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The non-coding RNAs as riboregulators

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ABSTRACT

The non-coding RNAs database (http://biobases.ibch.poznan.pl/ncRNA/) contains currently available data on RNAs, which do not have long open reading frames and act as riboregulators. Non-coding RNAs are involved in the specific recognition of cellular nucleic acid targets through complementary base pairing to control cell growth and differentiation. Some of them are connected with several well known developmental and neurobehavioral disorders. We have divided them into four groups. This paper is a short introduction to the database and presents its latest, updated edition.

INTRODUCTION

RNA plays a variety of structural, informational and regulatory roles in the cell. Some RNAs possess catalytic properties and act as ribozymes. 23S rRNA alone possesses peptidyltransferase activity and other RNAs as aptamers can bind small molecular compounds. In the last few years many data have accumulated showing that various non-translatable, non-coding RNA transcripts are present in different cells. They are lacking a protein coding capacity and it seems they exert their action mainly or exclusively at the RNA level. Functions of RNA as a genetic regulator (riboregulator) by acting either *in cis* or *in trans* have been demonstrated widely in bacteria and eukaryotes, including nematodes and mammals. Regulatory RNAs are synthesized in most cases by pol II, then polyadenylated and spliced without defined open reading frames (ORFs) (1). An analysis of non-coding RNAs (ncRNAs) origin, functions and properties gave us a basis for their partition into four groups.

(i) Gene regulators. They affect the activity of a gene by different mechanisms such as silencing, methylation and DNA–RNA or RNA–RNA interaction. This group includes: *Xist*, *roX*, *PAT-1*, *Tsix*, *XistAS*, *H19*, *IPW*, *NTT*, *DGCR5*, *KvLQTIAS*, *Nesp GNAS*, *SCA8*, *CMPD*, *lin-4*, *let-7*, *UBE3A*, *ZNF127AS*, *ScYc*, *DISC2*, *sok*. *CopA*, *RNAI*, *pnd*, *RNA-OUT*.

(ii) Abiotic stress signals. These RNAs are synthesized in response to stress (e.g. oxidation) and include: *gadd7/adapt15*, *adapt33*, *hsrω*, *G90*, *OxyS*, *DsrA*.

(iii) Biotic stress signals. This group consists of RNAs inducible by biologically active molecules (e.g. cytokinins). They are: *His-1, ENOD40*, *lbi*, *CR20*, *GUT15*.

(iv) Other RNAs within this group have a different origin and function. They include: *Bsr*, *BC1*, *BC200*, *SRA*, *meiRNA*, *UHG*, *Xlsirt.*

In comparison with the previous database new RNAs, especially antisense RNAs, are included (1). Their nucleotide sequences are stored and can be retrieved as separate files at: http://biobases.ibch.poznan.pl/ncRNA/. Below we characterize briefly those ncRNAs for which new data have appeared since last year's edition of the database (1).

DOSAGE COMPENSATION RNAs

One of the fundamental differences between male and female cells is the number of X chromosomes. The difference in its content and the requirement of equal expression of the chromosome X genes in both sexes led to the evolution of several types of dosage-compensation mechanisms. In mammals this process involves expression of specific genes, whose products (RNA transcripts) do not contain a long ORF (1–4). In *Drosophila*, compensation for the reduced dosage of genes located on a single male X chromosome involves doubling their expression in relation to their counterparts on female X chromosomes. This is an epigenetic process involving the specific acetylation of histone 4 at lysine 16 by histone acetyltransferase (MOF). It is part of the chromosome-associated dosage compensation complex comprising male-specific lethal (MSL) proteins and rox RNAs. The dosage compensation process involves products of the two genes *roX1* and *roX2*, which are male specific and do not encode proteins. It is already known that *roX1* RNA becomes associated with chromosome X at sites determined by binding of the *msl* (*m*ale *s*pecific *l*ethal) gene products, which leads probably to the remodeling of the chromatin and allows increased transcription. The *rox* RNAs are expressed in all somatic cells in males and in diploid cells they co-localize with the MSL proteins (5). A two-step process for recognition of the X chromosome by the MSL protein complex has been proposed (6). In the first step, MSL 1 and MSL 2 together recognize 30–40 sites distributed along the length of X, where they recruit other MSL proteins. Two of these sites encode *rox* RNAs, which are incorporated into the growing MSL complex. In the second step, the MSL complex can associate with chromatin entry sites on X

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and then spread along the chromosome *in cis* to locate all of the genes that utilize MSL-mediated dosage compensation (6–8).

H19 RNA

In adulthood a basal *H19* gene expression has been detected only in mammary gland, cardiac and skeletal muscles and to a lesser extent in kidney, adrenal gland and lung. The cells with a high *H19* level stopped their proliferation after 48 h when cultivated in a low serum-containing media, while the cells lacking *H19* continued their proliferation (9). It was suggested that *H19* RNA could be used as an adjuvant tumor marker for the diagnosis, staging and follow-up of patients with serious ovarian carcinoma (10,11).

The imprinting is the result of methylation of a 7–9 kb domain on the paternal allele of *H19*. The 5′ flank of the *H19* gene contains an imprinting mark region characterized by paternal allele-specific methylation. The evolutionarily conserved 42 bp element located upstream of the domain might play a role in imprinting and/or transcriptional regulation of *H19* (12). The G-rich repeat 1.5 kb upstream of mouse *H19* was shown to be present in rats but absent in humans, and is not essential for *H19* imprinting. The transcriptionally active maternal allele is unmethylated whereas the inactive paternal allele is methylated. The differentially modified region in the mouse *H19* locus extends over 2 kb at ~4 kb upstream of the gene promoter. A similar pattern of methylation is observed for the human *H19* gene. Its RNA contains four attachment sites for the oncofetal IGF2 mRNA-binding proteins with K_ds of 0.4–1.3 nM. These sites are located within a 700 nt segment encoded by exons 4 and 5 (13). Recently a chromatin boundary model of genomic imprinting has been proposed. It suggests that chromatin boundary elements (insulators) act *in cis* at an insulated gene and its regulatory portion thereby blocks transcription when placed between a gene and its enhancer. An insulator located upstream of the *H19* gene isolates *Igf2* from its enhancers. They are accessible to *Igf2* but not to *H19* when moved between two genes (upstream of the putative insulator). When the ICR is unmethylated on the maternally inherited chromosome, there are two nuclease hypersensitive regions that overlap with several short CG rich repetitive elements conserved in human and mouse. These repeats are the target for the conserved DNA-binding zinc finger protein CTCF. The CCCTC-binding factor interacts with the core insulator sequence via a domain containing 11 zinc fingers. The resulting insulator blocks activation of the maternal copy of *Igf2* by *H19*. The methylated ICR contains no hypersensitive sites and this prevents CTCF binding, thereby inactivating the insulator and allowing the *H19* enhancer to activate *Igf2* (14–21). Recently it was also demonstrated that *H19* gene expression is controlled by steroid hormones and this gene is highly expressed in hormone-sensitive organs when the hormonal stimulation is accompanied by morphological repair (22).

KvLQT1-AS

Beckwith–Wiedeman Syndrome (BWS) is a human disease characterized by tissue overgrowth, macroglossia, abnormal wall defects and predisposition to embryonal tumors. BWS is also connected to hypomethylation at KvDMR1, a maternally methylated CpG island within an intron of the *KvLQT1* gene. Imprinting control elements are proposed to exist within the *KvLQT1* locus. Recently an antisense transcript *KvLQT1-AS* or *LIT1* has been found. It is transcribed exclusively from the paternal allele in the reverse orientation with respect to the maternally expressed *KvLQT1* gene, but the function is still not known (23). It is thought to be a good target for methylation. *KvLQT1-AS*/(*LIT1*) could be an imprintor gene that competes with the target-imprinted gene *KvLQT1* for expression and is silenced by DNA methylation, probably RNA directed (24). Down-regulation of *KvLQT1-AS* expression during developmental relaxation of *KvLQT1* imprinting would support the notion of a functional role for antisense RNA transcription in *KvLQT1* imprinting (23–26).

SCA8

Spinocelebellar ataxia type 8 (SCA8) is a neurodegenerative disorder caused by the expansion of a CTG trinucleotide repeat, which is transcribed as a part of an untranslated RNA. It has been found that the *SCA8* gene is transcribed through the repeat only in the CTG orientation, as in the case for myotonic dystrophy (DM), but not in the CAG orientation, as in the other dominantly inherited ataxias: SCA1, 2, 3, 6 and 7. In these latter cases, the CAG expansion is translated into a polyglutamine tract that adds a toxic gain of function to the respective proteins, whereas the CTG expansions in DM and SCA8 are not translated. The RNA transcripts containing the SCA8 CTG are alternatively spliced and polyadenylated, and are finally expressed in various brain tissues. No extended ORF has been found (27,28). The SCA8 CTG repeat is preceded by a polymorphic but stable CTA tract with a $(CTA)_{1–21}(CTG)_n$ configuration. The affected individuals analyzed had either an uninterrupted CTG repeat tract or an allele with one or more CCG, CTA, CTC, CCA or CTT insertions. In addition, the *SCA8* repeat tract in sperm underwent contractions, with nearly all of the resulting expanded alleles having repeat lengths of <100 CTGs, a size that is not often associated with disease. These repeat contractions in sperm likely underlie the reduced penetrance associated with paternal transmission (29).

DEVELOPMENTAL TIMING RNA

The *Caenorhabditis elegans* heterochronic gene pathway consists of a cascade of regulatory genes that are temporally controlled to specify the timing of developmental events. The products of the heterochronic genes include transcriptional and translational regulators and two different cases of novel small translational regulatory RNAs. Other genes of the pathway encode evolutionarily conserved proteins (30–34).

The *C.elegans* heterochronic gene *lin-14* generates a temporal gradient of the LIN-14 and LIN-28 proteins to control stage-specific patterns of cell lineage during development. Down-regulation of LIN-14 is mediated by the lin-14 and lin-28 3′-untranslated region through interactions with *lin-4* RNAs of 22 and 61 nt (30–34).

Another heterochronic switch gene is *let-7*. Loss of its activity causes reiteration of larval cell fates during the adult stage, whereas increased *let-7* gene dosage results in precocious expression of adult fates during larval stages. *let-7* encodes a temporally regulated 21 nt RNA that is complementary to elements in the 3′ untranslated regions of *lin-14*, *lin-28*, *lin-41*,

lin-42 and *daf-12*. The sequential stage-specific expression of the *lin-4* and *let-7* regulatory RNAs trigger transitions in the complement of heterochronic regulatory proteins to coordinate developmental timing. *lin-4* and *let-7* are the only genes known to encode small RNAs that specifically regulate other genes. However, it is clear that *lin-14* is regulated by *lin-4* RNA at a step after transcription (35).

OTHER NON-CODING RNA TRANSCRIPTS

Non-coding antisense RNA has been found that overlaps with ubiquitin protein ligase (*UBE3A*) connected with Angelman syndrome, which is characterized by severe neurological features (36). It covers the 3′ half of *UBE3A* and additional sequence downstream. The 5′ end of the antisense transcript is 6.5 kb from the stop codon of *UBE3A* (37).

A novel locus in the human Prader–Willi syndrome region encodes the antisense ZNF127 AS gene (38,39).

A balanced translocation segregating with schizophrenia can disrupt the gene function. It was found that two novel genes are disrupted by translocation: DISC1, encoding a protein and DISC2, which is a non-coding RNA lying antisense to DISC1 (40).

The myoplasm is a localized cytoplasmic region that is involved in axis determination, gastrulation, muscle cell specification and the pattern of cell division during ascidian development. One-cell zygotes of the ascidian *Styela clava* contain 1.2 kb polyadenylated RNA (yellow crescent RNA), present throughout embryonic development and associated with the cytoskeleton. The *S.clava* yellow crescent RNA (Sc Yc RNA) has no long ORF. It contains a short ORF that encodes a putative peptide of 49 amino acids without significant homology to known proteins (41). It is localized in the cytoplasm and segregates to the larval muscle cells during cleavage. Probes containing the 3′ region of Yc RNA were used to identify maternal Yc-related RNAs. A cDNA clone encoding the ascidian Proliferating Cell Nuclear Antigen (PCNA) has a long 3′ untranslated region containing a 521 nucleotide segment with antisense complementary to the part of the 3′ region of Yc RNA. This suggests that differential cell proliferation in the embryo may be limited by localization of maternal PCNA in RNA and protein (42). The role of Yc RNA may be to promote PCNA mRNA degradation in the myoplasm.

ANTISENSE PLASMID RNAs

There is a great number of genes that mediate programmed cell death in bacteria. This function has been ascribed primarily to their ability to mediate plasmid maintenance by killing plasmid-free cells. At the same time bacterial chromosomes encode numerous proteins that are homologous to products of the plasmid harboring killer genes. Two types of loci that mediate plasmid stabilization by post-segregational killing have been described. One is the toxin–antitoxin gene system, which encodes a stable toxin and an unstable protein antitoxin. The other is regulated by antisense RNAs. The toxins are encoded by stable mRNAs, whose translation is inhibited by unstable antisense RNAs. Most of them have been identified in prokaryotic cells, mainly in their plasmids, transposons and bacteriophages. Transient structures in RNA can be functionally important, as the final structure of an RNA often depends on a specific folding pathway determined by the RNA itself. In RNA they play a very important role in the *hok–sok* system of plasmid R1. This locus encodes a very stable mRNA, which specifies the toxic Hok (host killing) protein that can kill the cells by damaging the membrane. Translation of *hok* is regulated by Sok RNA (suppression of killing), an unstable antisense RNA of 63 nt that is complementary to the *hok* mRNA leader (43–45).

Initiation frequency of plasmid R1 is controlled by antisense RNA (CopA), which binds to the leader region of the *repA* mRNA (CopT). The CopA–CopT binding process is viewed as a series of reactions leading to progressively more stable complexes. CopA and CopT are fully complementary and both RNAs contain a major stem–loop structure, which is essential for high pairing rates. The initial step involves a transient loop–loop interaction (kissing complex) between complementary hairpin loops (46,47).

An antisense RNA, RNAI, interacting with the preprimer, RNAII, controls replication in the plasmids of the Col1 family via initial and transient base pairing between complementary loops. From crystallographic data it turned out that the stem–loop structures have melted the duplex. In the hairpin conformation the RNA oligos bind the plasmid encoding the four-helix bundle protein rop (48).

Very similar interactions are also observed in other plasmids: pnd–pndB of R483 (144) and RNA-IN/RNA-OUT of IS10 (49).

DsrA RNA

The regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12 depends on the level of an unstable positive regulator, RcsA. The amount of RcsA protein is small because of its rapid degradation and low level of protein synthesis. The latter effect is due to transcriptional silencing by the histone-like protein H-NS. A small, 85 nt DsrA RNA, activates transcription by counteracting H-NS silencing when overproduced. DsrA contains regions of sequence complementary to at least five different genes: *hns*, *argR*, *ilvIH*, *rpoS* and *rbsD*. However, it acts *in trans* by RNA–RNA interactions with only two different mRNAs: hns and rpoS. H-NS is a major nucleoid-structuring, histone-like protein responsible for the silencing of a number of bacterial genes, and RpoS(G·) is the stationary phase and stress response sigma-factor of RNA polymerase. DsrA antagonizes HN-S function by decreasing the level of H-NS protein in the cell and increasing that of RpoS, especially at low temperature. It has opposite effects on these two targets, both mediated by RNA, with global regulatory consequences for the transcriptional state of the cell. Structure predictions using thermodynamic calculations suggest that DsrA consists of three stem–loop structures, the last one being the transcription terminator of DsrA. The complementary region, in *hns*, in the center of the molecule, resides within the predicted second stem–loop but in rpoS it occupies the predicted first hairpin and the base of the second stem. Footprinting in the presence or absence of hns RNA *in vitro* shows distinct DsrA–rpoS interaction involving stem–loop 1, whereas a two-part DsrA–hns pairing involves stem–loop 2. Double strand formation between DsrA and the two regions of *hns*, near the 5′ and 3′ ends of its coding region, results in a contiguous coaxial stack, looping out the middle part of the hns

mRNA, which is then exposed to nucleases. These interactions are proposed to circularize hns mRNA and provide a structural basis for DsrA activity at hns (50).

lbi RNA

The lbi (*l*ipopolysaccharide *b*iosynthesis *i*nterfering) RNA of phage Acm1, a non-translated RNA species of 97 nt, affects the biosynthesis of the O-specific polysaccharide of lipopolysaccharide in various *E.coli* strains and downregulates synthesis of a D-glucan component of the O-specific polysaccharide in *Klebsiella pneumoniae* serotype O1. lbi RNA consists of two consecutive stem–loop structures. The 5′-proximal hairpin–loop function is a key structural element in the mechanism leading to the inhibition of D-glucan biosynthesis due to its antisense interactions with cellular target RNAs (51).

THE DATABASE

The database provides access to the published ncRNAs. The files contain the nucleotide sequences as well as other information in EMBL format. The primary structures are available from a hypertext list for each RNA. All data can be retrieved as separate files. Any suggestions are welcome.

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