Stability of secondary structure of different ribosomal RNA types in *Plasmodium* species

Introduction

Plasmodium is a sporozoan parasite distributed throughout the world, with high prevalence in tropical and subtropical regions of South Africa, Asia, and South America. It causes the disease malaria, which is a deadly parasitic disease with high mortality and morbidity rate. About 229 million infected cases were reported and among them, around 409,000 infected people died from the disease in 2019[1].

Four species of *Plasmodium* are known to infect humans. Among them, *P. falciparum* is the most pathogenic to humans[3]. *Plasmodium* is a digenetic parasite, and its life cycle is completed in two different hosts, i.e., primary host (vertebrates, including humans) and secondary host (invertebrates, mosquitos). The parasite completes its asexual life cycle in the vertebrate host and its sexual life cycle in the mosquito[3].

The parasite is exposed to different environmental conditions at its various stages of development, such as different temperatures, PH, ions, and nutrients[1]. Its development is more complex because of many environmental changes in the different hosts. Research has shown that temperature is a decisive factor for parasite development during asexual reproduction including thermoregulation of rRNA expression and gametogenesis[6]. It develops in the vertebrate host at 37°C (in mammals) to 42 °C (in birds) while in mosquitoes it develops at 26°C-28°C[6], the ideal temperature for the invertebrate host. During the developmental stages, the human malarial parasite transcribes into three different 18S rRNA genes i.e., (A genes) expressed in the vertebrate host during asexual multiplication in RBCs, whereas (O and S genes) are expressed in the mosquito during the sexual life cycle. Recent studies have established that the RNA secondary and tertiary structure acts as a crucial role in the transcript expression of rRNA[1]. The A-type of rRNA is highly expressed in the signet ring and trophozoite stage of mature RBCs of human or vertebrate hosts[6]. After the infected blood meal, the A-type of the gene is transcribed in the oocyst after the zygote formation in the stomach wall of the female *Anopheles* mosquito[10].

Submitted by: Bhoj Bhat Chhetri Supervised by: Dr. Luiz Shozo Ozaki

Similarly, the O-type gene is expressed inside the oocyst after the complete disappearance of ookinete, and the S-type of genes are transcript after the oocyst matures with sporozoites and remains within the sporozoites until they are injected into the blood of the vertebrate host[7]. The malarial parasite released more free energy for the formation of a stable secondary structure in the vertebrate host because of the high-temperature comparison to the invertebrate host(mosquito) low temperature[5].

Plasmodium species of malaria parasite seen with having a correlation between proliferative stages of development and enhanced rRNA transcription and ribosome production[5]. Thus, this study focuses on the 18S ribosomal RNA gene, and their types A, O, and S expressed in different temperate hosts and Gibbs free energy release for the formation of the stable secondary structure. The prediction of the secondary structure of RNA at arbitrary temperatures would expand our knowledge of structure and evolution in the RNA world[11]. As GC composition is known to influence the structural energy, this inherent feature was split to obtain many derived parameters like the individual effect of G, individual effect of the number of C, total GC count independent of no. of bases, and GC content % as a fraction of composition and G/C ratio. Different loop types were considered to elucidate the interrelationship among different kinds of loops and structural energy[8].

Methods

Data Collection and data set preparation

18S ribosomal RNA FASTA sequences of different *Plasmodium* species were downloaded from NCBI (https://www.ncbi.nih.gov). Those sequences were checked for incomplete, partial, unconserved, and filtered with the help of Sequencher®. The final curated input database contains a complete sequence with more than 2000 nucleotides base pairs. Incomplete and short sequences below 2000 base pairs were discarded in this study.

Name of species	rRNA type	Seq length	Accession no.
P. vivax	A	2064	U03079
P. vivax	O	2064	XR003001206
P. vivax	S	2147	U07368
P. falciparum	A	2090	M19172
P. falciparum	O	2087	XR002273081
P. falciparum	S	2149	XR002273101
P. ovale	A	2104	AB182489
P. ovale curtisi	A	2104	KF696371
P. malariae	A	2157	XR003751948
P. gallinacaeum	A	2120	M61723

Table 1. Complete sequences of 18S rRNAof different species of *Plasmodium* (A, O & S types genes) (Source https://www.ncbi.nih.gov).

Multiple Sequence Alignment

Multiple Sequence Alignment was performed using 'ClustalW (www.Ebi.ac.UK/clustalw) and analyzed by ClustalW (Thompson et. al; 1994). The sequences for A-type genes are expressed during the asexual stage of the parasite in the vertebrate host, whereas O and S-type rRNA genes are expressed during the sexual stages in mosquitoes. The homologies and conserved sequences were identified during multiple sequences alignment.

Secondary Structure Prediction and Gibbs Free energy

The complete sequences are analyzed using RNAfold WebServer (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi) which calculates the Gibbs free energy and secondary structure of the input complete sequences of *Plasmodium* species.

Result

The secondary structure of selected Plasmodium species was predicted for the different 18S rRNA genes A, O, and S types. Gibbs free (ΔG) energy for the different 18S rRNA (A, O, and S) types was calculated in *P. vivax* and *P. falciparum*. In *P. vivax* the Gibbs free energy was released higher in S-type (ΔG=-600Kcalmole/mole) compared to A and O types (ΔG=-587 and ΔG=-586 Kcal/mole). Similarly, in *P. falciparum* Gibbs free energy was released higher in A-type (ΔG=-585Kcal/mole) compared to S and O-types (ΔG=-586 and ΔG=-587Kcal/mole). Comparatively, the highest Gibbs free energy was calculated(ΔG=-614Kcal/mole) in the A-type of 18S rRNA gene in birds (*P. gallinaecium*).

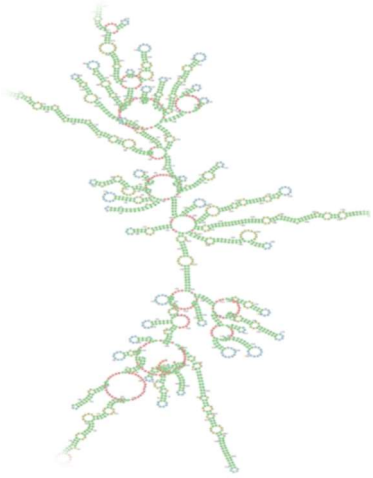


Figure 1: Secondary structure of 18s rRNA A- type gene of *P. vivax*

Figure 2: Secondary structure of 18S rRNA O-type gene of *P. vivax*

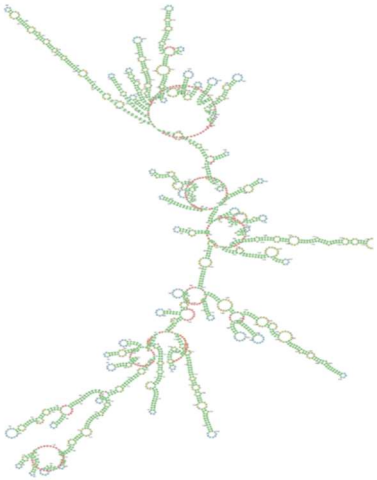


Figure 3: Secondary structure of 18S rRNA S-type gene of *P. vivax*

Figure 1,2,3. Comparison of the secondary structures of *P. vivax* 18 S rRNAs: Type A compared with type O and S. The helices are represented according to Watson-Crick, wobble (G*U) and unusual (G*A) base-pairing but in these views which is not observable. Regions of variable sequence are left mostly as nucleotides inline format. Insertions and missing nucleotides relative

to the A and S genes are color-coded and insertions in variable and conserved regions. Compensatory base pairs and differences in the O gene sequences are indicated by the symbol. Secondary structures for the *P. vivax* rRNAs are available from the Worldwide Website for RNA secondary structures (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi)

Species	rRNA type	Length	Free Energy (Kcalmol^{1})
P. vivax	A	2064	- 584
	O	2064	-583
	S	2046	-600
P. falciparum	A	2090	-560
	O	2087	-558
	S	2119	-526
P. ovale	A	2104	-586
P. ovale curtisi	A	2104	-594
P. malariae	A	2157	-587
P. Gallinacaeum(Bird)	A	2157	-614

Table 1:Comparison of Gibbs free energy released (ΔG) in the formation of the secondary structure of the different 18S rRNA types, A, O, and S, of different *Plasmodium* species.

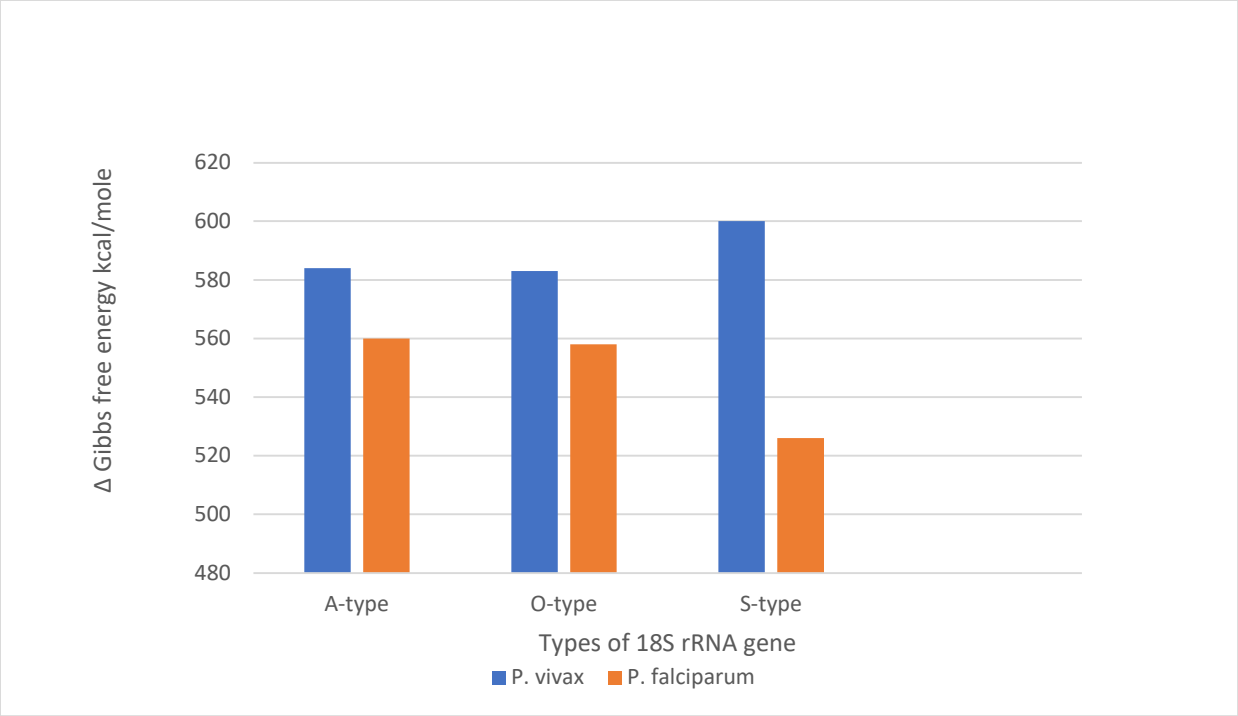
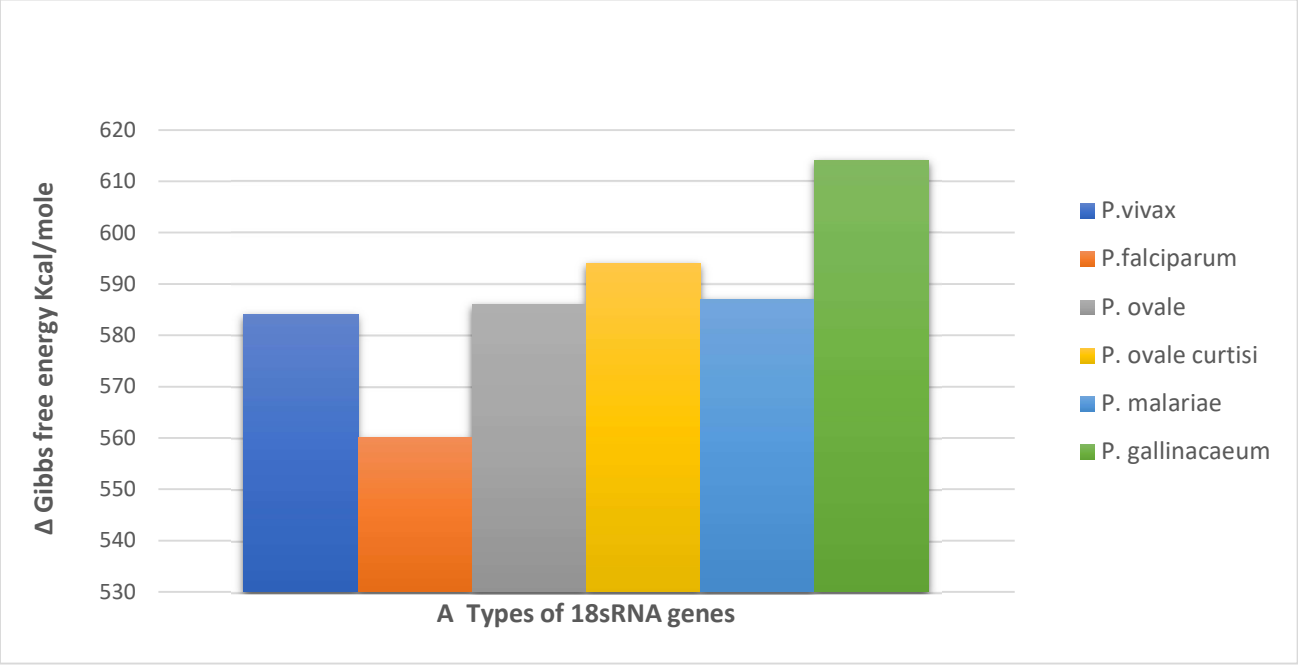


Figure 4. Gibbs free energy (ΔG) comparison between the different18S rRNA types of *P. vivax* and *P. falciparum.* The blue color represents the *P. vivax* and orange color represents the *P. falciparum*



Discussion

The Gibbs free energy (ΔG) calculated was higher for the S-type of rRNA gene in *Plasmodium vivax,* compared to O and A-type. However, in *Plasmodium falciparum* the ΔG was higher in A-type compared to O and S-type. We would expect a more stable or a higher ΔG of secondary structure formation for the A-type 18S rRNA due to be the one present at higher temperature in the vertebrate host as compared to the O and S type, present in the parasite at lower temperature of the invertebrate host (mosquito). This hypothesis was supported in only *Plasmodium falciparum* (Table 2).

The ribosome has a large structure made up of two subunits, i.e., a large subunit (LSU) and a small subunit (SSU). They are composed of many proteins in addition to the rRNAs, and their stability should be assessed as a whole, which then might reflect their stability more accurately in relation to the different temperatures of the hosts[9]. This study was focused on only the 18S rRNA. For future studies, we plan to identify complete sequences of 18S rRNA and their types A, O, and S in other *Plasmodium* as this conclusion was based on only two species of *Plasmodium.* We will also expand our research to encompass all genes from the large subunit and comprehensively depict the biology of secondary structure formation based on the host temperature.

References

1. Mathews, D. H. (2006). Revolutions in RNA secondary structure prediction. Journal of molecular biology, 359(3), 526-532.
2. McCutchan, T. F., Vidal, F., Lal, A. A., Gunderson, J. H., Elwood, H. J., & Sogin, M. L. (1988). Primary sequences of two small subunit ribosomal RNA genes from Plasmodium falciparum. *Molecular and biochemical parasitology*, *28*(1), 63-68
3. Chatterjee, S., Mukhopadhyay, P., Bandyopadhyay, R., Dhal, P., Biswal, D., & Bandyopadhyay, P. K. (2017). Molecular characterization and phylogenetic analysis of Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and Plasmodium cynomolgi. *Journal of Parasitic Diseases*, *41*(1), 230-236.
4. Escalante, A. A., & Ayala, F. J. (1994). Phylogeny of the malarial genus Plasmodium, derived from rRNA gene sequences. *Proceedings of the National Academy of Sciences*, *91*(24), 11373-11377.
5. Li, J., Gutell, R. R., Damberger, S. H., Wirtz, R. A., Kissinger, J. C., Rogers, M. J., ... & McCutchan, T. F. (1997). Regulation and trafficking of three distinct 18 S ribosomal RNAs during the development of the malaria parasite. *Journal of molecular biology*, *269*(2), 203-213.
6. Y., Qi, Y., Zhang, Y., Mu, Q., Zheng, G., Zhang, M., Chen, B., ... & Wang, X. (2022). RNA Secondary Structure Revealed Distinct Thermoregulation in. *Cellular and Molecular Basis in Parasitic Diseases Control: Research Trends*.
7. González-Cerón, L., Rodríguez, M. H., Ovilla-Muñoz, M. T., Santillan-Valenzuela, F., Hernández-Ávila, J. E., Rodríguez, M. C., ... & Villarreal-Trevino, C. (2020). Ookinete-specific genes and 18S SSU rRNA evidenced in plasmodium vivax selection and adaptation by sympatric vectors. *Frontiers in genetics*, 1362.
8. Banerjee, A. K., Arora, N., & Murty, U. S. N. (2007). Stability of ITS2 secondary structure in Anopheles: what lies beneath. *International Journal of Integrative Biology*, *1*(3), 232.
9. Li, J., Wirtz, R. A., McConkey, G. A., Sattabongkot, J., & McCutchan, T. F. (1994). The transition of *Plasmodium vivax* ribosome types corresponds to sporozoite differentiation in the mosquito. *Molecular and biochemical parasitology*, *65*(2), 283-289.
10. McCutchan, T. F., Vidal, F., Lal, A. A., Gunderson, J. H., Elwood, H. J., & Sogin, M. L. (1988). Primary sequences of two small subunit ribosomal RNA genes from Plasmodium falciparum. *Molecular and biochemical parasitology*, *28*(1), 63-68.
11. Lu, Z. J., Turner, D. H., & Mathews, D. H. (2006). A set of nearest-neighbor parameters for predicting the enthalpy change of RNA secondary structure formation. *Nucleic acids research*, *34*(17), 4912-4924.