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THE SPLICEOSOMAL PROTEIN SnRNP F BINDS TO BOTH U3 AND U14 CLASS OF snoRNA IN *Giardia lamblia*

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Abstract

Small nuclear Ribonucleo Protein F (snRNP F) is a spliceosomal protein that binds with U1, U2, U4/U6 and U5 small nuclear RNA (snRNA) to form spliceosomal complexes responsible for pre mRNA processing. This study reports the unusual interaction of giardial snRNP F with small nucleolar RNAs (snoRNA) that are responsible for pre rRNA processing. Electrophoretic Mobility Shift Assay was used to demonstrate the interaction of this protein with U3 and U14 class snoRNA of the early branching eukaryote *Giardia lamblia*. It was also evident from our study that snRNP F in *Giardia* is evolutionary distinct from its other eukaryotic orthologues.

Keywords: *Giardia lamblia*, snRNA, snoRNA, U3, U14.

1. Introduction

snRNP F belongs to a large family of Sm and Sm-like (LSm) proteins that have the conserved Sm motif (Hermann *et al*, 1995; Seraphin 1995). In eukaryotes the splicing of pre mRNA is carried out by the small nuclear Ribonucleo protein (snRNP) complexes U1, U2, U4/U6 and U5 (Burge *et al*, 1999; Yu *et al*, 1999). Each spliceosomal snRNPs consists of one snRNA (U1, U2, U4/U6 and U5) and proteins that are classified in two groups: the specific proteins that associate only with certain snRNP particle and seven Sm proteins (B/B', D1, D2, D3, E, F and G) that are common to each particle (Hermann *et al*, 1995). snRNP F along with six other Sm proteins assemble in a stepwise manner onto the single stranded Sm site element of the U1,U2, U4/U6 and U5 spliceosomal snRNAs, resulting in a doughnut shaped core RNP structure (Raker *et al*, 1999).

snoRNAs are a group of small non-coding RNAs (sncRNAs) that are known to be involved in the processing of pre rRNA or any other aspect of ribosome biogenesis (Maxwell *et al*, 1995; Gerbi 1995; Sollner-Webb *et al*, 1995). The snoRNAs are broadly categorised into two classes, C/D and H/ACA, based on the presence of conserved domains and their functional differences (Bachellerie *et al*, 1995; Cavallie *et al*, 1996; Kiss-Laszlo *et al*, 1996; Ganot *et al* 1997; Ni *et al*, 1997). U3 class of snoRNAs are characterized by the presence of two conserved C' & D boxes (Speckmann *et al*, 1999) and are involved in site-specific cleavage of pre rRNA (Clery *et al*, 2007). U14 class of snoRNAs has A and B conserved domains, along with the C and D conserved sequences (Jarmolowski *et al*, 1990; Huang *et al*, 1992) and are reported to function in the early cleavage of eukaryotic pre rRNA. It has been shown that in *S.cereisiae* inactivation of U14 snoRNA disrupts cleavage, leading to the formation of a 20S precursor RNA, instead of 18S RNA (Zagorski *et al*, 1988; Li *et al*, 1990). In *Giardia lamblia* U3 and U14 class of snoRNAs are represented by RNA H and RNA J respectively (Niu et al, 1994).

A 107 amino acid long putative orthologue of snRNP F protein has already been reported in *Giardia* (GL50803_4954). Our study shows that snRNP F (which normally associates with spliceosomal snRNAs in eukaryotes) of *Giardia* is evolutionary distinct from its other eukaryotic orthologues and binds with both RNA H and RNA J of the organism. Till date binding of snRNP F with any snoRNAs of eukaryotes is unreported.

2. Materials and Methods

According to previous reports, the Sm proteins are known to be evolutionary conserved throughout a diverse group of organism (Hermann *et al*, 1995). Sets of amino acid sequences of snRNP F from both distant and closely related organism of *Giardia lamblia* was obtained from NCBI database and aligned using MEGA4 software (Tamura *et al*, 2007) by CLUSTAL W method to examine the evolutionary position of this giardial protein. Phylogenetic tree was constructed separately using the alignment with two different methods (PhyML/Blosum62 model/aLRT validation & BioNJ/Poisson distribution model/ Bootstrap with 1000 replicates) using SeaView Graphical Representation Ver. 4 software.

To study the interaction of this evolutionary distinct snRNP F of *Giardia* with its snoRNAs, RNA J and RNA H, the genes were cloned in pET 33b and pGEM 4z vectors (see supplementary file for the details of plasmid construction and expression of snRNP F in *E.coli*). For expression of snRNP F, the transformed *E.coli* Bl21 cells were induced with 1mM IPTG at 25^{o}C for 5 hrs when most of the protein was found in the soluble fraction. The expressed protein was purified by Ni-NTA Superflo.

Interaction of the protein snRNPF with the snoRNAs was studied *in vitro,* by Electrophoretic Mobility Shift Assay (EMSA). Fluorescein labled, *in vitro* transcribed RNA J and RNA H were prepared by using T7 Maxiscript kit (Ambion) following the manufacturer's protocol. They were separately incubated for 30 mins at 0^oC with 2 µg of recombinant giardial snRNP F protein in a 20 µl binding reaction that contained binding buffer (10 mM Hepes-KOH, pH 8.0, 10% glycerol, 0.05% NP40, 1 mM EDTA, pH 8.0, 0.5 mM DTT, 10 mM KCl nad 5mM $MgCl_2$). After incubation, samples were electrophoresed on a 5% native PAGE (Acrylamide: Bis acrylamide, 29:1) in TAE buffer at 100 Volts at a temperature of 4^oC.

3. Results and Discussion

In the phylogenetic analysis, every tree showed similar Clustal distribution. Data obtained from each tree were combined manually to create a single tree where both the bootstrap values are placed beside the clusters (Figure 1). All the closely related organisms are distinctly placed within single clusters, whereas *Giardia* remained separated even from close organisms such as *Toxoplasma*, *Entamoeba*, *Trypanosoma* etc, thus confirming its evolutionary distance from other organism depending on this particular protein. It may be a reason for its functional dissimilarity towards binding with both the U3 and U14 class snoRNAs.

The *E.coli* BL21 cells containing the recombinant pET 33b plasmid with the snRNP F gene insert was induced with 1 mM IPTG at 25^oC for 5 hrs when the maximum protein was obtained in the soluble fraction. The crude soluble fraction of the protein was loaded into Ni^{2+} binding resin column. After unbound proteins were washed away, the target protein was recovered by eluting it with elution buffer containing 200 mM imidazole.

In the EMSA study, shift in bands corresponding to RNA J and RNA H bound snRNP F in the native PAGE clearly suggests the interaction of this protein with U3 and U14 class snoRNAs (Figure 2) which are involved in the processing of pre rRNA.

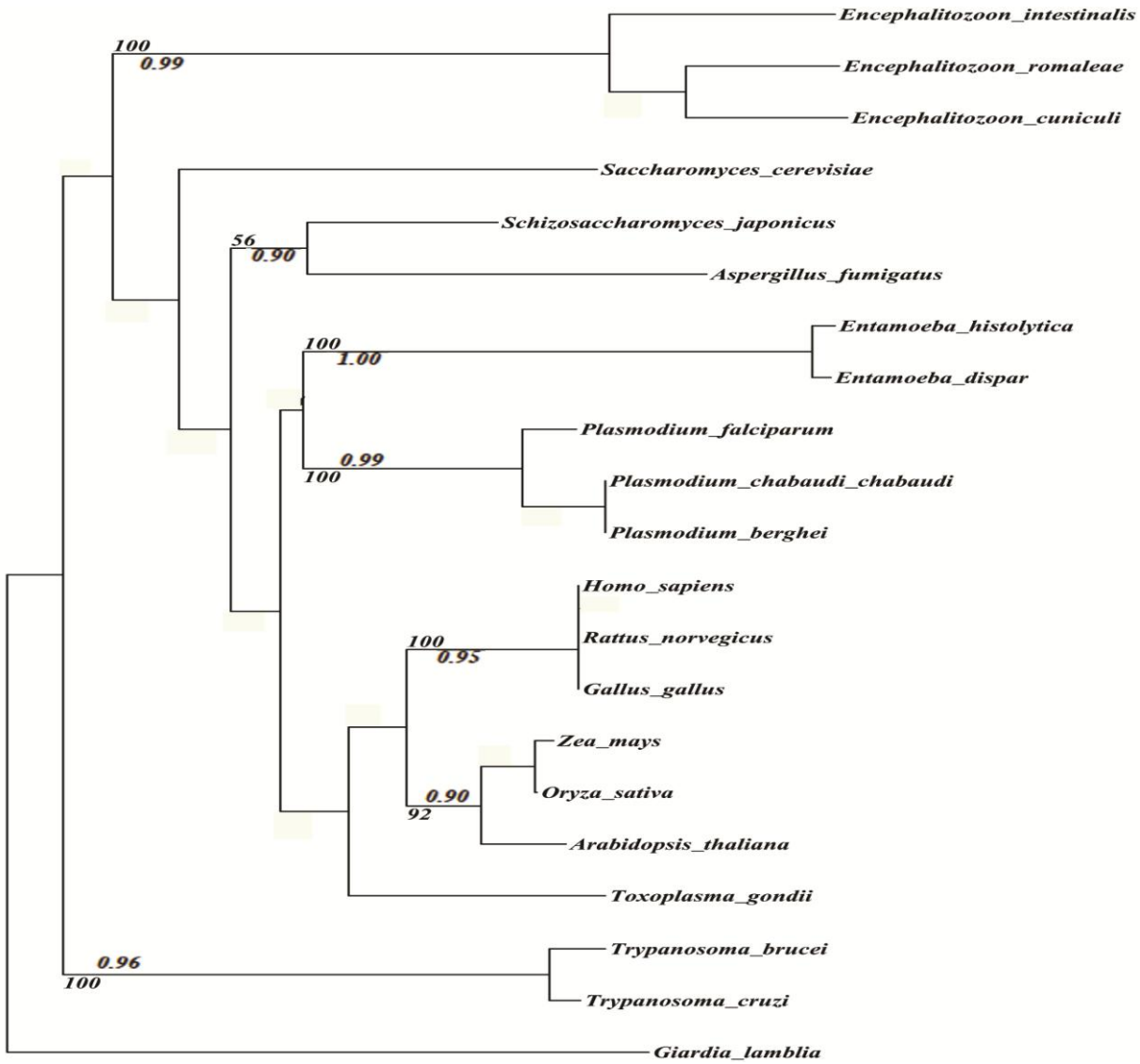

Figure 1. Phylogenetic tree showing clustal distribution using the alignment with two different methods (PhyML/Blosum62 model/aLRT validation & BioNJ/Poisson distribution model/ Bootstrap with 1000 replicates) using SeaView Graphical Representation Ver. 4 software and were manually merged to get this tree. Both the aLRT and Boostrap validation are given on the either side of the branch. The branch lengths are arbitrary and do not represents any evolutionary distances.

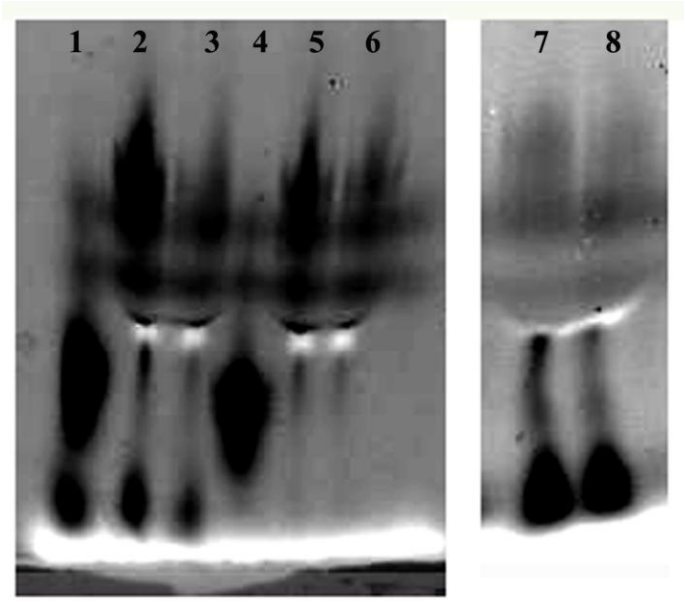

Figure 2. Electrophoteric Mobility Shift Assay (EMSA) to show snRNP F of *Giardia lamblia* binds with U3 and U14 class snoRNA. Lane 1: RNA J (U14); Lane 2: RNA J+snRNP F; Lane 3: RNA J+Fibrillarin (positive control, as fibrillarin is reported to bind with this RNA); Lane 4: RNA H (U3); Lane 5: RNA H+snRNP F; Lane 6: RNA H+Fibrillarin (positive control, as fibrillarin is reported to bind with this RNA); Lane 7: RNA J+BSA (negative control as BSA is not reported to bind with this RNA); Lane 8: RNA H+BSA (negative control as BSA is not reported to bind with this RNA). There is shift in bands in the lanes 2, 3, 5 and lane 6 from that in lanes 1 and 4 suggesting that snRNP F and fibrillarin (positive control protein) of *Giardia lamblia* binds with both the RNAs *in vitro*.

4. Conclusion

snRNP F usually binds with U1, U2, U4/U6 and U5 snRNA in eukaryotes to form the spliceosomal complex involved in pre-mRNA processing. In this study we have shown that the protein in *Giardia* is evolutionary distinct from its other eukaryotic orthologues and binds with both U3 and U14 class of snoRNAs that are involved in pre rRNA processing.

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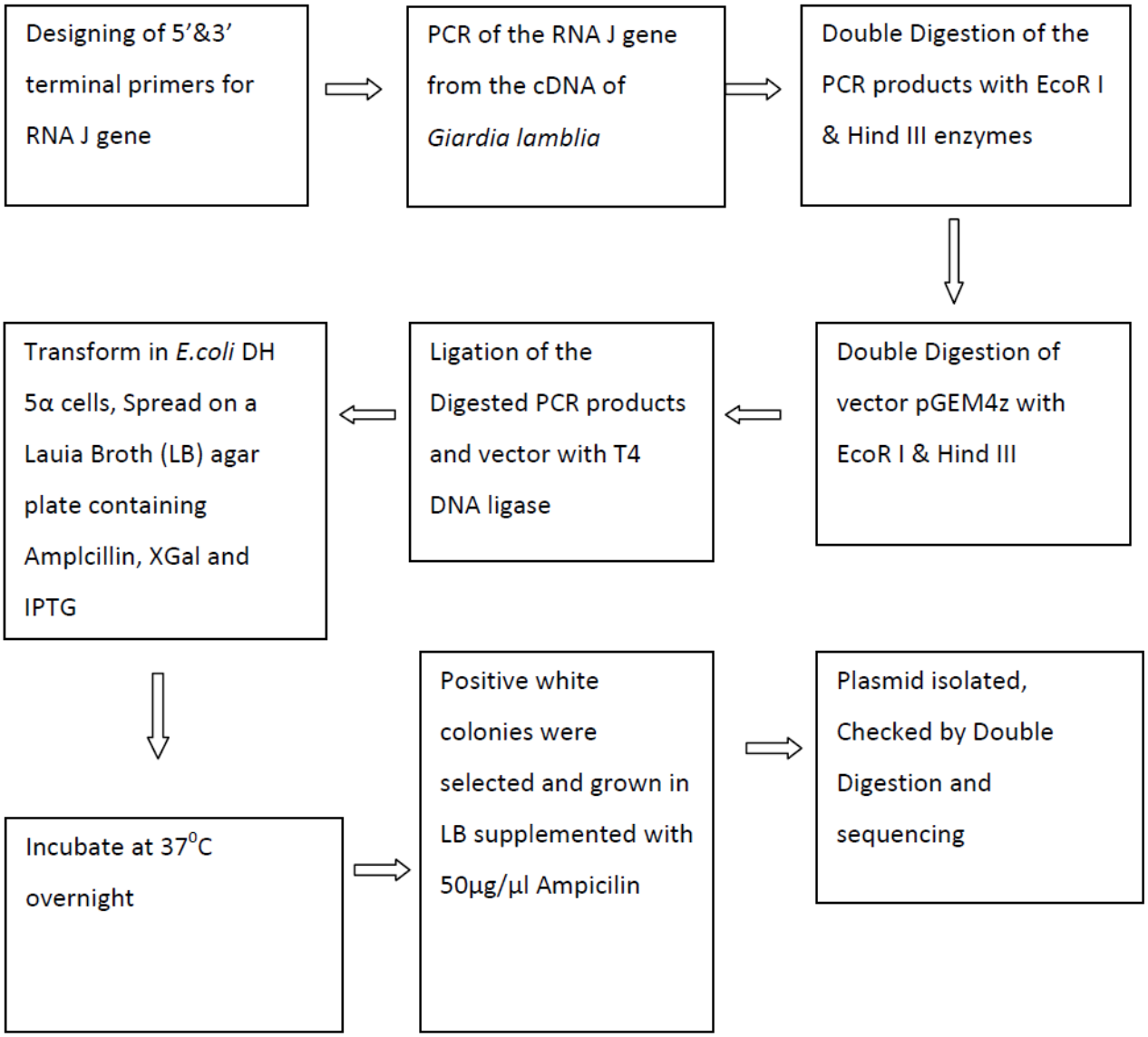
Annexure:

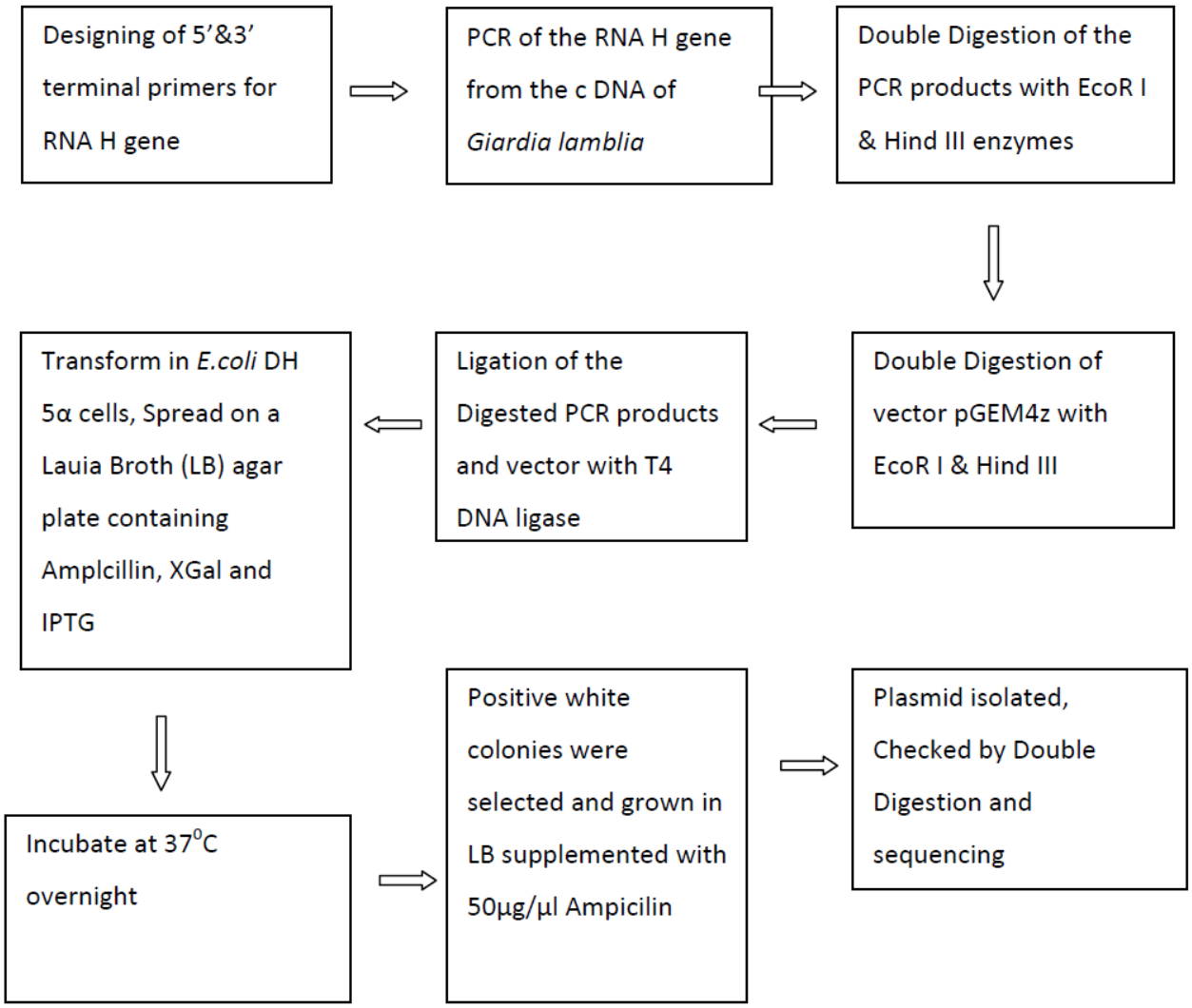
Table showing the sequence of primers used to clone the mentioned genes. Sequences in bold shows the restriction enzyme sites used for digestion.

Primers	Sequence	Restriction Enzyme
RNA J F (5' Terminal)	5'-CCG**GAATTC**AATGTAGCGAACCCACGC-3'	EcoRI
RNA J R (3' Terminal)	5'-GGG**AAGCTT**ATTAAGTAAGGAAGGCTCG-3'	HindIII
RNA H F (5' Terminal)	5'-AA**GAATTC**ACTGCCTCTCCTGAGGCAGATG-3'	EcoRI
RNA H R (3' Terminal)	5'-CCC**AAGCTT**GAATTCAGAATACGACAAACTTCG-3'	HindIII
snRNP F F	5'-CG**GGATCC**AATGGCGACAAACG-3'	BamHI
snRNP F R	5'-TTG**CTCGAG**CGGCTACACACTATTCG-3'	XhoI

Cloning of RNA J:



Cloning of RNA H:



Cloning and Expression of snRNP F: