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Detection of differentially expressed growth dependent noncoding RNAs in *Sulfolobus solfataricus*

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Noncoding RNA mediated gene regulation exists in all the three kingdoms of life including prokaryotes, eukaryotes and archaea. In prokaryotes, the ncRNAs typically bind to the 5' end of the messenger RNAs to facilitate its activation or repression. The repression of gene expression in eukaryotes by ncRNAs is regulated by its binding to the 3'-untranslated region. A significant amount of information is available on the ncRNA mediated gene regulation in eukaryotes and prokaryotes. However, the data on ncRNA interference mechanisms in archaea are largely based on bioinformatics predictions. More than 300 noncoding RNAs have been bioinformatically predicted to exist in the hyperthemophilic archeon *Sulfolobus solfataricus*. The main objective of this study was to ascertain the growth dependent differential expression of certain ncRNAs in *S. solfataricus*. The northern blot analysis confirms that certain ncRNAs are differentially expressed at particular phases of growth while others are constitutively expressed in all phases of growth. One of the three ncRNAs, RNA 22 was expressed constitutively whereas the other two ncRNAs, RNA 43 and RNA 115 was expressed at specific points of growth. In addition, we also attempt to predict the putative mRNAs that are targeted by specific ncRNAs. In conclusion, our study states that in term with the constitutive expression of ncRNAs 22, the predicted targets include a drug resistance transporter and transposase protein which should be down regulated during the normal growth of Sulfolobus. The second candidate, ncRNA 43 was specifically expressed at the late log phase with putative targets that includes critical metabolic proteins. The third RNA analyzed was ncRNA 115. The expression of ncRNA 115 was at the mid-log phase with the predicted target being the Translation recovery factor (TRF). These predictions with functional classifications of the mRNAs relevant in specific growth points out that ncRNAs plays significant role in gene regulation in *S. solfataricus*.

Key words: Noncoding RNA, Sulfolobus, Archaea, gene regulation.

INTRODUCTION

Control of RNA stability is routinely performed by cells to mount an effective regulation of gene expression, thereby modulating the synthesis of proteins in response to the physiological need. Non coding RNAs (ncRNAs) are present in all the three kingdoms of life. Apart from messenger RNAs (mRNAs), ncRNAs are not functionally

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translated to proteins and exert their activity at RNA level (Gerbi et al., 2003). In the Eukaryotic cells, microRNAs (miRNAs) and short interfering RNAs (siRNA) act as regulators of critical cellular processes. The siRNAs are usually derived from double-stranded RNA and function in cleavage of the target mRNA by RNA interference (RNAi) (Meister and Tuschl, 2004). In contrast, the miRNAs encoded from the genome are part of the ribonucleoprotein complexes. Specific binding of the ribonucleoprotein complexes to the 3′ untranslated region (UTR) of mRNAs leads to translational repression and/or mRNA decay (Filipowicz et al., 2008). At variance to eukaryotes, the bacterial regulation of mRNA is done by small regulatory RNAs (sRNAs) that predominantly bind to the 5′ UTR in mRNAs (Bell and Jackson, 1998). Although the clade of Archaea bacteria lacks specific nucleus and other organelles similar to prokaryotic bacteria, they show intense similarity to eukaryotes in molecular functioning such as transcription, translation and DNA packaging (Waters and Storz, 2009). Although bioinformatics analysis has revealed the presence of significant number of ncRNAs in archaea bacteria, it was only recently shown experimentally that these predicted ncRNAs could be involved in gene regulation. Recently, another study reported the presence of a large number of small RNAs in *S. solfataricus* P2 which are of approximately 20 nt in length based on deep sequencing (Xu et al., 2012). Nevertheless, the first experimental evidence of ncRNA mediated gene regulation was shown by Märtens et al. (2013). It was shown that a specific noncoding RNA 257 and its four orthologues (RNA-257$_{1-4}$) regulate the inorganic phosphate transporter protein coding mRNA (SSO1183) in the hyperthermophilic archaeon *S. solfataricus* (Märtens et al., 2013). Interestingly, the levels of RNA-257 orthologues were dependent on the amount of inorganic phosphate present in the growth media. A surplus amount of phosphate in the media leads to an increased level of RNA-257 and a reduced level of SSO1183 and vice versa in a phosphate depleted condition.

In a recent study, around 300 ncRNAs were predicted with precise Transcription Start Site (TSS) in *S. solfataricus* by the technique of whole-genome sequencing and strand-sensitive 5'-end determination (Wurtzel et al., 2010). The data obtained from the 5'-end determination approach corresponds to real start of the transcripts in the native cells. The TSS was predicted by taking into account the highest number of 5'-end reads followed by the detection of TATA motifs placed approximately 26 bp upstream of the TSS, which is a hallmark of most archaeal genes (Reiter et al., 1990; Zillig et al., 1998). Archaeal mRNAs usually have 3' UTRs of significant sizes (Brenneis et al., 2007). Interestingly, it was also reported that the ncRNA mediated gene regulation in archaea bacteria is achieved by its interaction to the 3' UTR of mRNAs, similar to eukaryotes (Tang et al., 2005). Apart from most of the available bioinformatics data on the probable interaction of ncRNAs with the 3' UTRs of mRNAs in archaea bacteria, the only study that has experimentally shown ncRNA interaction with target mRNA was the RNA-257 interaction with SSO1183 mRNA in the 3' UTR and its subsequent degradation *in vitro* (Märtens et al., 2013).

In this study we attempt to experimentally prove the existence of three ncRNAs predicted in different stages of growth of *S. solfataricus*. In addition, we also attempt to predict the putative target mRNAs of these three ncRNAs with an outlook into its possible interaction regions.

MATERIALS AND METHODS

Archaeal strains and growth conditions

The *S. solfataricus* P2 strain was used for the growth curve determination and isolation of total RNA. *S. solfataricus* P2 was grown at 75°C and pH 3.0 in Brock's medium (Brock et al., 1972). The media was supplemented with 0.2% NZamine and 0.2% D-arabinose. The culture media was inoculated with *S. solfataricus* P2 strain and was grown aerobically by shaking at 160 rpm in a rotary shaker. The samples were withdrawn for 7 days for the assessment of growth.

Isolation of total RNA and northern blotting

Total RNA from *S. solfataricus* P2 was extracted at different phases of growth corresponding to OD_{600} (0.3, 0.6, 1.0 and 1.6). The cells were pelleted at 2300 x *g* for 10 min followed by the addition of 1.0 ml Trizol (Invitrogen). The resuspended pellet in Trizol was incubated at 65°C for 10 min followed by phenol/chloroform extraction. The nucleic acids were precipitated with the addition of 96% ethanol. DNA was removed by treatment with DNase I. The concentration of RNA was determined with Nanodrop 8000. For northern blot detection of specific noncoding RNA, 1 µg of the total RNA was separated on an 8% polyacrylamide/urea gel and then transferred to nylon membranes. After UV- crosslinking, the membrane was incubated with either the [^{32}P]-5′-end labelled oligonucleotides as shown: 5′-GGCAACAACAACAGAGTGGCGGA-3′ specific for ncRNA 22, the oligonucleotide 5′-GTATGCAATAATAATAGCAGCAG-3′ specific for ncRNA 43 or the oligonucleotide 5′-GGAAAAGAAGGTGATTAGATTCAA-3′ specific for ncRNA 115. The 5S rRNA was probed with oligonucleotide 5′-CACTAACGTGAGCGGCTTAAC-3′ and served as loading control. The incubation of the membrane with the labeled primers was done at 50°C overnight. The membrane was washed following incubation and specific RNAs were detected by scanning (Typhoon 9400, Amersham) of the exposure cassette.

Target prediction of ncRNAs

Putative targets of the three ncRNAs 22, 43 and 115 (Table 1) were done by Blast analysis. The conditions for the Blast analysis is as follows: The program selection was optimized for discontinuous Mega blast. The algorithm was set for Match/Mismatch scores of 2, -3 and the Gap costs Existence: 2, Extension: 4. Following blast analysis, the predicted target sequences were scrutinized for possible interaction 'pockets' with ncRNA by IntaRNA RNA-RNA prediction tool (Busch et al., 2008). The interaction temperature was set to 75°C with a minimum 'seed pair' of 4. The best ncRNA

Table 1. Features of the studied ncRNAs.

ncRNA	Strand/orientation	Coordinates 5' end	Coordinates3' end	Length
22	+	581783	581907	124
43	+	990534	990681	147
115	-	2397330	2397165	165

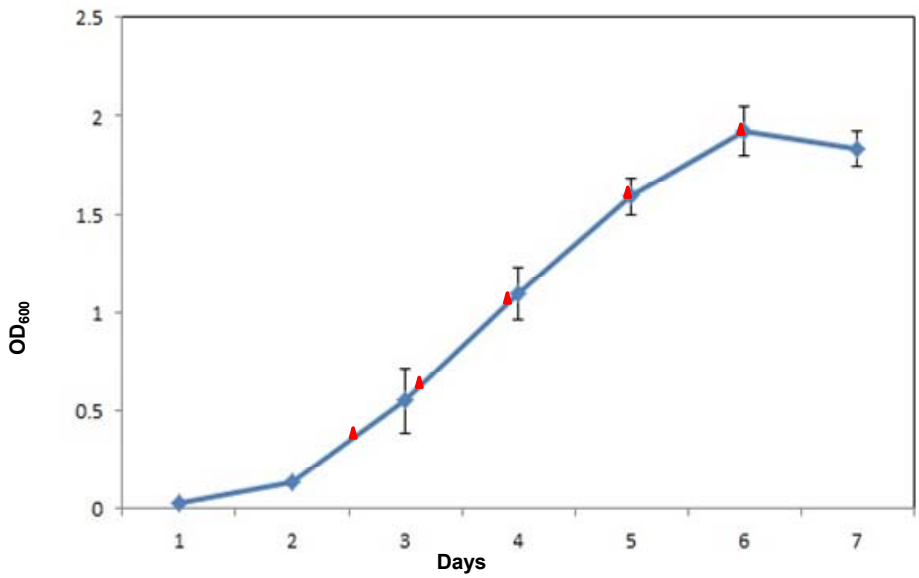


Figure 1. Growth curve of *Sulfolobus solfataricus* P2 in full growth medium. The time-points at which total RNA was extracted is shown as red triangle. The OD600 of the cultures were measured spectrophotometrically at a wavelength of 600 nm. The total RNA was extracted at OD600 of 0.3, 0.6, 1.0 and 1.6. The cell doubling time was approximately 8-10 h. The growth curve is the average of three independent experiments. The error bars shows the standard deviation.

targets were selected that had high hybridization energy between the ncRNA and the target. The interaction site of ncRNA with the target RNA was preferentially the 3' untranslated region or the internal ORF. The target genes with annotated functions were of special interest.

RESULTS AND DISCUSSION

Constitutive expression of ncRNA in *S. solfataricus* P2

In order to find the expression of specific ncRNAs predicted (Wurtzel et al., 2010), RNAs were chosen on four criteria: (a) should be of detectable length possibly larger than 100 base long, (b) ncRNAs should have a strong transcriptional start site and relative abundance as predicted by the sulfolobus transcriptome analysis software (http://www.weizmann.ac.il/molgen/Sorek/Sulfolobus_solfataricus_transcriptome/), (c) ncRNAs should not be present in multiple copies in the sulfolobus genome as seen in RNA257 (Märtens et al., 2013) and (d) should possibly interact with the 3'-UTR or internal ORF of the target RNA.

Based on these criterions we selected three potential RNAs designated as ncRNA 22, ncRNA 43 and ncRNA 115. Initially, we extracted total RNA from *S. solfataricus* P2 grown on a complete media at different phases of growth as shown in Figure 1. Total RNA was extracted until late log (OD_{600}: 1.9) phage of growth. To determine the expression of ncRNA 22, first aim was to determine the expression levels of ncRNA 22 based on the Transcripitional Start Site Mapping on the Sulfolobus transcriptome described by Wurtzel et al. (2010). A maximum read of 17915 was seen in the TSS of ncRNA 22 corresponding to the genome co-ordinate of 581783. As shown in Figure 2A, there is a strong TSS corresponding to ncRNA 22, which will be sufficient to enable its detection by northern blotting. The experimental detection of ncRNA 22 was done by the hybridization

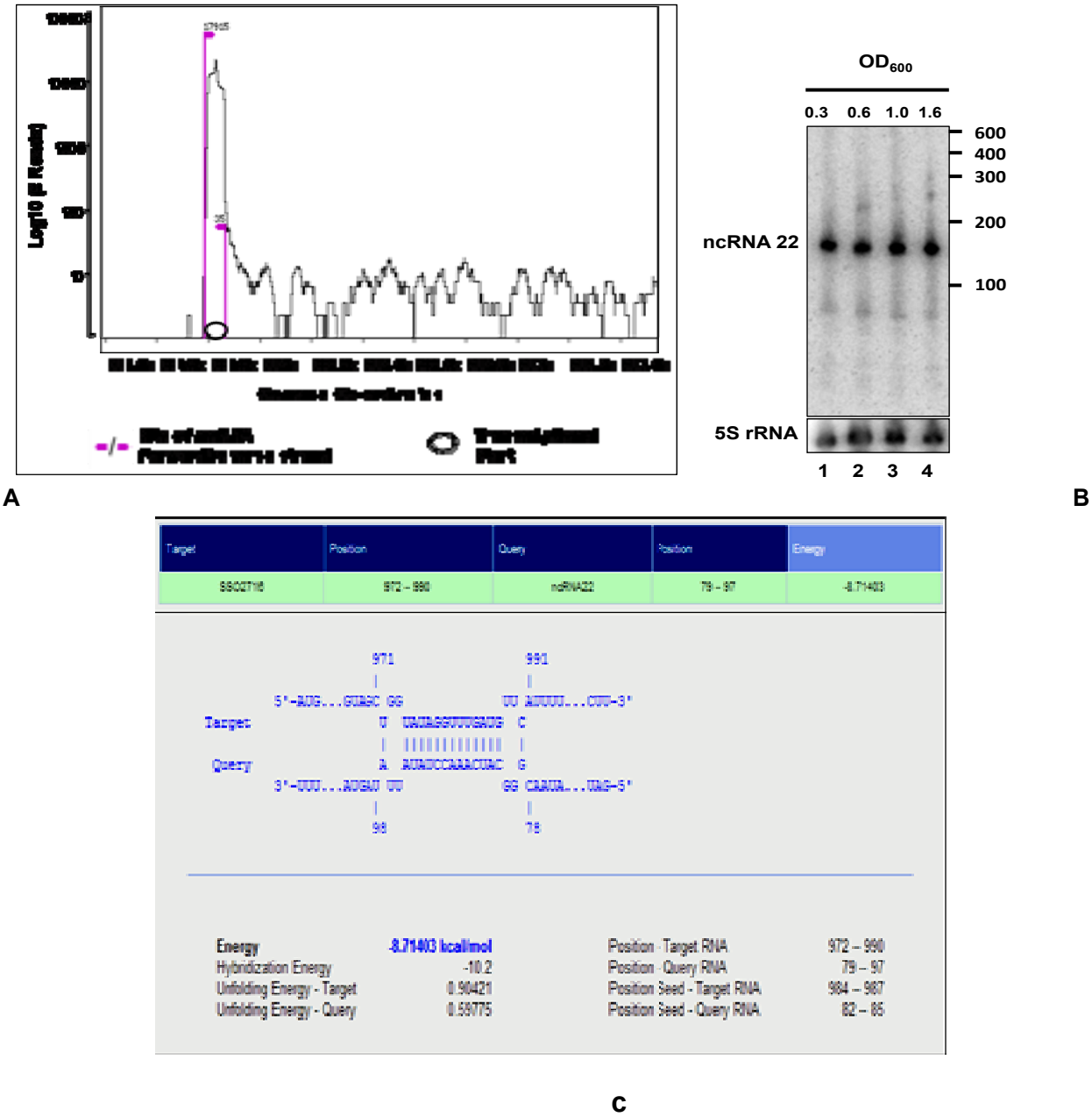


Figure 2. The transcriptional start and abundance of ncRNA22 **(A)** The transcriptional start site of ncRNA 22 is shown at position 581783 in the genome co-ordinate. The number of reads corresponding to the transcripitional start is 17915. **(B)** The constitutive expression of ncRNA in all phases of growth ranging from lag to late log phase (Lane 1-4). A strong abundant expression of the ncRNA 22 is seen. The bottom lane (5S rRNA) represents loading control. **(C)** The picture shows the hybridization of ncRNA 22 with SSO2716. SSO2716 encodes for a drug resistance transporter. The ncRNA 22 hybridizes within the ORF of SSO2716 with hybridization energy of -8.71 kcal/mol. The hybridization was done at 75°C, which is the optimal growth temperature of *Sulfolobus solfataricus* P2.

of ^{32}P labeled specific primer sequences as described above. As shown in Figure 2B, ncRNA 22 was constitutively expressed in all phases of growth ranging from early log phase to late log phase (Figure 2B: Lane 1-4). The prominent band seen on the northern blot corresponds to the predicted size of ncRNA 22. However, a lower band of around 70 nt was seen on the northern blot consistently present in all the phases of growth. These fragments to which the primer binds could be degraded fragments from the complete RNA. We cannot also exclude the possibility of a shorter transcript synthesized during transcription. Nevertheless, our

Table 2. Predicted targets of ncRNA 22.

S/N	ncRNA	ncRNA length (bases)	Target mRNA	Target mRNA length (bases)	Interaction site of ncRNA	Interaction site of Target mRNA	Hybridization energy at 75°C (kcal/mol)	Target mRNA Function
1	22	124	SSO1960	867	52-60	620-628 (internal ORF binding)	-4.4	Hypothetical Protein
2	22	124	SSO2946	726	8-14	794-800 (3' end binding)	-3.9	Unknown function
3	22	124	SSO0283	1630	49-63	1676-1690 (3' end binding)	-7.8	Putative Thermosome
4	22	124	SSO0290	1356	25-39	477-491 (internal ORF binding)	-4.6	Inorganic Phosphate Permease
5	22	124	SSO2676	765	54-62	534-542 (internal ORF binding)	-5.6	Hypothetical Protein
6	22	124	SSO0299	943	33-65	194-228 (internal ORF binding)	-9.4	Transketolase Domain Protein
7	22	124	SSO0520	315	32-60	218-242 (internal ORF binding)	-10.1	Hypothetical Protein
8	22	124	SSO1010	996	26-39	1221-1237 (3' end binding)	-6.6	FAD-dependent pyridine nucleotide-disulphide oxidoreductase
9	22	124	SSO1336	1272	29-37	255-263 (internal ORF binding)	-7.8	Transposon ISC1904
10	22	124	SSO2138	408	113-122	299-308 (internal ORF binding)	-5.0	Daunorubicin resistance ABC transporter ATPase subunit
11	22	124	SSO2209	1059	11-20	455-464 (internal ORF binding)	-6.1	Glycosyl Transferase Family 2
12	22	124	SSO2277	1486	54-63	383-392 (internal ORF binding)	-7.2	ATPase like protein
13	22	124	SSO2435	729	54-65	362-372 (internal ORF binding)	-5.1	Uroporphyrin-III C-methyltransferase
14	22	124	SSO2716	1410	79-97	972-990 (internal ORF binding)	-8.71	Drug resistance transporter, EmrB/QacA subfamily
15	22	124	SSO3153	1272	8-20	582-594 (internal ORF binding)	-8.3	DNA binding domain protein, excisionase family transposase

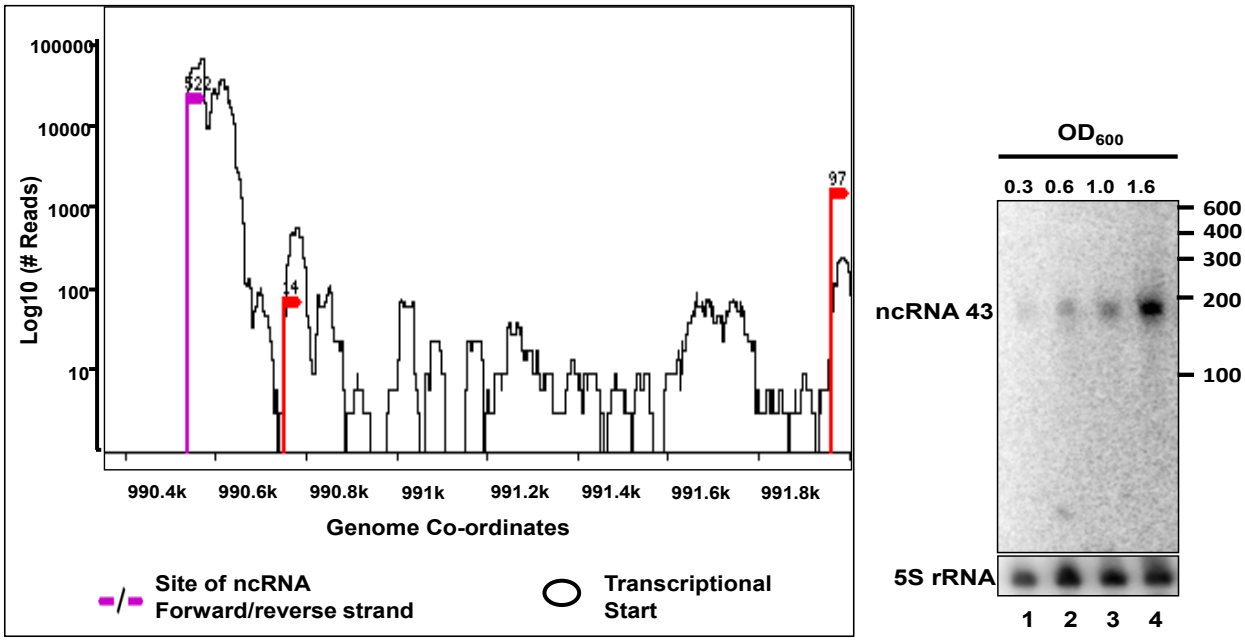
expected size of the whole ncRNA 22 corresponding to the prominent band and was only accounted for further analysis. The expression pattern of ncRNA 22 is similar even in the stationary phase with RNA extracted at OD_{600} of 1.9 (Data not Shown). The predicted mRNA targets of ncRNA 22 are shown in Table 2. A total of 15 putative target RNAs were listed out from BLAST analysis followed by IntaRNA prediction of the interaction between target mRNAs and ncRNA 22. The interaction prediction with IntaRNA was done at 75°C, the optimal growth temperature of *S. solfataricus*. Interestingly the hybridization energy of ncRNA 22 interaction with SSO2716 was -8.71 kcal/mol (Table 2, Figure 2C). SSO2716 encodes for a drug resistance transporter, which belongs to the EmrB/QacA subfamily. The Emr locus in *E. coli* encodes membrane translocases that also include multi drug resistant proteins of Gram-positive bacteria (Miyazono et al., 2007; Lomovskaya and Lewis, 1992). An archaeal homologue of EmrR, StEmrR was identified from *S. tokodaii*. Although structurally different from their bacterial counterparts, both the proteins were functionally similar in contributing to a phenotype resistant to multiple antibiotic drugs (Miyazono et al., 2007). Another interesting candidate target mRNA predicted to be down regulated by ncRNA 22 is the transposase protein encoded by SSO3153 (Table 2). All transposase enzymes possess a nuclease activity that contributes to their functionality of excising transposon DNA and subsequently integrating it into a new location. The transposase encoded by SSO3153 falls in the family of IS605. Apart from other transposon families, IS*605* elements (Kersulyte et al., 2002) do not have inverted sequences at their ends but they possess imperfect palindromic (IP) sequences located close to the

transposon ends. Another peculiar feature of IS605 family of transposases is its preference for integration in the 3′ of a specific four or five nucleotide (nt) sequence rather than random (Barabas et al., 2008). Transposon mutagenesis is a dominant mechanism of mutation in *S. solfataricus*. Spontaneous mutations that were created by IS elements arose with variable frequencies of between 10^{-4} and 10^{-5} per plated cell (Martusewitsch et al., 2000). It is speculated that even small differences in growth conditions or conditions that cause stress reactions might induce transpositions (Schleper et al., 1994). The constitutive expression of ncRNA 22 is therefore conceivable and a predicted high energy of hybridization (-8.3 kcal/mol) with SSO3153 relates to the possibility of an effective repression of the transposase by ncRNA 22. This is the first time to the best of our knowledge that the possibility of a regulation of transposase by ncRNA has been speculated in *Sulfolobus solfataricus*.

Growth phase dependent expression of ncRNAs

In contrast to ncRNA 22 which is constitutively expressed in all phases of growth, ncRNA 43 and 115 was seen to be expressed at a specific point of growth in *S. solfataricus*. Specifically, a strong Transcription Start Site was predicted for ncRNA 43 in the sulfolobus transcriptome (Figure 3A). The predominant expression of ncRNA 43 was seen in the late log phase (Figure 3B: Lane 4). Interestingly, no significant expression of the ncRNA 43 was observed in the mid-log phase or early stationary phase of growth. In order to look for the putative mRNA targets of ncRNA 43, a BLAST search followed by IntaRNA prediction was done. As shown in Table 3, 14 target RNAs were predicted. The predicted hybridization energy of the ncRNA 43 to the target RNA SSO0607 encoding for MarC protein was -8.0 kcal/mol (Figure 3C). A recent study by (McDermott et al., 2008) has pointed out that in *E. coli*, *marC* is divergently transcribed from marRAB, an operon involved in conferring resistance against multiple antibiotics (Cohen et al., 1989; George and Levy, 1983), oxidative stress (Ariza et al., 1994) and organic solvents (Asako et al., 1997). However, no function could be attributed to the MarC protein (McDermott et al., 2008). Interestingly, other potential target RNAs predicted to hybridize with ncRNA 43 were SSO2401, SSO2440 and SSO3194 coding for Ketopantoate hydroxylmethyltransferase, Glutamine synthetase and Glyceraldehyde 3-phosphate dehydrogenase, respectively. All these three proteins are involved in critical regulatory functions. Ketopantoate hydroxylmethyltransferase and Glyceraldehyde-3-phosphate are indispensable for energy metabolism. Ketopantoate hydroxylmethyltransferase is the enzyme encoded by *PanB* what catalyzes the conversion of Ketoisovalerate to Ketopantoate, which is in turn converted to Pantoate, which is a precursor in Coenzyme A biosynthesis (Hüser et al., 2005). Coenzyme A is involved in the oxidation of pyruvate in the citric acid cycle. The Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) in contrast catalyzes the conversion of Glyceraldehyde 3 phosphate to 1,3-bisphosphoglycerate in the glycolytic pathway. Interestingly, ncRNA 43 which is predicted to target mRNAs that encode Ketopantoate hydroxylmethyltransferase and Glyceraldehyde 3 phosphate dehydrogenase is expressed at the late log phase of OD_{600} 1.6. It is understandable that the metabolic processes including energy production are kept at a minimum pace in the stationary phase of growth and targeting important enzymes in the energy production cycle is the most potent way of down regulating the metabolic pathways. In addition to these metabolic proteins, Glutamine synthetase (GS) mRNA encoded by SSO2440 is also targeted by ncRNA 43. Glutamine synthetase is an important enzyme in living cells, where it controls the use of nitrogen for synthesis of DNA or amino acids. It is known that during stationary phase, all the vital processes including protein synthesis and DNA replication are slowed down. It is therefore conceivable that the mRNA encoding for GS is targeted by ncRNA 43 and may be subsequently degraded thereby checking the production of GS.

Similar to ncRNA43, another ncRNA that was found to be expressed in a specific growth phase of *S. solfataricus* is ncRNA 115. In contrast ncRNA 22 and 43, ncRNA115 is transcribed in the reverse orientation as shown in Figure 4A. In addition, the abundance of the ncRNA 115 as judged from the transcriptome analysis was lower (3340 Reads). In order to look for the expression of ncRNA 115, northern blotting was done on total RNA extracted at different phases of growth. ncRNA 115 was specifically expressed in the mid log phase at an OD_{600} of 1.0 (Figure 4B). There appears to be two transcription products which are detected on the northern blot. Both of the transcripts appear to be in the range of the predicted ncRNA 115 with only a few base differences between the two transcripts. Interestingly, both the transcripts seem to be equally transcribed. We could not affirm if there are two copies of the gene encoding ncRNA 115 in the *Sso* genome in varying lengths. Also we cannot exclude a longer transcript being generated from the same transcriptional start site. At this point it is unknown to us why there are two transcripts in ncRNA 115. The target of ncRNA 115 was predicted to be the translation recovery factor (Trf) encoded by SSO2509. Function of the Trf protein was recently described by Märtens et al. (2014). Trf directly interacts with aIF2/aIF2γ and facilitates its release from leaderless mRNAs thereby restoring translation during outgrowth of cells from stationary phase. It was earlier shown that during stationary phase, aIF2γ was able to bind to the 5' end of mRNAs and protect them from degradation in *S. solfataricus* (Hasenöhrl et al., 2008). In this scenario, this is evident



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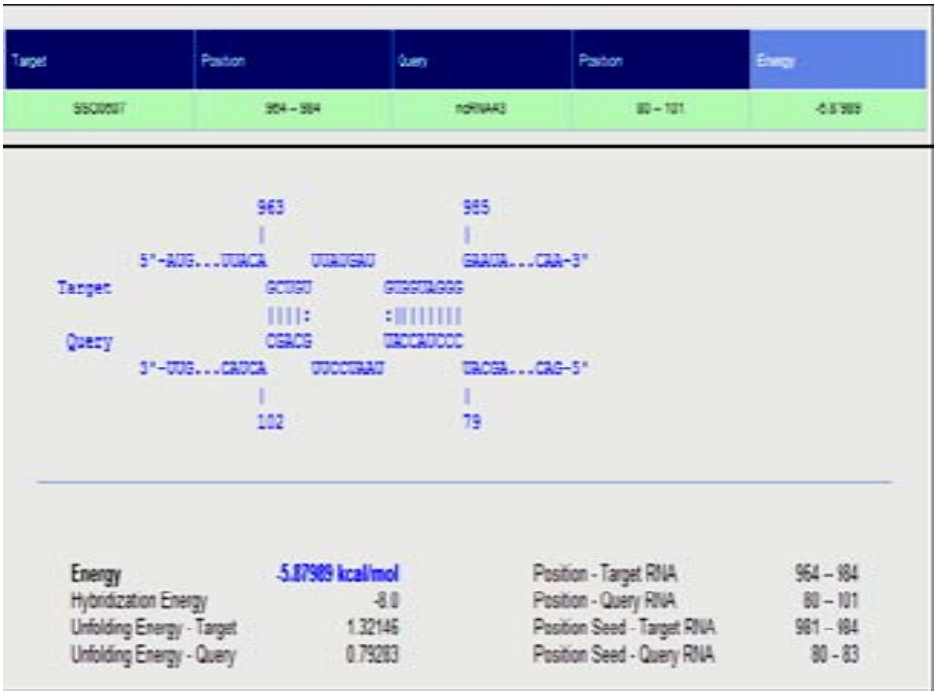

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Figure 3. The ncRNA 43 is expressed at the late log phase of growth. **(A)** The transcripition start site of ncRNA 43 is at position 990534 extending up to position 990681 in the genome co-ordinate. **(B)** The differentially expressed ncRNA 43 in different phases of growth is shown. A minimal expression of the RNA was seen in the early and mid-log phase as shown in lanes 1-3. A strong expression of ncRNA 43 was seen in the late log phase as shown in lane 4. The expression of the RNA was further subsided in the stationary phase (lane 5). The 5S rRNA represents the loading control. **(C)** The IntaRNA prediction of the interaction of ncRNA 43 with SSO0607 coding for multiple antibiotic resistance (MarC) related protein. The hybridization energy is -5.87 kcal/mol. The ncRNA 43 inteacts within the ORF of SSO0607.

Table 3. Predicted targets of ncRNA 43.

S/N	ncRNA	ncRNA length (bases)	Target mRNA	Target mRNA length (bases)	Interaction site of ncRNA	Interaction site of Target mRNA	Hybridization energy at 75°C (kcal/mol)	Target mRNA function
1	43	147	SSO2168	1033	138-150	119-131 (internal ORF binding)	-6.5	Peptide ABC Transporter
2	43	147	SSO2846	354	75-88	280-293 (internal ORF binding)	-5.1	Putative Glucosyl transferase
3	43	147	SSO0569	1446	75-88	280-293 (internal ORF binding)	-5.1	prolyl- tRNA synthetase
4	43	147	SSO0607	609	80-101	964-984 (3' end binding)	-8.0	multiple antibiotic resistance (MarC)-related protein
5	43	147	SSO3231	978	112-134	521-543 (internal ORF binding)	-5.8	Peptidase M48Ste24p
6	43	147	SSO0244	1410	1260-1273	1260-1273 (internal ORF binding)	-5.9	FAD dependent oxidoreductase
7	43	147	SSO0645	849	75-82	561-568	-5.5	Translin lysine biosynthesis enzyme LysX
8	43	147	SSO1137	516	29-37	239-247	-4.7	Hypothetical Protein
9	43	147	SSO1859	936	67-82	177-192	-6.6	peptidase M48 Ste24p
10	43	147	SSO2323	924	28-38	898-908	-6.4	Flagellin, putative
11	43	147	SSO2372	543	138-146	251-259	-4.7	Putative RNA-binding protein (contains KH domains)
12	43	147	SSO2401	684	37-50	414-427	-7.4	Ketopantoate hydroxymethyl-transferase (panB)
13	43	147	SSO2440	1278	73-98	623-647	-7.7	Glutamine synthetase (glutamate ammonia ligase)
14	43	147	SSO3194	1530	78-86	691-699	-6.9	Glyceraldehyde-3-phosphate dehydrogenase

that the Trf is expressed in the early to mid-log phase, helping in resumption of translation by removing the mRNA bound aIF2. The ncRNA 115 binds specifically to the 3' UTR of SSO2509 with hybridization energy of -8.4 kcal/mol (Figure 4C). Interestingly, ncRNA 115 was seen to be expressed at the mid-log phase. The role of Trf protein is predominantly required in the out growth phase and is least required once normal rate of translation resumes. Trf protein expression should also be regulated in the stationary phase where aIF2 should be bound to mRNAs for its protection. Hence, we speculate that the regulation of Trf protein synthesis is performed in two ways. In the first instance, immediate blockage of the Trf expression is done by ncRNA 115 by binding to the SSO2509. After the immediate cessation of translation of SSO2509, there could be another mechanism which keeps the level of Trf under check during the stationary phase. One could also not rule out the possibility of another ncRNA which may bind to SSO2509 and block the translation.

Conclusion

Here we show and discuss the expression and targets of three ncRNAs from *S. solfataricus*. The target RNAs were identified by Blast analysis followed by the interaction mapping with IntaRNA. There are open questions that are relevant to this work such as the experimental mapping of the target mRNA degradation upon interaction with ncRNA. Is there a reduction in the abundance of the target mRNA at the point of expression of ncRNAs? An experimental analysis by northern blotting or RT-PCR to detect the decrease in the amount of target RNA upon hybridization with ncRNAs will clearly prove our hypothesis that ncRNAs are effective gene regulators in the hyperthermophilic archeon *S. solfataricus*. To the best of our knowledge this is the first study that represents a divergent expression pattern of noncoding RNAs in *S. solfataricus*.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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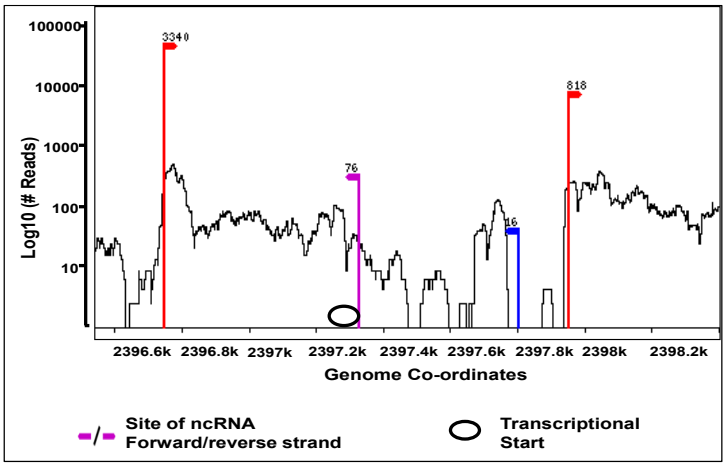

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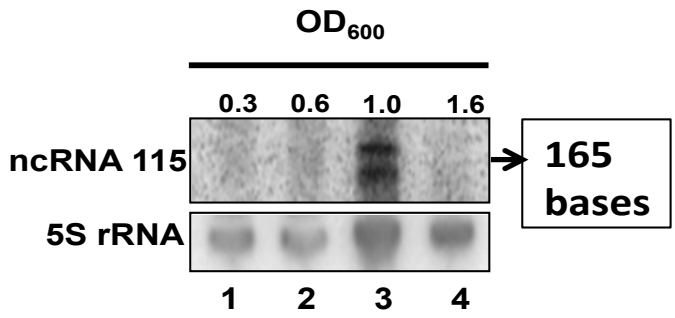

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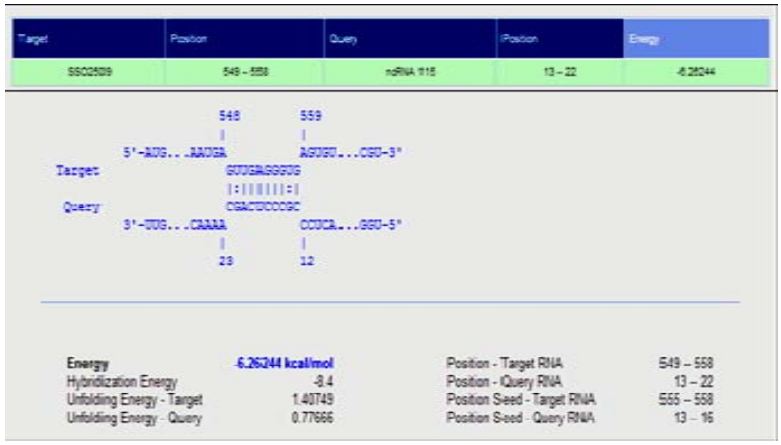

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Figure 4. The ncRNA 115 targets the mRNA coding for translation recovery factor (Trf). **(A)** The transcription start site of ncRNA 115 is shown at position 2397330 to 2397165 in the genome co-ordinates. The ncRNA 115 is encoded in the reverse orientation in the Sulfolobus genome. The reads corresponding to the transcripition start site is 76, which represents that the ncRNA 115 is not as abundantly expressed as ncRNA 22 or ncRNA 43. **(B)** The ncRNA 115 is expressed specifically at the mid-log phase of growth (Lane 3). Two bands of the RNA are seen with low expression abundance. The lower lane represents loading control (5S rRNA). A digital normalization of the bands is done and is shown as graph below. The graph represents the fold change in the intensity of ncRNA 115 in comparison to 5S rRNA. **(C)** The target of ncRNA was predicted to be the translation recovery factor (Trf) encoded by SSO2509. The IntaRNA hybridization profile of ncRNA 115 and SSO2509 is shown. The hybridization energy is -6.26 kcal/mol. The interaction with ncRNA 115 interaction occur approximately 200 bases downstream of 3'-end in SSO2509.

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