and insertion sequence (IS) elements are mobile genetic entities. Transposition frequency must be under tight control to avoid mutations and chromosomal rearrangements caused by these 'jumping genes'. Many transposons ensure extremely low transposition frequencies by employing several levels of control. In the bacterial transposon Tn*10*, the best studied case so far, the transposase gene (*tnp*) is encoded within the IS*10* element. Silencing of the low-abundance *tnp* mRNA is accomplished by RNA-OUT. RNA-OUT is an antisense RNA whose structure consists of a single stem–loop. Complementarity between the single-stranded 5′ end of *tnp* mRNA and the loop region of RNA-OUT permits initiation of binding, which then progresses to a fully paired structure. Ultimately, this RNA duplex sequesters the *tnp* ribosome-binding site (RBS) and blocks translation. Additional levels of control keep transposition frequencies low. Transcription of the *tnp* mRNA occurs only in the absence of full methylation at GATC sequences located in the promoter region. Thus, synthesis of the mRNA only initiates within a short time interval immediately after passage of a replication fork, before the hemi-methylated DNA is again fully methylated. Furthermore, *tnp* mRNA that has escaped RNA-OUT binding becomes inactivated *in cis* by a 3′ terminal *fbi* (fold-back-inhibition) sequence that base pairs with and sequesters the *tnp* translation initiation site.

At least one other mobile genetic element, IS*30*, appears to use antisense control. In this case it was suggested that the antisense RNA-mediated block of Tnp synthesis may be accomplished by formation of an RNA duplex within the coding region. As a consequence, translating ribosomes would be blocked at this point, and the shorter, incomplete Tnp proteins produced are presumed inactive.

Antisense Control of Bacterial and Phage Gene Expression

Although regulatory RNA species have been difficult to detect in larger genomes, by direct experiments or sequence analysis, many small RNAs have over the years been demonstrated to be antisense regulators of gene expression in bacteria, mostly in *Escherichia coli* and in bacteriophages.

Bacterial antisense RNA systems

Several of the chromosomally encoded antisense RNAs have been linked to stress responses. For example, the 93-nucleotide MicF RNA is induced by a variety of environmental conditions, including temperature shifts, high osmolarity and superoxide stress; the 109-nucleotide

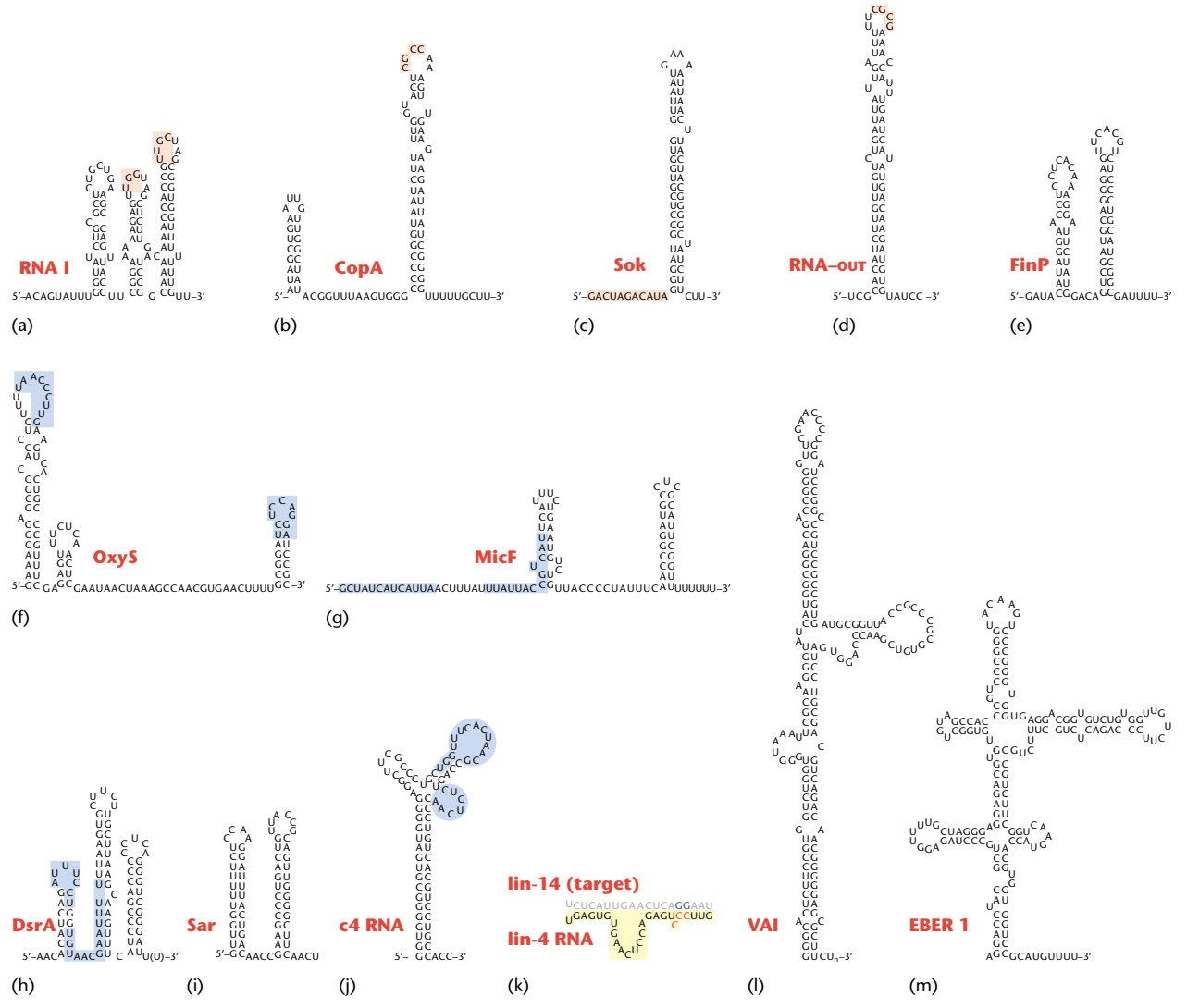

Figure 2 Experimentally determined or predicted secondary structures of regulatory RNAs. (a, b) Antisense RNAs that control copy numbers of plasmid R1 (a) and of plasmid ColE1 (b). (c) Antisense RNA of the *hok/sok* killer system of R1. (d) Antisense regulator of IS*10* transposition. (e) Antisense regulator of conjugation of plasmid F. (f, g, h) Stress response regulators OxyS (f), MicF (g) and DsrA (h) from *Escherichia coli*. (i, j) Antisense RNAs controlling lysogeny in *Salmonella typhimurium* phage P22 Sar (i) and coliphages P1/P7 (j). (k, l, m) The short antisense RNA lin-4 in its proposed complex with one of the lin-14 target elements (grey letters; repeat 4) (k), and regulatory RNAs from adenovirus (l) and Epstein–Barr virus (m). The RNAs a–c and i are fully complementary to their target RNAs, RNA-OUT (d) is fully complementary only in its 5′ half. Pink boxes indicate examples of known sites at which target binding initiates. Blue boxes show regions of target complementarity in '*trans*'-encoded antisense RNAs. Only one of two such regions is shown for DsrA (h).

OxyS RNA is induced by oxidative stress. Most regulatory RNAs are subject to minor trimming of 1–5 nucleotides by various exonucleases, while others are generated by processing of primary transcripts. For example, DicF RNA (53 nucleotides) is generated by RNAase E and RNAase III-dependent processing of a polycistronic transcript.

Unlike the antisense control RNAs of plasmids, many bacterial regulatory antisense RNAs are encoded *in trans*, at genetic loci other than those of the target genes. Therefore, complementarity is incomplete, and regulation is mediated by the formation of partial and imperfect duplexes formed between antisense and target RNAs. In many cases, inhibition occurs at the level of initiation of translation: the antisense RNA binds to a 5′ region of the target mRNA and blocks ribosome binding. MicF RNA interferes with the translation of *ompF*, encoding an outer membrane porin. DicF is predicted to sequester part of the translation initiation region of its target, the mRNA encoding the cell division protein FtsZ.

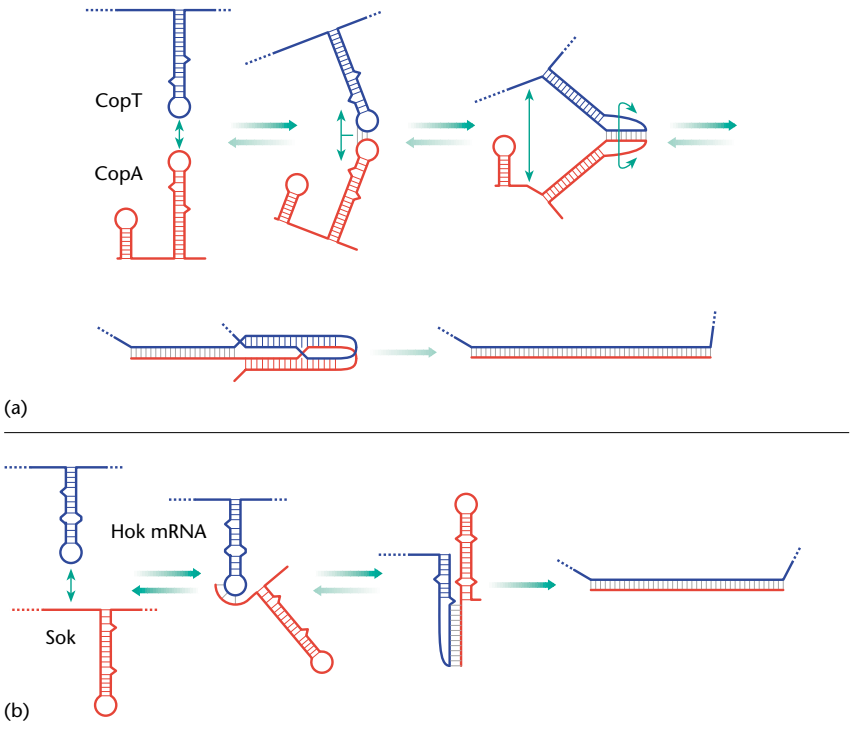

Figure 3 Two examples of antisense-target RNA binding pathways. (a) The proposed binding pathway of CopA to CopT, the target of plasmid R1. Binding initiates between loops on top of bulged stems, proceeds by helix extension (asymmetrically) and results in the major inhibitory complex (lower, left). The unidirectional broken arrow indicates that subsequent duplex formation is slow and biologically not important. (b) The binding pathway of Sok and *hok* mRNA. Here, binding initiates between the single-stranded 5′ tail of Sok and a target stem–loop. A complicated binding intermediate (third structure from left) suffices for inhibition, but is converted to a full duplex.

Other RNAs appear to regulate by more than one mechanism (**Figure 4**). OxyS acts pleiotropically, affecting the expression of multiple genes in *E. coli*, and protects against DNA damage. Molecular characterization of the regulation of two target genes indicates two distinct mechanisms. OxyS inhibits translation of the stationary phase sigma factor RpoS by modulating the activity of Hfq, an RNA-binding protein required for *rpoS* translation. A second target is the *fhlA* gene. Translation of FhlA, a transcription factor, is directly inhibited by OxyS through pairing with two short sequences, one of which overlaps the *fhlA* Shine–Dalgarno (SD).

A plasmid-encoded regulatory system, yet involved in host cell metabolism, is found in *Vibrio anguillarum*. The iron transport genes *fatA* and *fatB* are negatively regulated by an antisense RNA, RNAα. It appears that the accumulation of RNAα under iron-rich conditions leads to destabilization of the *fatA-fatB* transcript.

Activation by antisense RNA

Most antisense RNAs inhibit the expression of their target genes. However, at least in two cases, an activation mechanism has been demonstrated. The *agr* locus of *Staphylococcus aureus* provides the first example: intramolecular base pairing within the 5′ segment of the alpha toxin (*hla*) mRNA sequesters the ribosome-binding site and thereby results in a translationally inert message. The activator RNAIII is a 514-nucleotide untranslated RNA that pairs with the self-inhibitory target RNA segment (anti-SD). As a consequence, the SD region is freed, and translation of *hla* mRNA is activated. The second example is provided by the *E. coli* 87-nucleotide DsrA RNA. DsrA both upregulates the sigma factor RpoS and downregulates the transcriptional silencer H-NS (**Figure 4**). Base pairing of DsrA with an anti-SD segment of *rpoS* mRNA appears to promote translation, whereas interaction with the *hns* mRNA results in inhibition of translation.

Control of lytic cycle/ lysogeny in bacteriophages

A greater variety of mechanisms is observed in bacteriophages. Phages are streamlined parasites that often employ several levels of regulation for optimized switches between different lifestyles. In many cases, regulatory proteins are responsible for the major decisions, and antisense RNAs are used to fine-tune gene expression for

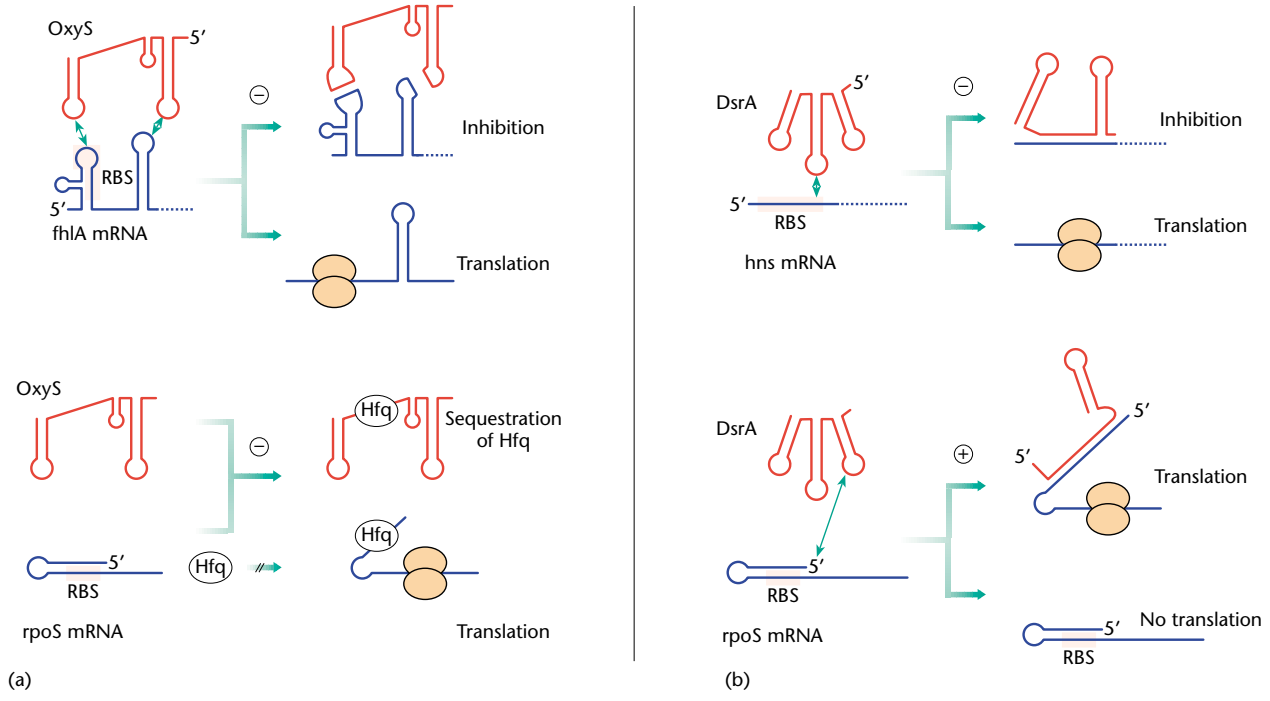

Figure 4 Models for different regulatory mechanisms used – the OxyS and DsrA cases. (a) Two proposed mechanisms for OxyS function. OxyS inhibition of *fhlA* translation is mediated by an antisense effect (upper), and OxyS repression of *rpoS* translation is due to binding of the translational activator Hfq. (b) Two antisense mechanisms proposed for DsrA: binding of DsrA to the *hns* mRNA blocks translation, and DsrA binding to an anti-RBS segment of the *rpoS* mRNA activates translation.

appropriate control. The *cII* gene of phage λ, a key player in the lysis–lysogeny decision, displays these properties. The *cII* mRNA is negatively regulated by OOP RNA, a short (77-nucleotide) phage-encoded antisense transcript. In the absence of polyadenylation, OOP destabilizes the *cII* message by creating a substrate for RNAase III cleavage, resulting in 100-fold reduction of *cII* expression. A second, less well-defined antisense system in λ appears to control the *q* gene.

C4 RNA of phages P1 and P7 is transcribed as an internal segment of the antirepressor (*ant*) mRNA. Processing of the primary transcript by RNAase P permits the accumulation of the free, *trans*-acting C4 RNA. Thus, both antisense and target RNA are initially part of the same transcript. The mature antisense RNA inhibits Ant synthesis by occlusion of an RBS involved in *ant* expression. This event additionally entails premature termination of transcription of *ant* mRNA and further helps to maintain the lysogenic state. The same mechanism appears to be used by phage P4. In the *Salmonella* phage P22, two modes of antisense regulation have been documented. Ant synthesis is negatively controlled by Sar RNA which acts *in trans* to interfere with ribosomal binding at the *ant* mRNA.

The second case of antisense control in P22 involves Sas RNA. Many phages encode immunity determinants that prevent the growth of a second superinfecting phage belonging to the same class. One such gene is *sieB*, whose gene product aborts the lytic development of some phages. P22 is insensitive to *sieB*, since it encodes a function that permits escape from phage exclusion. It appears that two genes are encoded by the *sieB* mRNA, so that approximately equal amounts of two proteins with antagonistic functions are synthesized: SieB (exclusion factor) and Esc (escape factor). Superinfecting P22 synthesizes an antisense RNA, Sas, that allows the phage to bypass the SieB-mediated exclusion. The mechanism can be envisioned as a translational switch: Sar RNA blocks *sieB* translation, whereas Esc synthesis is unaffected.

The only reported case of antisense control in archaea so far was found in ΦH, a prophage of *Halobacterium salinarium*. This phage requires translation of a transcript, denoted T1, for lytic growth. In the presence of the phage-encoded immunity function, T_{ant} antisense RNA, the complex formed between antisense and target RNA becomes a substrate for an unidentified RNAase which cleaves immediately outside the duplex region, i.e. the mRNA is destabilized and lysogeny is maintained.

Viral regulatory RNAs

In response to infections by viruses that generate double-stranded RNA (dsRNA) either as their genetic material or as replication intermediates, many eukaryotic cells shut down translation as a defence against the parasite. Upon infection, the protein kinase R (PKR) which is associated with ribosomal protein L22 is activated by dsRNA to phosphorylate, and thereby inactivate, the translation initiation factor eIF2α. As a countermeasure, the Epstein–Barr virus encodes two small, highly structured RNAs, EBER 1 and 2, that bind to PKR with high affinity and inhibit kinase activity. Similarly, the adenovirus VAI RNA binds PKR, resulting in a conformational change of the protein that precludes its association with the ribosome and thus inhibits kinase activity. In both cases, eIF2α is not phosphorylated in response to viral infection, translation is not inhibited, and the infected cell produces viral progeny. VAI RNA has also been shown to impair ADAR1, the deaminase which modifies adenine residues to inosines in dsRNA. The physiological effect of this is, at present, unknown.

Herpesvirus saimiri causes malignant transformation of T cells. Small viral RNAs similar to small nuclear RNAs (snRNAs) appear to prolong the half-life of some intrinsically unstable oncogene and growth factor mRNAs and thus contribute to cell transformation.

Antisense Control of Eukaryotic Gene Expression

In eukaryotes, RNAs of the antisense type have so far only been found fortuitously, and their regulatory roles have, in most cases, remained unsubstantiated. Only a small number of such RNAs have unambiguously been demonstrated to be regulators (**Table 1**). In *Caenorhabditis elegans*, the lin-4 RNA displays partial complementarity to seven sequence elements in the 3′ untranslated region (UTR) of the *lin-14* and *lin-28* genes. These genes are involved in larval stage development. Hybridization of the short (22-nucleotides) processed *lin-4* transcript with mature *lin-14* mRNA results in imperfect duplexes, four of which contain a bulged C residue (**Figure 1**). This structure is required for translational downregulation of LIN-14 protein synthesis during nematode development. Recent experiments suggest the involvement of a protein in this process.

In *Dictyostelium*, expression of the prespore vesicle protein PSV-A is regulated at the level of mRNA stability: *psv-A* mRNA is constitutively transcribed throughout development but destabilized by a differentially regulated antisense transcript originating from the same chromosomal locus. Antisense RNA-mediated mRNA destabilization occurs mostly in the cytoplasm. In mammalian cells, expression of the translation elongation factor eIF2α is also regulated on the level of RNA stability. In this case, nuclear instability of the pre-mRNA is mediated by a differentially expressed antisense transcript originating from a promoter located in the first intron of the eIF2α gene.

Antisense mechanisms in eukaryotes

The mechanisms by which regulatory antisense RNAs exhibit their function in eukaryotes are mostly elusive. Often, the simultaneous synthesis of sense and antisense transcripts results in very low abundance of both, and the expected antisense-target RNA hybrids are usually undetectable in the cell. Therefore, destabilization has been attributed to targeting of dsRNA by a double strand-specific RNAase (dsRNAase). An enzyme with such properties has been partially purified from human cells. The activity was found in the nucleus as well as in the cytoplasm. A similar activity has been isolated from *Dictyostelium discoideum*. However, this dsRNAase is predominantly cytoplasmic and is distinct from the mammalian enzyme.

In plants, antisense transcripts are believed to target mRNA for degradation, as indicated by many studies in which artificial antisense RNA genes have been introduced. Short antisense RNAs can also be generated by RNA-dependent RNA polymerase (RdRp) from aberrant sense RNAs. In addition to interference with mRNA, such RNAs (including aberrant sense transcripts, e.g. lacking polyA tails) can mediate methylation of homologous DNA sequences in the plant genome, thus silencing gene expression. It may be expected that natural RNA regulators use similar pathways.

In the case of the bFGF gene (encoding basic fibroblast growth factor) in vertebrates, a partial overlap between the 3′ segment of the bFGF mRNA and an antisense transcript has been reported. Even in the noncoding region, these genomic sequences have been conserved throughout evolution and, hence, suggest functional importance. It appears that binding between these two complementary sequences either shuts off or modulates translation of the protein; since dsRNA is a target for ADAR1, the enzyme may alter the coding capacity of an mRNA in a sense–antisense complex. Deamination of bFGF mRNA has recently been demonstrated *in vivo*.

In the *c-erbAα* locus in mammals, an antisense RNA is transcribed that appears to inhibit splicing and thus may regulate alternative RNA processing. In mice, a nervous disorder (myelin deficiency) results from a tandem duplication and partial inversion of the *mbp* gene. The abundantly expressed antisense transcripts accumulate in the nucleus and impair either processing or the transport of the mRNA to the cytoplasm.

Similar to prokaryotes, transposition in eukaryotes may be controlled by antisense RNAs. In several species of

Drosophila, an antisense RNA of the micropia retrotransposon is specifically transcribed in testes. Even though a mechanism is not established, complementarity of this RNA to the coding region of reverse transcriptase and RNAase H suggests that downregulation may help to restrain the mobility of the element.

A search of the literature shows many reports in which the existence of antisense RNAs in eukaryotes has been postulated. It is, however, unclear whether the identification of such transcripts *per se* implies a regulatory function. So far, the evidence in these cases is, at best, circumstantial. These are, for example, genes encoding dopa decarboxylase in *Drosophila*, N-myc, c-myc, α1(I) collagen in chicken, the gonadotrophin-releasing hormone (in rat hypothalamus), Hoxa-11, Hoxd-3 and many others.

Other Cellular Regulatory RNAs

A number of untranslated non-antisense RNAs are involved in regulatory aspects of metabolism in prokaryotes and eukaryotes. One such example is the 363-nucleotide tmRNA, also known as 10Sa RNA. One segment of this RNA folds into a transfer RNA (tRNA) mimic structure, whereas the remaining segment encodes a peptide tag which is a substrate for C-terminal-specific proteases. When the translation of 3′ truncated mRNAs (these will lack the normal stop codon and cannot terminate normally) is arrested, the empty ribosomal A site is bound by aminoacylated tmRNA. After peptide bond formation and translocation, translation continues on the tmRNA coding sequence, resulting in addition of the peptide tag to the growing polypeptide chain. Thus, abberant proteins translated from defective mRNAs are targeted for degradation and blocked ribosomes are rerouted to the active ribosome pool.

Some regulatory RNAs act by mechanisms other than antisense. For example, the activity of CsrA protein, which negatively regulates glycogen biosynthesis, is directly inhibited by the binding of CsrB RNA. CsrB contains several imperfect repeats of seven nucleotides that mediate the binding of $\approx$ 18 molecules of CsrA protein. Some small RNAs, like 6S and Spot 42 RNA, were discovered more than 25 years ago. Yet, their biological roles and their mechanisms of action are still elusive. Two other untranslated RNAs, 4.5S and the M1 subunit of RNAase P, are well-characterized. They are not considered regulatory; their biological roles in protein secretion and tRNA processing, respectively, are described elsewhere.

In eukaryotes, many untranslated, putative regulatory 'orphan RNAs' may be serendipitous and nonfunctional readthrough products of adjacent genes, yet others may be *bona fide* regulators of so far unknown function. RNAs whose roles were not understood until some time after their discovery include the eukaryotic snRNAs (discussed elsewhere), the bacterial tmRNA, Xist (RNA involved in sex determination in mammals), and gRNAs. These examples and others document the general problems in identifying functional, noncoding RNAs and defining their biological roles. Another interesting case is the 'inside-out' *uhg* gene in which most introns encode snoRNAs, whereas the function of the noncoding, spliced RNA product is unknown. In *Dictyostelium*, the synthesis and/or accumulation of a noncoding polII transcript (*dutA*) is strictly regulated during development, but the implied regulatory function has not been elucidated.

Summary

The rapid progress in the identification of small, regulatory RNAs with regulatory functions indicates that we may only have seen the tip of the iceberg. It is apparent from the recent progress of the genome projects that many small, regulatory RNAs may be encoded in bacterial genomes. It will be a challenge to investigate the functions of these orphan RNAs.

Further Reading

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