U-turns and regulatory RNAs
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Conventional antisense RNAs, such as those controlling plasmid replication and maintenance, inhibit the function of their target RNAs rapidly and efficiently. Novel findings show that a common U-turn loop structure mediates fast RNA pairing in the majority of these RNA controlled systems. Usually, an antisense RNA regulates a single, cognate target RNA only. Recent reports, however, show that antisense RNAs can act as promiscuous regulators that control multiple genes in concert to integrate complex physiological responses in Escherichia coli.

Introduction
Antisense RNAs are small, non-translatable, regulatory entities usually transcribed from eubacterial chromosomes, bacteriophages and accessory genetic elements [1]. Antisense RNAs regulate their target RNAs by different mechanisms including translational repression, target RNA degradation and RNA folding interference [1]. Usually, antisense RNAs act as post-transcriptional regulators in processes where fast adaptive responses are appropriate.

The majority of known antisense RNAs are regulators that form paired complexes with their complementary target RNAs. In several cases, the extensive base pairing between antisense and target RNA does not occur within a feasible time-frame. Consequently, many antisense RNA regulated systems have evolved structural features that mediate target RNA inactivation upon formation of only a limited number of interstrand base pairs [2–7]. Despite clear diversities in binding pathways, the rate-limiting step in complex formation between an antisense RNA and its target is always an initial base-pairing interaction between two loops, or a loop and a single-stranded RNA tail. Comparative sequence analysis of the recognition loops in antisense RNAs and their targets revealed the presence of a common structural motif that specifies so-called U-turns [8••]. U-turns are structural elements present in the anticodon loops of tRNAs, where they facilitate rapid codon–anticodon interaction. Thus, the U-turn loop structure is a general binding-rate-enhancer that promotes RNA-RNA pairing. Usually, an antisense RNA regulates only one cognate, complementary target RNA. However, newly described regulatory RNAs encoded by the Escherichia coli chromosome act as promiscuous regulators that control or modulate the functions of multiple RNAs.

Here we review the importance of the U-turn motif in antisense RNA regulation and the multifaceted regulatory properties of several small RNAs.

Rapid RNA–RNA pairing mediated by U-turn loops
The effects of antisense RNAs on their target RNAs are proportional to the rate of complex formation. In vitro bimolecular reactions yield maximum RNA pairing rate constants of $10^6–10^7$ M$^{-1}$s$^{-1}$ [9–11]. Thus, the interaction between RNA molecules is inherently constrained and not simply diffusion limited. From such studies, it was inferred that antisense RNAs have evolved structural elements that reduce constraints and ambiguity of the pairing reactions. Recently, a comparative sequence analysis of antisense RNA recognition loops revealed the presence of a ubiquitous YUNR (where Y = pyrimidine, U = uracil, N = any nucleoside, R = purine) sequence motif [8••] (see Figure 1). Strikingly, the YUNR motif of different antisense and target RNAs are located at the same relative positions within the recognition loops (Figure 1). This argues for a conserved function of the motif in the initial recognition steps. The motif was proposed to configure a U-turn loop structure similar to that observed in tRNA anticodon loops [8••]. The U-turn is characterised by a sharp turn in the phosphodiester-backbone between the invariant uracil and the N base stabilised by three non-Watson–Crick interactions involving the invariant uracil ribonucleoside [12–17,18•]. The nucleobases on the 3’ side of the turning phosphate is presented in an A-form structure creating an unpaired Watson–Crick surface predisposed for binding to a complementary set of nucleotides. Furthermore, the U-turn architecture retracts the phosphodiester backbone within the loop, thereby decreasing the local electronegative potential surrounding the bases presented. This, in turn, promotes the pairing to the complementary bases by reducing backbone repulsion in the initial recognition step [19,20].

The activity of the hok/sok locus of plasmid R1, which mediates plasmid maintenance by killing of plasmid-free cells, is regulated by Sok antisense RNA [21]. The target loop in hok mRNA contains a U-turn structure that is recognised by the single-stranded tail of the complementary Sok antisense RNA [8••]. Substitutions of loop nucleotides that disrupt the YUNR U-motif in hok mRNA adversely affect the kinetics of RNA pairing, whereas substitutions that maintain the motif are silent [8••]. Structure probing analyses support the formation of a U-turn loop structure in the antisense-binding loop of the mRNA [8••]. A U-turn structure was also proposed to be present in the IncI-antisense-binding loop of repZ mRNA.
of plasmid ColIl-P9 [22•]. Here, the UpU dinucleotide of the rUGGGCG hexa-nucleotide loop sequence was shown to enhance the rate of intermolecular binding to the Incl antisense RNA and to promote formation of an intramolecular pseudoknot structure. The UpU dinucleotide sequence does not appear to interact with the complementary nucleotides in IncI. Instead, this sequence serves a pivotal structural purpose in presenting the subsequent sequence, rGGCG, for high affinity intramolecular base pairing. Furthermore, kinetic analyses of several antisense/target RNA pairs including RNAI/RNAII of ColE1 [23], CopACopT of R1 (EGH Wagner, personal communication) and RNA-IN/RNA-OUT of IS10 [24] support a similar rate-enhancing effect of the UYNR U-turn motif. This suggests that, although the binding pathways and the mechanisms of target RNA inhibition are different for different systems, UYNR U-turn loop structures act as rate-of-binding enhancers in the majority of the naturally occurring antisense RNA-regulated gene systems (Figure 1). Since most control units appear unrelated in structure and function, the ubiquitous presence of U-turns represents an interesting example of convergent evolution at the mechanistic level. Furthermore, two distinct processes involving RNA–RNA recognition, mRNA decoding and antisense RNA gene control, have evolved the U-turn loop configuration independently. Clearly, the U-turn loop structure is a unique element in RNA–RNA recognition.

OxyS RNA
Respiratory organisms have evolved mechanisms that counteract the damaging production of reactive oxygen species [25]. In E. coli, the transcriptional activator, OxyR, induces the expression of a number of genes including a small 109 nucleotide RNA, OxyS [26]. The transient synthesis of OxyS affects more than 40 genes including the stress σ-factor of RNA polymerase, σE, encoded by the rpoS gene. OxyS RNA represses translation of rpoS mRNA despite the lack of obvious complementary sequence between the two RNAs. Translation of rpoS requires the small RNA-binding protein Hfq ([27]; see also Noguiera and Springer, this issue, pp 154–158). To activate translation of rpoS, Hfq presumably counteracts a negatively acting secondary structure in the rpoS mRNA [27]. OxyS RNA was proposed to bind the Hfq protein via its single-stranded, A-rich linker region (see Figure 2a) and, thereby, counteracts the Hfq effect on rpoS translation, perhaps via formation of a translationally inactive rpoS/Hfq/OxyS ternary complex [28••]. If true, OxyS converts Hfq from a translational activator into a co-repressor. This observation suggests that gene control by regulatory RNAs can be exerted in the absence of sequence complementarity to a target RNA and thus expands the repertoire of regulatory mechanisms used by antisense RNAs.

The fhlA gene encodes a transcriptional activator of genes necessary for the assembly of the formate-hydrogenylase complex [29]. Expression of OxyS from a multi-copy plasmid negatively regulates FhlA production at the post-transcriptional level [30•]. A short segment of seven nucleotides centred in the transcriptional terminator hairpin loop of OxyS RNA is complementary to the Shine & Dalgarno (SD) region of fhlA mRNA (Figure 2a). Mutational analyses and toeprinting experiments support a
model in which OxyS pairs to the complementary bases in the leader of the fhlA transcript, thereby preventing ribosome binding at the translational initiation region (TIR). Intriguingly, OxyS regulates rpoS and fhlA expression by different mechanisms involving separate regions of OxyS (Figure 2a). Secondary structure predictions of the fhlA mRNA leader suggest that the OxyS target forms a part of a six-nucleotide YUNR U-turn loop structure presented by a short unstable stem (T Franch, unpublished data). This U-turn loop may enhance the kinetics of OxyS/fhlA pairing consistent with the observed high level of fhlA repression by OxyS.

Highly reactive hydroxyl radicals generated by Fe$^{2+}$ ions are hazardous to cell components, especially DNA. A region of 11 nucleotides in OxyS overlapping with the region responsible for fhlA regulation is complementary to the single-stranded SD region of the exbB mRNA (T Franch, unpublished data), which encodes a component promoting ferric-siderophore uptake via TonB and the iron/B$_{12}$ subset of ABC membrane transporters [31]. Consequently, OxyS might reduce in vivo iron concentration by repression of exbB synthesis during oxidative stress. Thus, OxyS could help further to reduce detrimental DNA lesions and maintain the integrity of biomolecules, an effect consistent with the proposed role of OxyS as a protector of mutagenesis upon oxidative stress [26].

Upon challenge with superoxide, the SoxRS transcription factors activate numerous genes involved in the defence against oxidative stress in E. coli. Interestingly, SoxS also activates transcription of another antisense RNA, MicF, that is responsible for repression of the outer-membrane OmpF porin synthesis [32]. Thus, OxyS and MicF act by distinct mechanisms to increase resistance to various compounds.

**DsrA RNA**

The *cps* genes that encode factors involved in the synthesis of the capsular polysaccharide colanic acid are regulated by the RscC–RscB phosphorelay system and the auxiliary positive regulator RcsA [33]. Multiple copies of a region closely linked to the rcsA gene increase rcsA-dependent capsule synthesis [34]. This region encodes a small, stable 85 nucleotide RNA, known as DsrA, that folds into a compact secondary structure with three consecutive stem-loops (Figure 2b). DsrA-mediated activation of capsule polysaccharide production largely mimics the effect observed in strains devoid of H-NS, a major histone-like protein responsible for silencing of numerous bacterial genes, including rcsA [34]. Since overproduction of DsrA induced the proU and papA, also known to be silenced by H-NS, DsrA was proposed to promote rcsA transcription by reducing H-NS activity. DsrA exhibits 13 base-pairs of complementarity to a region immediately downstream of the AUG start codon of *hns* [35••]. Nucleotide substitutions in DsrA in the region of complementarity abolished DsrA repression of H-NS activity, whereas introduction of compensating mutations in the *hns* gene restored DsrA regulation [35••]. These data support a model in which DsrA represses expression of *hns* by direct RNA–RNA interaction, either by ribosome exclusion at the TIR or by DsrA facilitated decay of the *hns* mRNA. Since H-NS represses transcription of rpoS, DsrA indirectly stimulates expression of *σ* via its repression of *hns* translation.

Positive regulation is unusual in antisense control circuits and have thus far only been proposed for the stimulation of the *hla* alpha-toxin mRNA by the RNAIII regulator of the *agr* virulence locus in *Staphylococcus aureus* [36]. As described below, DsrA RNA may also active as a positive regulator of gene expression.
The level of σ^S is inversely correlated with growth rate and virtually no σ^S is present at optimal growth conditions at 37°C. In contrast, σ^S accumulates during exponential growth at low temperatures (20°C) coinciding with optimal synthesis of DsrA [37].

Surprisingly, DsrA exerts both H-NS-dependent and -independent control of rpoS expression [37]. Two short regions of 8 and 13 nucleotides in DsrA are complementary to an element in the rpoS mRNA that is responsible for cis-inhibition of rpoS translation (Figure 2b). Mutational analyses of DsrA and the complementary element in rpoS mRNA verified that DsrA activates rpoS translation by direct RNA–RNA interaction [35*, 38**]. The Hfq protein is required for translation of rpoS. Since Hfq is believed to bind to the same region as DsrA in the rpoS transcript [27], DsrA may act either separate from or in combination with Hfq to stabilise a translationally active configuration of the rpoS mRNA. The dual actions of DsrA that ultimately promote RpoS production show that DsrA is a highly flexible regulator that may serve an important function as an activator of the stress-response network at reduced temperatures.

The regulation of the cellular content of σ^S is highly complex and involves control of rpoS transcription and translation, and σ^S proteolysis. Moreover, translation of rpoS mRNA is regulated by at least three factors: DsrA and Hfq stimulate rpoS translation, whereas OxyS inhibits rpoS translation. The effect of OxyS appears counterintuitive, since it stimulates the expression of several genes that protect against oxidative stress. Since OxyS is expressed when OxyR is activated, OxyS may act to prevent redundant and wasteful activation of genes that are under positive control of both OxyR and σ^S [28**]. Thus, OxyS and DsrA are interesting examples of promiscuous antisense RNAs that integrate different global regulatory networks into an appropriate physiological response.

CsrB RNA

Recently, a 61 amino acid protein, CsrA, and a ~360 nucleotide RNA, CsrB, were identified as important components in the regulation of carbohydrate metabolism in E. coli [39,40]. High levels of csrA expression promote the activity of several enzymes of the glycolytic pathway and repress genes responsible for glucogenesis and glycolgen biosynthesis [39,41]. Furthermore, a csrA^- strain exhibits altered motility, adherence and cell morphology phenotypes [39,42*].

Studies on the glgCAP operon, which encodes genes required for glucogen biosynthesis, have shown that CsrA facilitates the decay of the glgCAP mRNA by binding to the TIR of glgC [43,44]. The CsrB RNA consists of 18 imperfect repeats of seven nucleotides that are located in loops of predicted hairpin structures (see Figure 2c). Overexpression of CsrB yields a csrA^- phenotype and antagonises CsrA by the formation of a globular CsrA/CsrB ribonucleoprotein complex that consists of approximately 18 monomers of CsrA [40]. The stoichiometry of the complex indicates that CsrA recognises and binds the imperfect repeats of CsrB via its KH RNA-binding motif. A model has been proposed in which CsrB acts as a sink or a decoy molecule for CsrA and thereby controls the concentration of free CsrA [40,42*]. Although a detailed knowledge on the regulation of CsrA and CsrB synthesis is still lacking, it is plausible that the ratio between the CsrA protein and the CsrB RNA determines the expression of a wide array of CsrA regulated genes ([42*]; see also Noguiera and Springer, this issue, pp 154–158).

A similar two-component system encoded by rsmA (csrA homolog) and rsmB (csrB homolog) is present in the plant pathogenic enterobacteria, Erwinia carotovora [45,46]. During exponential growth, RsmA negatively regulates production and secretion of extracellular enzymes, quorum-sensing signals and other components necessary for virulence [45]. The RsmB RNA counteracts RsmA by formation of a ribonucleoprotein similar to that observed for CsrA/CsrB [46]. The well-documented control of virulence factors by RsmA/RsmB could indicate that the homologous system CsrA/CsrB might have a related, undisclosed function in pathogenesis in E. coli. This, in turn, could explain the altered adherence and surface properties observed for static csrA^- cells [39].

Conclusions

Conventional antisense RNAs such as those controlling plasmid replication and maintenance, regulate the function of a single, cognate target RNA. Such antisense RNAs or their targets contain YUNR motifs that specify dynamic U-turn loop structures required for fast bi-molecular interaction. The evolution of similar U-turn loop structures in tRNAs and antisense RNAs argues that the intrinsic properties of the U-turn loop configuration facilitate rapid bi-molecular RNA–RNA interaction. In contrast, the OxyS and DsrA RNAs are promiscuous regulators, each modulating the expression of several or even many target genes. The negative regulation by these RNAs, exerted by short stretch base pairing to their targets, resembles the regulation by conventional antisense RNAs. However, the extraordinary complex negative and positive control of rpoS translation shows that OxyS and DsrA are highly versatile regulators of multiple target genes. The promiscuous regulation by OxyS and DsrA is important for the adaptive responses to adverse growth conditions, such as oxidative stress and low temperatures, and appears to be an integral part of global stress response networks. We anticipate the discovery of additional regulatory RNAs in E. coli and other prokaryotic organisms.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
• of outstanding interest


A comparative analysis of recognition loops of antisense RNA-regulated gene systems revealed the presence of a YURN U-turn motif. Using hok/sok as a model system, mutational analysis and structure probing data support the presence of a U-turn structure that enhances the rate of Sok-antisense-binding to its target in the hok mRNA. A similar function as a rate enhancer was inferred for other antisense-RNA-regulated gene systems.


The function of the invariant uracil ribonucleoside of the tRNA anticodon loop is investigated. The uracil contributes to U-turn stability of the anticodon loop by two non-Watson-Crick interactions and strong base-stacking effects. These properties promote the anticodon loop interaction with a ribosome/mRNA complex.


A comprehensive mutational analyses of the binding of antisense Inc to its target in the repz mRNA suggest that the iU4 dinucleotide of the recognition loop in repz is important for fast pairing to Incz. The authors propose that the six-nucleotide target loop forms a U-turn configuration. In the accompanying paper [47] they show that the U-turn loop structure is also important for intramolecular pseudoknot formation in the repz mRNA.


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30. Altuvia S, Zhang A, Argaman L, Tiwari A, Storz G: The Escherichia coli OxyS regulatory RNA represses poS translation by blocking ribosome binding. EMBO J 1998, 17:6069-6075. OxyS represses hfa encoding a transcriptional activator of genes required for formation of the formate-hydrogenlyase complex. Seven nucleotides centered around the loop of the terminator hairpin of OxyS is complementary to the Shine & Dalgarno (SD) region of hfa. toeprinting and mutational analyses showed that OxyS precludes ribosome binding at the SD of hfa.


The small RNA, DsrA, exhibits partial sequence is complementary to the leader of ppoS mRNA and to a segment of the hns gene immediately downstream of the AUG start-codon. DsrA appears to interfere with translation initiation of hns, whereas the translation of poS is stimulated by DsrA. DsrA is complementary to an element in the poS mRNA that occludes the TIR and thus enhances poS translation by binding to this element.


38. Majdalani N, Cunning C, Sledjeski D, Elliot T, Gottesman S: DsrA RNA regulates translation of RpoS message by an anti-antisense mechanism, independent of its action as an antisilencer of transcription. Proc Natl Acad Sci USA 1998, 95:12462-12467. Previously, Gottesman and co-workers described that the effect of DsrA on rpoS was most pronounced at low temperatures where the secondary structure stability in rpoS mRNA is augmented. In this paper, mutational analysis of a base-pair in the rpoS secondary structure and the corresponding nucleotide in DsrA provide compelling evidence for the mechanism of DsrA stimulation of rpoS synthesis.


42. Romeo T: Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB. Mol Microbiol 1998, 29:1321-1330. This review describes the effects of CsrA/CsrB on the carbohydrate metabolism, cell surface, motility and adherence properties of *E. coli*. Furthermore, the importance of the homologous system RomA/RomB in plant pathogenesis in *E. corotovora* is described.


