sRNATarBase: A comprehensive database of bacterial sRNA targets verified by experiments

YUAN CAO,[1,2,3] JIAYAO WU,[1,3] QIAN LIU,[1] YALIN ZHAO,[1] XIAOMIN YING,[1] LEI CHA,[1] LIGUI WANG,[1] and WUJU LI[1]

[1]Center of Computational Biology, Beijing Institute of Basic Medical Sciences, Beijing 100850, China
[2]Department of Clinical Laboratory, The 90th Hospital of Jinan, Jinan, Shandong 250031, China

ABSTRACT

Bacterial sRNAs are an emerging class of small regulatory RNAs, 40–500 nt in length, which play a variety of important roles in many biological processes through binding to their mRNA or protein targets. A comprehensive database of experimentally confirmed sRNA targets would be helpful in understanding sRNA functions systematically and provide support for developing prediction models. Here we report on such a database—sRNATarBase. The database holds 138 sRNA–target interactions and 252 noninteraction entries, which were manually collected from peer-reviewed papers. The detailed information for each entry, such as supporting experimental protocols, BLAST-based phylogenetic analysis of sRNA–mRNA target interaction in closely related bacteria, predicted secondary structures for both sRNAs and their targets, and available binding regions, is provided as accurately as possible. This database also provides hyperlinks to other databases including GenBank, SWISS-PROT, and MPIDB. The database is available from the web page http://ccb.bmi.ac.cn/srnatarbase/.

Keywords: sRNA; sRNA targets; database; experimental supports

INTRODUCTION

Bacterial sRNAs are an emerging class of small regulatory RNAs, 40–500 nt in length, which play a variety of important roles in many biological processes through binding to their mRNA or protein targets. These processes include regulating the expression of outer membrane proteins (Guillier and Gottesman 2006; Valentin-Hansen et al. 2007), iron homeostasis (Massé et al. 2005, 2007; Vecerek et al. 2007), quorum sensing (Lenz et al. 2005; Tu and Bassler 2007), and bacterial virulence (Romby et al. 2006; Toledo-Arana et al. 2007). For example, it has been found that *MicF*, a 93-nt sRNA, can inhibit the expression of *OmpF*, an outer membrane protein (Axmann et al. 2005; Vogel and Papenfort 2006; Prévost et al. 2007; Urban et al. 2007; Song et al. 2008; Desnoyers et al. 2009; Papenfort et al. 2009). Although the functions of some sRNAs have been obtained, there are still many sRNAs with functions waiting to be elucidated. Additionally, more sRNAs have gradually been found using high-throughput experimental technologies and bioinformatics methods (Livny et al. 2006, 2008; Pichon and Felden 2008; Huang et al. 2009; Sharma and Vogel 2009; Backofen and Hess 2010). Determining the functions of bacterial sRNAs will become an important part of sRNA biology.

Bacterial sRNAs can be divided into two classes according to their mode of action (Vogel and Wagner 2007). The first class bindings to protein targets and thereby modifies the activity of their target proteins, while the second class binds to the mRNA targets and regulates expression or stability of their target genes at the post-transcriptional level. Therefore, identification of sRNA targets is very important in determining sRNA functions. Additionally, according to the gene positions of bacterial antisense sRNAs and their targets (Wagner 2009), the sRNAs can also be categorized: *cis*-encoded sRNAs contain an overlap between the antisense RNA gene and the target gene, and in *trans*-encoded sRNAs, the antisense RNA gene is separate from the target gene.

In their recent review, Vogel and Wagner (2007) systematically summarized the experimental and bioinformatics approaches for the discovery and validation of sRNA targets. Although sRNA targets should be finally tested by experiments, computational methods still provide a time-saving and less labor-intensive way to identify sRNA targets. For example, in a recently published paper (Richter et al. 2010),

[3]These authors contributed equally to this work.

Reprint requests to: WuJu Li, Center of Computational Biology, Beijing Institute of Basic Medical Sciences, Taiping Road 27, Haidian District, Beijing, 100850, China; e-mail: liwj@nic.bmi.ac.cn; fax: 86 10 68213039.

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Richter and coworkers applied IntaRNA (Busch et al. 2008), a model for prediction of sRNA targets, to identify mRNA targets for the sRNA *Yfr1* in the genome *Prochlorococcus* MED4, which contains more than 1700 mRNA-encoding genes. Initially, the program IntaRNA was used to find the candidate targets for the sRNA *Yfr1*. Then, the top 10 candidate targets were tested using a GFP-reporter system, and two targets were found. Without a prediction model, however, a large number of experiments would need to be carried out for the identification of *Yfr1* targets. This example shows that prediction models for sRNA targets play a key role in elucidating sRNA functions.

Several prediction models have been developed (Zhang et al. 2006; Busch et al. 2008; Tjaden 2008). In principle, the process of developing models is to first extract the rules from a training data set composed of known sRNA targets and then to apply the rules to predict sRNA targets for experimental validation. Therefore, from a machine-learning point of view, it would be better to have as many samples as possible in the training data set. However, the model IntaRNA, containing the largest number of samples in the training data set among the aforementioned models, had only 18 samples for parameters optimization (Busch et al. 2008). Therefore, it is essential to collect as many sRNA targets as possible to construct a comprehensive database of sRNA targets. This will not only be helpful in understanding sRNA functions systematically, but will also provide a benchmark data set for constructing prediction models.

When we initiated this project, we also noticed two other databases, RegulonDB (Gama-Castro et al. 2008) and sRNAMap (Huang et al. 2009), which include 49 and 60 sRNA–target interactions, respectively. However, the accurate binding regions between sRNAs and their targets, which play a key role in developing models, were not provided (Busch et al. 2008; Tjaden 2008). Additionally, no experimentally confirmed negative samples were included, which are necessary for the construction of prediction models, at least from a machine-learning point of view, To provide detailed information on sRNA targets, we have systematically and manually collected experimental data on sRNA–target interactions from peer-reviewed papers and developed a database for sRNA targets called sRNATarBase. The database contains 10 entries for activated targets, 128 entries for repressed targets, and 252 entries for which no interaction has been reported. The numbers of involved sRNAs, targets, and genomes are 68, 227, and 17, respectively.

RESULTS AND DISCUSSION

Construction of database

After obtaining the detailed information for each experimentally confirmed sRNA–target pair (interaction or noninteraction), we constructed a table file in CSV format that was then imported into a MySql database. To ensure that the data were imported accurately, we performed quality control several times. The template file in CSV format can be downloaded from the database homepage. The Web interface for the database was designed in PHP language.

Database access

Users can search sRNA targets through the Web interface (Fig. 1A) using the following fields: ID, organism, sRNA name, target name, target type, regulation, direct support, indirect support, PMID, or a combination of fields. The meaning of each field is as follows:

ID

Each database entry is assigned an ID, defined by "SRNAT," followed by a five-digit number. For example, "SRNAT00001" stands for the interaction of sRNA *DsrA* and mRNA target *hns*. There are 390 entries in the database. The IDs were named SRNAT00001, SRNAT00002, . . ., SRNAT00390.

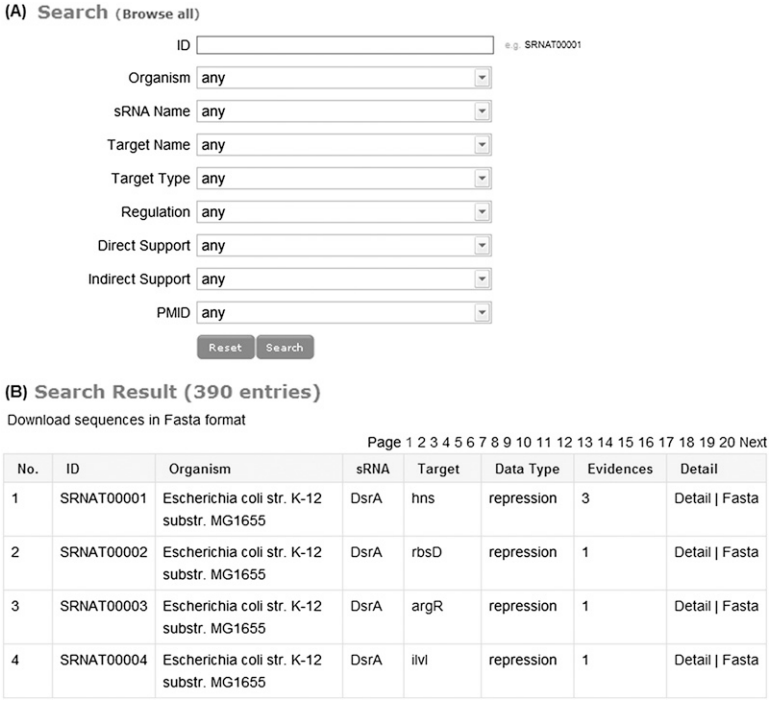


FIGURE 1. (*A*) The query interface for the database, from which users can search sRNA targets using a particular field or a combination of fields. (*B*) The part of the search results using default values, from which users can check detailed information on sRNA targets, such as sRNA sequence, target sequence, and supporting experimental protocols by clicking the hyperlinked "Detail."

Organism

There are 17 genomes involved. The distribution of the database entries across the 17 genomes is given in Table 1. The *Escherichia coli* str. K-12 genome contains the maximum number of entries, 230.

sRNA name

The number of involved sRNAs is 68. Users can select any one of them to search the database. For example, if sRNA *RybB* is selected and other fields use the default values, then five entries—SRNAT00038, SRNAT00040, SRNAT00041, SRNAT00268, and SRNAT00320—will be obtained.

Target name

The number of involved targets is 227. Users can select any one of them to search the database. For example, if target mRNA *OmpF* is selected and other fields use the default values, one interaction entry, SRNAT00014 (MicF-OmpF), and eight no-interaction entries will be obtained.

Target type

Two kinds of sRNA targets, mRNAs and proteins, are provided in our database because present studies show that sRNAs function primarily by binding mRNAs or proteins. The numbers of entries for mRNA and protein targets are 379 and 11, respectively.

Regulation

Three regulation types are provided for the interaction of sRNA–mRNA or protein targets, including repression, activation, and no interaction. ''Repression'' means that the expression of mRNA targets was repressed, the stability of mRNA targets was decreased, or the activity of protein targets was down-regulated (Altuvia et al. 1998; Romeo 1998; Massé et al. 2005). ''Activation'' indicates that the expression of mRNA targets was activated, the stability of mRNA targets was increased, or the activity of protein targets was up-regulated (Majdalani et al. 1998; Prévost et al. 2007). ''No interaction'' shows that the expression level of mRNA targets, the stability of mRNA targets, or the activity of protein targets was basically not affected by sRNAs.

Direct support

Only those targets that were confirmed by at least one of the following experimental protocols (Vogel and Wagner 2007; Frohlich and Vogel 2009; Sharma and Vogel 2009): point mutation of mRNA, point mutation of sRNA, mRNA reporter gene, sRNA reporter gene, sRNA deletion, sRNA knockout, and in vitro footprinting, were considered to be true targets. Because these protocols provided direct support for sRNA–target interaction, these data can be applied to construct models for the prediction of sRNA targets.

TABLE 1. Distribution of sRNATarBase entries across 17 genomes

Genome	NCBI code	Number of papers	Number of entries
E. coli str. K-12 substr. MG1655	NC_000913	60	230
E. coli O127:H6 str. E2348/69	NC_0011601	4	8
Salmonella enterica subsp. *enterica* serovar *Typhi* str. CT18	NC_003198	5	1
S. enterica subsp. *enterica* serovar *Typhimurium* str. LT2	NC_003197	12	53
Staphylococcus aureus subsp. *aureus* str. MW2	NC_003923	1	1
S. aureus subsp. *aureus* str. NEWMAN	NC_009641	2	2
Vibrio cholerae O1 biovar El Tor str. N16961	NC_002505	5	23
	NC_002506		
Vibrio harveyi ATCC BAA-1116	NC_009783	2	4
	NC_009784		
Pseudomonas aeruginosa PAO1	NC_002516	3	30
Pseudomonas fluorescens	DQ_137846	1	1
P. fluorescens SBW25	NC_012660	1	4
P. fluorescens strain CHA0	NC_002516	1	2
Neisseria meningitidis MC58	NC_003112	1	5
Azotobacter vinelandii DJ	NC_012560	2	13
Prochlorococcus marinus subsp. *Pastoris* str. CCMP1986	NC_005072	1	6
Listeria monocytogenes EGD-e	NC_003210	1	5
Bacillus subtilis subsp. *subtilis* str. 168	NC_000964	2	2
Total	17	91	390

For each genome, related information is provided, including NCBI code, the number of associated papers, and the number of database entries. Since the papers, with PubMed IDs 15242645, 16359331, 17264113, 17608792, 18399940, 18619465, and 18953042, provide the information for two genomes, and the papers with PubMed IDs 17383221, 17427289, and 19333007 provide information for three genomes; these papers were counted two or three times, respectively. Thus, the total number of involved papers is 91.

Indirect support

Many experimental protocols, such as 2D-PAGE and microarray, can be used to detect gene expression level. These can also be used to decipher possible sRNA targets. However, the distinction between primary targets and secondary targets cannot be determined by gene expression only (Vogel and Wagner 2007). Therefore, all experimental protocols listed in this search field provide only indirect support for sRNA targets.

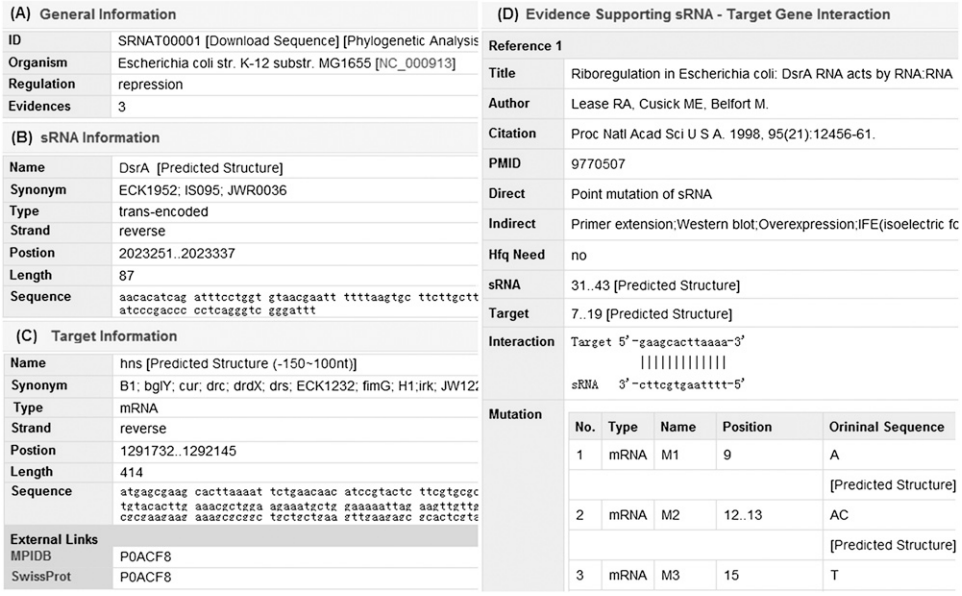
PMID

PMIDs are the indexes of papers stored in the PubMed database. Here we provide a PMID field for a user to quickly determine which entries in the sRNATarBase are associated with the given PMID. For example, one entry, SRNAT00001, can be obtained by setting up the PMID field as ''10954740'' and other fields by their default values. The number of involved papers is 91. The other role of the PMID is to let a user easily find the papers related to some particular database entry.

If a user searches the database using default values, all entries will be displayed and sorted by sRNA target ID (Fig. 1B). Additionally, all database entries can be downloaded by clicking ''Download sequences in Fasta format'' under ''(B) Search Result (390 entries)'' (Fig. 1B). Each entry in Fasta format is depicted by a description line containing the sRNA target ID, organism name, sRNA name, target name, and regulation type separated by the sign ''|'', followed by sRNA sequence, hyphens ''--'', and target sequence. The detailed information as follows for each entry can also be obtained by clicking on the hyperlinked ''Detail'' (Fig. 1B).

First, general information is provided (Fig. 2A), including entry ID, links to BLAST-based phylogenetic analysis, and GenBank for genome information (Benson et al. 2009) and regulation type. According to Zhang et al. (2006), Busch et al. (2008), and Tjaden (2008), a particular sRNA–mRNA target interaction is often conserved in closely related bacteria. To provide comprehensive support for the sRNA research community, we present the BLAST-based procedure for conservation analysis of sRNA–mRNA target interaction. When a user clicks the hyperlinked ''Phylogenetic analysis,'' a new web page will be displayed (Fig. 3). This page contains the corresponding entry ID, genome name, sRNA information, mRNA information, options for the user to choose closely related bacteria, and parameters for BLAST comparison. Here we want to emphasize that the sequences for both sRNA and mRNA cannot be changed, but the user can set up the range for BLAST analysis. Because the binding region on the mRNA target is often located in the flanking region around the initial start codon, we include the upstream 150 nt of the mRNA target. Additionally, the maximum allowed number of closely related genomes is 20. Finally, the parsed BLAST results will be provided for each selected genome, from which the user can check the upstream, downstream, and overlapping genes of the entry.

Second, sRNA information is provided (Fig. 2B), which involves name, link to the predicted secondary structure, type (*trans*-, *cis*-encoded, or protein binding), strand, position on the genome, and sequence.



(A) General Information

ID	SRNAT00001 [Download Sequence] [Phylogenetic Analysis
Organism	Escherichia coli str. K-12 substr. MG1655 [NC_000913]
Regulation	repression
Evidences	3

(B) sRNA Information

Name	DsrA [Predicted Structure]
Synonym	ECK1952; IS095; JWR0036
Type	trans-encoded
Strand	reverse
Postion	2023251..2023337
Length	87
Sequence	aacacatcag atttcctggt gtaacgaatt ttttaagtgc ttcttgctt atcccgaccc cctcagggtc gggatttt

(C) Target Information

Name	hns [Predicted Structure (-150~100nt)]
Synonym	B1; bglY; cur; drc; drdX; drs; ECK1232; fimG; H1;irk; JW12
Type	mRNA
Strand	reverse
Postion	1291732..1292145
Length	414
Sequence	atgagcgaag cacttaaaat tctgaacaac atccgtactc ttcgtgcgc tgtacacttg aaacgctgga agaaatgctg gaaaaattag aagtttttg ccccaagaag aaagccccgc tcctcctgaa gttgaagaac gcactcgtg
External Links	
MPIDB	P0ACF8
SwissProt	P0ACF8

(D) Evidence Supporting sRNA - Target Gene Interaction

Reference 1	
Title	Riboregulation in Escherichia coli: DsrA RNA acts by RNA:RNA
Author	Lease RA, Cusick ME, Belfort M.
Citation	Proc Natl Acad Sci U S A. 1998, 95(21):12456-61.
PMID	9770507
Direct	Point mutation of sRNA
Indirect	Primer extension;Western blot;Overexpression;IFE(isoelectric fc
Hfq Need	no
sRNA	31..43 [Predicted Structure]
Target	7..19 [Predicted Structure]
Interaction	Target 5'-gaagcacttaaaa-3' ||||||||||||| sRNA 3'-cttcgtgaatttt-5'
Mutation	

No.	Type	Name	Position	Orininal Sequence
1	mRNA	M1	9	A
				[Predicted Structure]
2	mRNA	M2	12..13	AC
				[Predicted Structure]
3	mRNA	M3	15	T

FIGURE 2. Detailed information is displayed for a particular database entry, including general information (*A*), sRNA information (*B*), target information (*C*), and supporting information (*D*). Additionally, the links to the databases NCBI (*A*,*B*,*D*), MPID (*C*), and SWISS-PROT (*C*) are provided from which further information on the sRNA and its target can be accessed.

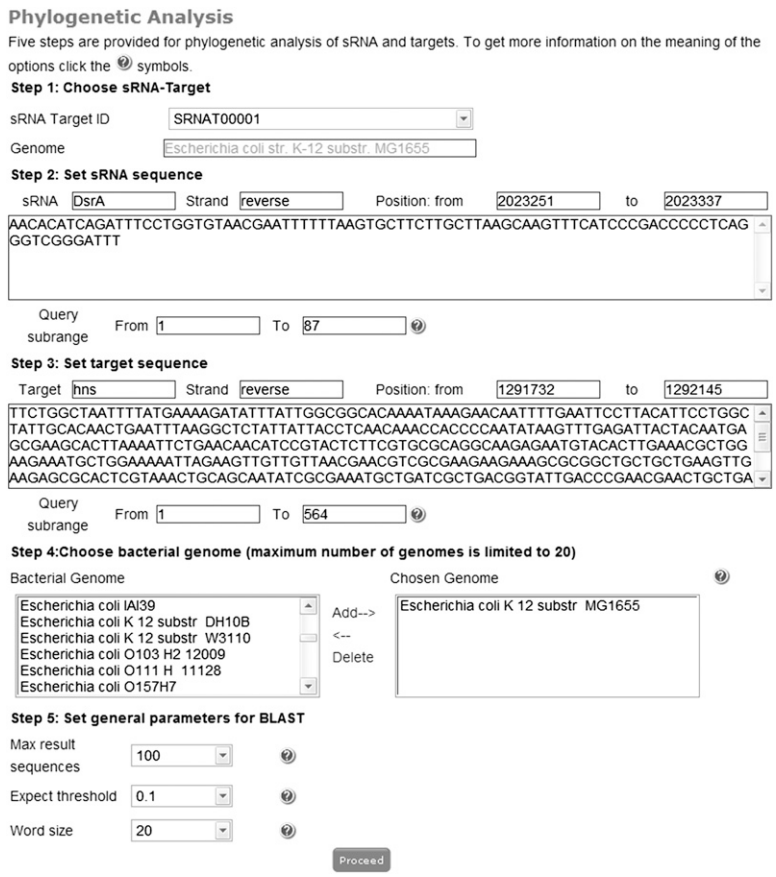


FIGURE 3. The interface of BLAST-based phylogenetic analysis of an sRNA–mRNA target interaction is provided, through which the conservation of sRNA–mRNA target interaction or their binding regions can be checked among the closely related bacteria.

Third, target information, including target name, link to its predicted secondary structure, strand, position on the genome, and external links to the databases MPIDB (Goll et al. 2008) and SWISS-PROT (UniProt Consortium 2010) is provided (Fig. 2C). Here the secondary structure of the flanking region −150–100 around the initial start codon of the target is predicted because in the sRNATarBase, of the 95 entries containing binding regions, 91 of them are located in this region.

Fourth, the evidence supporting the sRNA–target interaction is listed (Fig. 2D). This includes information on references, direct support, indirect support, binding positions, and their mapping on the predicted secondary structures, interaction regions, and available mutations. Here the RNAfold program is used to predict secondary structures of both sRNA and mRNA (Hofacker 2003), and VARNA is applied to draw RNA secondary structure. VARNA is a comprehensive and flexible tool to display RNA secondary structure (Darty et al. 2009). Many functions, such as rotation, linear drawing, and circular drawing, can be selected on the right-click menu. For example, Figure 4 shows the binding region of sRNA DsrA with its target hns on the predicted secondary structure. From Figure 4, the users can also check the binding information of the sRNA with other available targets.

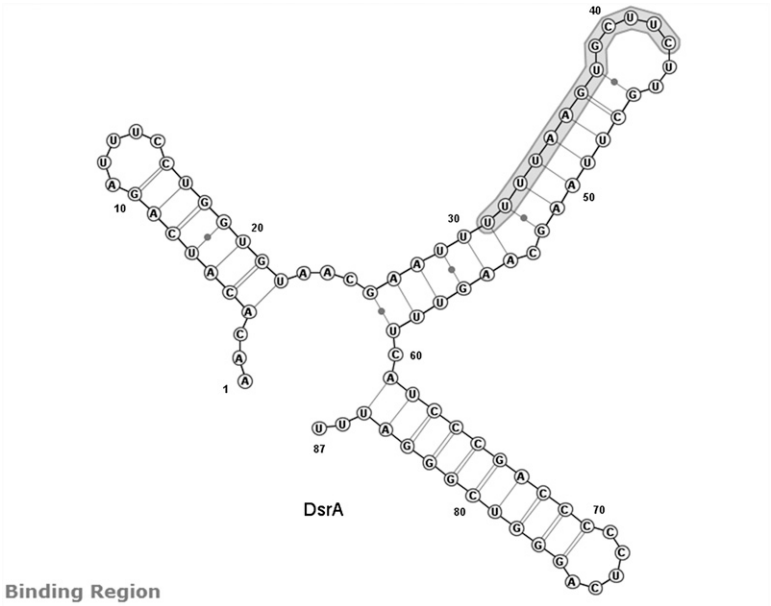
To keep the database updated for the sRNA research community, sRNATarBase allows users to submit newly identified sRNA targets to the database in two ways. The first is to enter the information for each sRNA–target entry individually in the submission form. The second is to submit many sRNA–target entries by filling in the related information in a template file in CSV format, which can be downloaded from our web page. After passing examination by the Web Administrator, the related data will be put into the database. At the same time, we will also continue to scan the literature to update the database.

Other functions

To provide better support for the sRNA research community, we also provide the following functions, including BLAST comparison, browsing and downloading the database, and prediction of sRNA targets. For BLAST comparison, the BLAST database includes all sRNA sequences from the sRNATarBase. When a query sRNA sequence is compared with the BLAST database, all hits will be listed, including the sRNA name, sRNA length, BLAST score, identity, E-value, and Link containing detailed comparison information. The database entry can be obtained by clicking the hyperlinked sRNA name. Additionally, all database entries can be browsed individually or downloaded entirely. We presently provide two formats, CSV and Fasta, for a user to download the data. And because the prediction of sRNA targets plays a key role in elucidating sRNA functions, we integrated the web server developed by our laboratory, sRNATarget, into the database interface (Zhao et al. 2008; Cao et al. 2009) for prediction of sRNA targets.

Future directions

Here we describe the database, sRNATarBase, for sRNA targets verified by experiments. Compared to other databases (Gama-Castro et al. 2008; Huang et al. 2009) in sRNA targets, the characteristics of sRNATarBase are as follows: First, our database not only contains more entries but also provides available binding regions between sRNAs and their targets as well as available mutation information. Second, we also provide no-interaction entries. From a machine-learning point of view, both positive samples



Binding Region

The same sRNA region which is basepairing to mRNA targets supported by experiments is listed below.

No.	ID	sRNA	Target	Data Type	sRNA Binding Position	Reference
1	SRNAT00001	DsrA	hns	repression	31..43	9770507
2	SRNAT00001	DsrA	hns	repression	31..54	10954740
3	SRNAT00006	DsrA	rpoS	inducement	10..32	9770508

FIGURE 4. The predicted secondary structure of sRNA *DsrA* and its binding information with target *hns*, as well as the binding region list of sRNA *DsrA* with other targets are displayed, from which the structure features of the binding regions between an sRNA and its targets can be explored.

(activation and repression) and negative samples (no interaction) are necessary, which can be used to construct models directly. In fact, we have even applied part entries of the database to develop two prediction models, sRNATargetNB and sRNATargetSVM (Zhao et al. 2008; Cao et al. 2009). The classification accuracy, sensitivity, and specificity were 93.03%, 40.90%, and 93.71% for sRNATargetNB; and 80.55%, 72.73%, and 80.65% for sRNATargetSVM, respectively. Obviously, to provide better support for the prediction of sRNA targets, the accuracy, sensitivity, and specificity should be improved. Third, BLAST-based phylogenetic analysis of sRNA–mRNA target interaction is provided; this can be used to check the conservation of sRNA–mRNA targets or their binding regions in closely related bacteria. Fourth, the mapping of binding regions on the predicted secondary structures of both sRNAs and mRNA targets is provided, which can be used to explore the structure characteristics of binding regions. In summary, the above features make sRNATarBase a comprehensive database for sRNA targets.

In the future, we will focus on three points. The first is to continue collecting sRNA targets from the literature so that more entries can be included in our database. The second is to develop more accurate prediction models using the database. The third is to incorporate the predicted targets of all known sRNAs into the database so that we can provide comprehensive support for the sRNA research community.

MATERIALS AND METHODS

To construct the sRNATarBase database, we queried PubMed using the related keywords, such as ''sRNA targets'' or ''sRNA,'' and read the resultant papers (before May 2010). Finally, 91 papers were chosen for extraction of sRNA targets, and 390 entries of sRNA–target interaction were obtained. Table 1 summarizes the distribution of these 390 entries among 17 genomes. The detailed information for these entries, such as supporting information, sRNA sequences and their secondary structures, mRNA target sequences and their secondary structures, available binding regions, and BLAST-based phylogenetic analysis, has been provided in our database.

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