Arina Dana Omer

Department of Biochemistry and Molecular Biology
University of British Columbia
2146 Health Sciences Mall
Vancouver, British Columbia, Canada V6T 1Z3
Phone: (604) 822-7582
E-mail:arina@interchange.ubc.ca

To: Yves Brun,
Systems Biology/Microbiology Faculty Search
Department of Biology, Indiana University
Jordan Hall 142, 1001 E 3rd Street, Bloomington, IN 47405-7005

Dear Dr. Brun,

Please accept this letter and enclosed curriculum vitae, in application for the position of Assistant Professor in your department, as listed on the Science Career web site.

I hold a Ph.D. in Biochemistry and Molecular Biology and I am an instructor in the Department of Biochemistry and Molecular Biology at the University of British Columbia.

Over the past ten years, my research has centered on the study of archaeal organisms using the hyperthermophilic *Sulfolobus solfataricus* as model. My investigations of protein-RNA complexes that mediate gene function at post-transcriptional level, allowed me to identify unexpected links between distinct pathways of the archaeal RNA metabolism.
Some of the highlights of my work are summarized below, in:

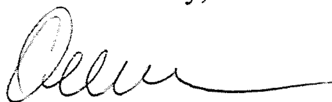
- The identification and characterization of small nucleolar-like RNAs (sno-like RNAs) in Archaea. (*Omer et al., Science 288, 517, 2000)*
- The development of the first *in vitro* system to dissect the mechanism RNA directed-post-transcriptional methylation of RNA targets. (*Omer et al., Proc. Natl. Acad. Sci. USA, 99, 5289, 2002*)
- The identification of novel classes of non coding RNAs with a broad range of regulatory functions. (*Zago et al., Mol Microbiol. 55, 1812, 2005)*

My teaching ability has developed through my experience as an instructor at the University of British Columbia and as a supervisor of my own research group.

Combining a strong interest in the pursuit of scientific discovery with the enthusiasm of sharing my knowledge through teaching, I am confident that I can make a valuable contribution to the prestige of your department.

Thank you for your consideration and I look forward to hearing from you.

Yours sincerely,



Arina Omer

Teaching interests

I currently teach the 301 Biochemistry laboratory in the Biochemistry and Molecular Biology Department, UBC. The course introduces to undergraduate students the basic biochemical and molecular biology approaches to study fundamental cellular processes. In the lecture component of this course, students are taught the biochemical/biophysical principles underlying practical laboratory procedures. In the laboratory section, students gain experience in executing laboratory experiments, writing laboratory reports and critically reading research articles. During the past year my aim was to diversify the topics covered in this course such that students are exposed to the most prevailing techniques employed any biochemical research laboratory. The topics covered in the course include: amplification of a DNA fragment using PCR; cloning, plasmid extraction and DNA fragment analysis by digestion with restriction enzymes; manual/automatic DNA sequencing and computer-aided analysis of the DNA sequence; in vitro transcription/translation; expression and initial purification of a recombinant protein; protein purification / analysis of proteins using chromatographic (gel filtration and ion exchange) and electrophoretic techniques (zone, SDS-PAGE), respectively; studies of the enzyme activity; bacterial growth and carbon source-dependent regulation; studies of nucleic acid (RNA) – protein interactions by EMSA.

As leader of my own research group I encourage and support students to develop into experienced and confident researchers. Currently, I mentor one graduate and three undergraduate students. Over the past three years I trained six students. While working with me, students learn to identify a scientific topic, to design and execute experimental procedures, to interpret and critically analyze experimental results, to draw and transmit the conclusions of their work to the scientific community in the form of oral presentations or research articles.

The courses I envision to teach in the future stem from my research interests and include topics on microbiology, nucleic acid structure and function, with emphasis on the RNA biology, functional and comparative genomics. I am enthusiastic about both laboratory-oriented and theoretical courses and think that both have an important place in the curriculum. I also enjoy one-on-one teaching and advising.

RNA chaperones and Small non coding RNAs reveal unexpected connections among distinct pathways in the archaeon *Sulfolobus solfataricus*; Perspectives to pursue in *Bacillus subtilis*

Arina Dana Omer

PREMISES

Over the past years, it became increasingly apparent that biological function is achieved through multiple interactions between proteins or proteins and nucleic acids, within dynamic macromolecular machines that exchange components and disintegrate cyclically, throughout the life time of any given cell. Deciphering the principles that govern assembly of macrocomplexes and their connectivity in the network of metabolic and regulatory pathways promise to answer important questions pertaining to cell function, development, evolution and disease.

Although remarkable progress has been made in mapping the molecular protein-protein interactions in model eukaryotic and prokaryotic cells, much less information has been accumulated about the involvement of RNA in the regulatory network. This changed with the discovery that RNAs lacking protein-encoding potential (termed non coding RNAs, or ncRNAs) play central roles in transcription regulation, processing and modification of ribosomal RNA, chromatin structure, telomere and transcript stability, protein export and more recently in genetic imprinting, cell differentiation and development.

The main obstacle in the characterization of novel ncRNAs is the absence of diagnostic sequence elements (like the beginning and termination signals defining ORFs) that would allow prediction of their genes by simple genome analysis. As an alternative, search programs have been developed that key on conserved sequence or structural motifs, combine these with searches for phylogenetic conservation among closely related species and ultimately, with the experimental validation (1, 2). Clues regarding the function of novel ncRNAs are often based on the observation that most act by antisense interactions with complementary RNA targets. Many naturally occurring ncRNAs exhibit either full-length complementarity, like siRNAs (small interfering RNAs) or more often, merely short stretches of complementarity to the RNA target, like spliceosomal snRNAs (small nuclear RNAs) and modification guide snoRNAs (small nucleolar RNAs) (3,4). In some instances, the hallmark feature of ncRNAs – their association with proteins, has been exploited in affinity purification and mass spectrometry studies, allowing accurate identification of individual proteins that physically interact with novel ncRNAs. As an example, these approaches have been successfully used to discover hundreds of snoRNAs in the eukaryotic nucleolus and, as demonstrated by my work, in archaeal cells (4, 5).

A major objective that emerged recently in the eukaryotic RNA research is the integration of independent functional studies into a larger framework, in the aim of characterizing links between different aspects of RNA metabolism and understanding how these contribute to the coordination of metabolic processes using the yeast model. In contrast, in Archaea, information on different RNA pathways is very scarce and efforts are primarily directed towards the characterization of RNA macrocomplexes, as prerequisite step to the detailed analysis of their network interactions. In this context, the advances made in snoRNA research in Archaea, point out intricate and unexpected connections among biological processes.

SnoRNAs are divided mainly into two distinct classes. The C/D box class of snoRNAs guides the 2'O-methyl-ether formation in ribose and the H/ACA class that guides isomerization of uridine to pseudouridine (4, 6). Both reactions occur post-transcriptionally, at specific sites in rRNA and rely on complementary base-pairing

between the antisense or guide region(s) in the snoRNA and the cognate target region in rRNA (7).

Each snoRNA family associate with specific sets of proteins and form discrete small ribonucleoprotein particles (snoRNPs) that provide structural stability to the antisense snoRNA and supply the methyltransferase or pseudouridylase activity.

It was found that, in contrast to Bacteria, archaeal genomes encode orthologs of all eukaryal snoRNA-associated proteins. For the class of C/D box snoRNAs these are: Fibrillarin, Nop5 (related to two eukaryotic proteins, Nop56 and Nop58) and the ribosomal protein L7Ae (homologue to the eukaryotic 15.5 kDa protein). My earlier work has directly demonstrated that Fibrillarin is the C/D box RNA associated-methyltransferase enzyme (8). Interestingly, the archaeal aL7Ae protein is an integral component of three functionally distinct macromolecular ribonucleoprotein complexes: the 50S large ribosomal subunit, the C/D box modification particles and as more recently discovered the H/ACA box particles. Similar to the eukaryotic model, L7Ae recognizes and binds to a characteristic RNA structure termed the kink-turn or K-turn motif and present in numerous RNAs (9). L7Ae stabilizes the k-turn RNA structure and functions as molecular RNA chaperone. My pioneering work used recombinant versions of the archaeal C/D box-associated proteins and *in vitro* transcribed C/D box RNAs, to reconstitute an sRNP core complex that possessed site specific 2'O-ribose methylation activity when provided with S-adenosyl methionine (SAM) and a short rRNA target (Figure 1)(8). We have also shown that in hyperthermophilic Archaea, tRNAs are also targets for modification by the C/D box guided machinery. Our *in vitro* methylation system has been recently expanded to explore the interaction with full length tRNA targets and we have investigated the specific guide-target interaction requirements of the reaction (10).

Although my previous work allowed assignment of novel function s to a large number of previously uncharacterized genes, has clarified mechanistic aspects of the methylation machinery and has demonstrate that the archaeal system is an excellent model to study the structure and function of the RNA-protein complexes, several fundamental questions remain unanswered. These include: (i) How many sno-like RNAs or in general ncRNAs are present in Archaea and in particular, in *Sulfolobus solfataricus*? (ii) Do sno-like RNAs play additional regulatory roles in mRNA stability, translation or other biological processes? (iv) What mechanisms are used by Archaea to wire the coordinated function of various RNA pathways?

RESEARCH PLAN

1. FUNCTIONAL CHARACTERIZATION OF NOVEL SMALL RNAs ASSOCIATED TO THE RIBOSOMAL PROTEIN L7AE, IN THE ARCHAEON *S. solfataricus*.

Immunoprecipitation studies performed in my lab, resulted in the observation that the core protein aL7Ae associates with a larger and more heterogeneous class of RNAs (ranging in size from 50-300 nucleotides) than either aNop5 or Fibrillarin. In the attempt to identify novel archaeal sno-like RNAs and other ncRNAs that might interact with L7Ae, we constructed a specific library of cDNA sequences from *Sulfolobus solfataricus* corresponding to the RNA transcripts associated with the L7Ae protein. To our surprise,

from a total of 45 different clones only six corresponded to canonical C/D box representatives and a single clone displayed the characteristics of an H/ACA box snoRNAs (11).

Despite their lack of recognizable sequence and structural motifs, most of the reminder clones were interacting efficiently with the recombinant L7Ae in gel retardation assays, suggesting that these RNAs contain a functional K-turn binding motif. Based on this result, we define a novel class of non-coding RNAs that function in association with the L7Ae protein, but are distinct from the C/D or the H/ACA box class of modifying RNAs. Remarkably, the action span of the L7Ae protein is much larger than initially anticipated, serving as platform in the building of functionally distinct complexes, and suggesting unprecedented crosstalk between RNA-mediated pathways. This study sets the stage for further investigations aimed to define the function of these novel RNAs and their associated protein partners.

The initial characterization of these RNAs included the determination of the location with in the *Sulfolobus solfataricus* genome relative to protein coding ORFs.

RNAs encoded between ORFs: Do Archaea use an antisense-based gene silencing mechanism? In addition to the new C/D and H/ACA representatives, this group includes two unprecedented RNA species. These correspond to RNAs that display full-length complementarity to two novel canonical C/D box members. These anti-C/D box RNAs are transcribed in the opposite direction form the DNA that encodes the authentic C/D box sRNA and lack L7Ae affinity for L7Ae protein binding. Knowing that the authentic C/D box RNAs bind L7Ae, the presence of the antisense sequences in our library could be explained by direct RNA-RNA interaction between sense and antisense sequences within an L7Ae-contatining particle. At first glance this situation parallels the complex mechanism of gene silencing mediated by interfering RNAs in Eukaryotes and raises the interesting possibility that a homologous mechanism is used by Archaea to regulate gene expression and/or function. In strong support to this hypothesis comes the recent crystal structure solved for the Argonaute homologue from the archaeon *Pyrococcus furiosus* (12). This protein is the signature component of eukaryotic RNA-induced silencing complexes. Making use of the recent advances for *in vivo* probing in *Sulfolobus*, further investigations are directed to decipher the possible regulatory role of the anti-C/D box RNA for the expression, stability or function of the cognate C/D box RNA. A regulatory system for gene silencing/RNA interference in Archaea would provide novel insights into the function, biochemistry and evolution of the homologous eukaryotic system.

RNAs overlapping annotated ORFs: Is L7Ae repressing translation of certain RNA messages? Several RNAs in this group overlap the 5', 3' or internal regions of predicted ORFs. The Northern and L7Ae binding analysis results, suggest that the RNAs recovered in this group are fragments of longer mRNAs that bind directly, and probably are regulated by, the interaction with the L7Ae protein.

(A) RNAs overlapping the 5'UTR region of protein encoding genes: Does L7Ae control the transposition event in Sulfolobus?

We found a number cDNAs that contained a K-turn motif and were derived from the 5'UTR of unstable mRNAs. The most interesting of these is the mRNA that encodes the L7Ae protein. We suspect that, similar to other ribosomal proteins, L7Ae auto-regulates its own expression by blocking translation initiation on the mRNA. Mutational

and toe-printing analyses have identified the K-turn forming sequence in the L7Ae pre-mRNA. To determine the function of this regulatory element, we have constructed mutant mRNAs, in the L7Ae-binding RNA motif. Preliminary results using this and wild type mRNA in a homologous *S. solfataricus* transcription/translation system indicate an inhibitory role of L7Ae protein in mRNA translation. The analyses can be extended to other mRNAs that have a K-turn motif in the 5'UTR; knowing that many of these mRNAs encode transposase proteins I speculate involvement of L7Ae in transposition regulation.

(B) RNAs overlapping the 3'UTR region of protein encoding genes: Is L7Ae implicated in the mechanism of Seleno-cysteine insertion in Archaea?

This subdivision includes RNAs fragments encoded in 3'-end of ORFs, overlapping the coding and the 3'-UTR, respectively. Two of these RNAs interact efficiently not only with the L7Ae but strikingly also with the other C/D box specific proteins, a Nop5 and Fibrillarin. Interestingly, the L7Ae binding site (K-turn structure), as confirmed by mutation, overlaps the ORF termination codon. This finding suggests a role of the L7Ae protein in the translation termination event, either by promoting mRNA release and translation stop, or more intriguingly, by masking the termination codon and therefore preventing ribosome release. The second hypothesis has similarities to the mechanism used by eukaryotic organisms to incorporate the rare amino acid Seleno-Cysteine into specific polypeptides. It is demonstrated that the presence of a typical secondary structure (SECIS element) in the 3'-end of the mRNA, is recognized first by a specific SECIS-binding protein and is followed by the binding of the ribosomal protein L30 causing the translating ribosome to pause until a Se-Cys-tRNA is charged onto the ribosome (13). As a result the UGA codon that usually signifies termination of the reading frame is in some cases read as Se-Cys. This argument is strengthened by the observation that the eukaryotic SECIS-element contains a K-turn structure that mediates L30 protein binding. The prospect that Archaea evolved the same L7Ae protein to accomplish Se-Cys incorporation enlarges the spectrum of functions associated with this protein.

(C) 7S RNA fragments: Is L7Ae a component of the signal recognition particle?

The most abundant representative in the L7Ae cDNA library corresponds to a fragment of the 7S RNA, the component of the signal recognition particle (SRP). Although this fragment displays no binding affinity for the L7Ae protein, we found that the full-length 7S RNA contains a highly specific K-turn motif that mediates L7Ae binding. The SRP is an evolutionarily conserved ribonucleoprotein complex responsible for the co-translational targeting of secreted proteins to membrane-bound SRP receptors. The role of this complex could be divided in three main activities: i) recognition and binding of the peptide signal sequence as this emerges from the translating ribosome, ii) temporary elongation arrest of the peptide chain and iii) docking to the membrane – bound SRP receptor and transfer of the peptide/ribosome complex to specific channels for protein translocation (14).

Based on sequence and functional homology, only two of the six protein components present in eukaryotes, have been characterized in the archaeal SRP, rising questions about the composition of the functional particle in Archaea (15). In light of the recent three dimensional reconstruction of an eukaryotic SRP within the functional

context of the stalled ribosome structure, it appears striking that SRP68/72, two of the SRP proteins with essential role in the elongation arrest activity, are absent from the archaeal SRP (16). Our finding that in *Sulfolobus* the 7S RNA can interact directly with L7Ae may compensate for the absence of SRP68/72 homologs. This hypothesis is supported by two observations. The location of the K-turn motif responsible for L7Ae binding in archaeal 7S RNA corresponds to the region involved in the interaction of eukaryotic 7S RNA with SRP68/72 heterodimer. Moreover, in the eukaryal SRP-ribosome complex, the same RNA region, protected by the SRP68/72 heterodimer and designated hinge 1, adopts a highly kinked conformation that is essential for the translation arrest activity. The hinge region involves an RNA domain that includes the long range interaction of nucleotides around positions 100 and 250 in the secondary structure of the eukaryotic 7S RNA. Our mutational analysis maps the K-turn structure at this location in *Sulfolobus* 7S RNA. Taken together, these findings reveal a possible direct role of L7Ae for the function of the archaeal SRP. We suggest that the L7Ae protein is the functional analogue of the eukaryal SRP68/72 and that binding promotes or stabilizes the kinked structure of the 7S RNA. To test this assumption, we will perform immunoprecipitation experiments using antiserum prepared either against the L7Ae or against one of the demonstrated SRP-specific proteins; the precipitates will be analyzed by Western blotting for the presence of L7Ae together with the SRP-specific proteins. Ultimately, the validation of our presumption will require the use a reconstituted translation-SRP system that promises to be available shortly in Archaea.

(D) RNAs antisense to annotated ORFs: Are there archaeal microRNAs that control gene expression in concert with the L7Ae?

There are five RNAs in the library that are encoded on the opposite strand of various predicted ORFs, predominantly encoding transposases. Two are complementary to the 5'-end, two are complementary to the 3'-end, and one is complementary to the central part of the ORF. The L7Ae protein has affinity for the 3'-antisense RNAs, but failed to interact with either the 5'- or the internal antisense RNAs. In prokaryotes, the regulation of gene expression mediated by antisense RNA is well documented but the detailed mechanism underlying the reaction and the protein components required in the system remain largely obscure. *In vitro* transcription/translation systems have been developed for *Sulfolobus* and represent a powerful tool for studying aspects of gene regulation *in vitro*. I will take advantage of these systems and test the ability of individual antisense RNAs to affect the mRNA stability and translation efficiency.

2. IDENTIFICATION AND CHARACTERIZATION OF ncRNAs THAT ASSOCIATE WITH THE SM-LIKE PROTEINS IN *S. solfataricus*.

Sm and Sm-like (Lsm) proteins represent a large family of RNA-binding proteins found in all domains of life and involved in a number of essential RNA-processing pathways. In Eukaryotes, Sm proteins are key players in pre-mRNA splicing, histone mRNA processing, telomere replication and mRNA degradation (17). In Bacteria, the Sm homologue is the Hfq protein (Host factor for Qbeta phage RNA replication). The importance of Hfq, as a global regulator of gene expression, became increasingly apparent in a variety of vital physiological processes that includes oxidative stress response, bacterial virulence, bacteriocin production and nitrogen fixation (18). Similar to the eukaryotic Sm members, Hfq associates with several non-coding RNAs and modulate

the stability and/or the translational efficiency of multiple mRNA targets. Another similarity to eukaryotic Sm proteins is the loose sequence specificity of Hfq for its RNA substrate, including solely a preference for A/U rich RNA tracks, as identified in 35 non-coding RNAs that associate with Hfq (19).

All members of the Sm/Lsm/Hfq protein family form hexa- or heptamers rings in Bacteria and in Eukaryotes, respectively. In active form, two such multimeric rings associate leaving a central cavity, where the interaction with the RNA substrate occurs (17).

Database searches of the available genomic sequences identified Sm/Lsm homologues in all Archaea and the crystal structure of one such particle has been solved (20). However, unlike the case in Bacteria or Eukaryotes, the identity of archaeal RNA substrates bound by Lsm proteins remains largely unknown.

Recent efforts in my lab are aimed at the identification of RNAs targeted by Lsm proteins, in *Sulfolobus*. In this genus, two Sm-like homologs have been identified and designated SM1 and SM2. We generated anti SM1-polyclonal antibodies and used them in immunoprecipitation experiments. The co-immunoprecipitated RNAs were purified, radioactively labelled and gel separated, allowing visualization of numerous RNAs ranging from 25 to more than 100 nucleotides. Preliminary analysis of the first 30 entries from the SM1 associated RNA library has revealed the presence of three categories of RNAs: longer mRNAs fragments, stable known ncRNAs and novel ncRNAs whose genes are located between predicted ORFs. One very interesting finding is the detection of two ncRNAs in both the L7Ae- and in the SM1-associated library. Efforts are now directed in my lab to delineate the RNA binding site(s) recognized by SM1 and to explore the possibility of interaction between SM1 and the L7Ae protein. Additionally, the availability of specific antibodies against the SM1 protein allows the identification of other proteins that may physically interact with SM1in *Sulfolobus*. Further comparative sequence analysis will be used to detect homologous SM1-specific RNAs in other sequenced archaeal genomes and to define the consensus RNA motif recognized by archaeal Sm-like proteins.

3. FUNCTIONAL CHARACTERIZATION OF A PREDICTED L7Ae HOMOLOGUE ENCODED BY B*acillus subtilis*

Phylogenetically, the archaeal L7Ae protein belongs to a larger family of proteins that includes other ribosomal proteins (human ribosomal proteins S12 and yeast ribosomal protein L30) as well as several non-ribosomal proteins of which one is an uncharacterized protein from the gram positive *Bacillus subtilis* (Bsu). Based on the RNA chaperone function demonstrated for the archaeal L7Ae, we infer that the authentic L7Ae homologue, if present in *Bacillus subtilis*, should function in association with non-coding RNAs.

We have expressed and generated polyclonal antibodies against the L7Ae homologue from *Bacillus subtilis*. Immunoprecipitation experiments are performed in the laboratory in the aim to access and characterize novel families of non-coding RNAs in *B. subtilis*. This technique proved to be a highly powerful tool allowing enrichment of specific ncRNAs that functionally interact with a given antigen, as we have previously demonstrated by the characterization of archaeal C/D box methylation guides and of the recent ncRNAs set associated to L7Ae. Preliminary results indicate that several small

RNA species, varying in size from 40 to 130 nucleotides, become enriched in anti-Bsu L7Ae precipitates. Following cloning and sequencing of the entries in the Bsu L7Ae library it will be possible to define the characteristic features of the associated RNAs. These features will serve as criteria to retrain existing search bioinformatics programs and will allow expanding the collection of ncRNAs in identified in bacterial genomes.

Summary: The immediate aim of this research is to complete the characterization and to understand the correlation between the structure and the function of the novel ncRNAs that can be detected in functional screens. The screens use antibodies directed against proteins with demonstrated global RNA chaperone activity; as such, the libraries are essentially uncontaminated by non specific sequences and virtually every clone points to novel and interesting biochemistry and physiology. Detection of L7Ae and Lsm-associated novel ncRNAs in *Sulfolobus* will greatly expand our knowledge and serve as a model for future investigations with other prokaryotic organisms. These studies will enhance our appreciation on the strategies used by hyperthermophilic Archaea to thrive and to adjust in extreme environments. In addition, because of the thermostability of its proteins and RNP complexes, *Sulfolobus* has proven to be an excellent model for understanding complex and poorly understood eukaryotic processes as exemplified by our characterization of archaeal C/D box RNP complexes. Every indication suggests that our current understanding of the ncRNA world in prokaryotic systems is only a small part of a much larger picture that is likely to penetrate into virtually every aspect of microbial physiology.

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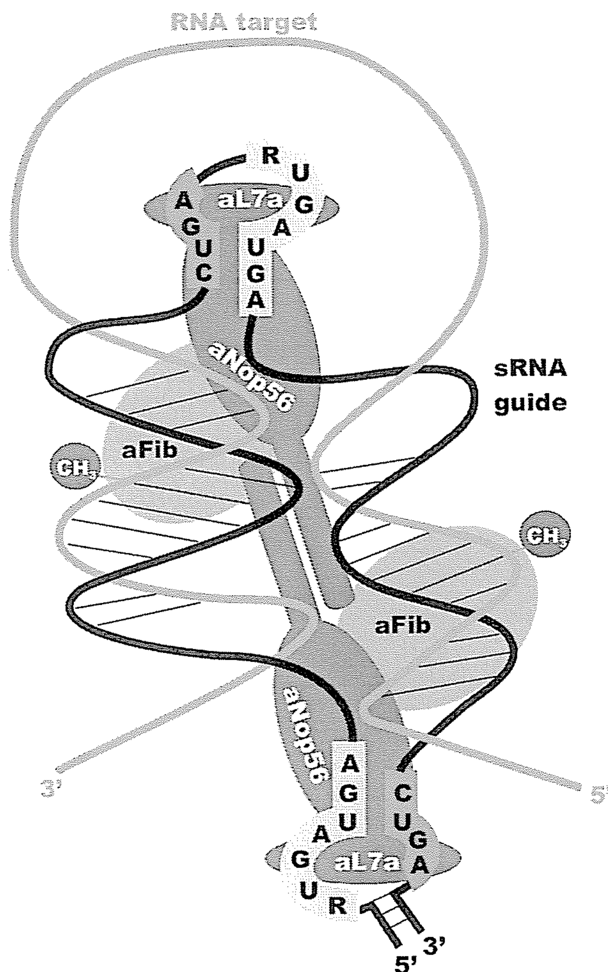

Figure 1. Features of archaeal C/D box RNA guides and their RNA targets. (A) A cartoon of the predicted structure of an archaeal, double guide C/D box sRNP complex is shown. The sRNA (dark line) contains two consensus sequence motifs designated C/D boxes that form K-turn folds at the end and in the middle of the molecule. These folds serve as binding sites for the nucleation protein aL7Ae. A dimer of Nop5/Fbrillarin binds symmetrically to the structure. The target RNA (light grey line) is complementary to the guide regions of the sRNA that are located immediately upstream of the respective D boxes. Methylation is directed to the nucleotides in the target RNA that forms a Watson Crick base pair five nucleotides upstream of the start of one of the D boxes. This is the "N-plus five" rule. The Fibrillarin enzyme uses S-adenosylmethionine as substrate to catalyse the methyl transfer.