Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 47 (2008) 45–53
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Evolution and phylogeny of the heterogeneous cytosolic SSU rRNA genes in the genus *Plasmodium* ☆

Yuriko Nishimoto [a,b], Nobuko Arisue [c], Satoru Kawai [d], Ananias A. Escalante [e], Toshihiro Horii [c], Kazuyuki Tanabe [f], Tetsuo Hashimoto [a,g,*]

[a] *The Institute of Statistical Mathematics, Minami-Azabu, Minato-ku, Tokyo 106-8569, Japan*
[b] *Department of Biosystems Science, Graduate University for Advanced Studies (Sokendai), Hayama, Kanagawa 240-0193, Japan*
[c] *Department of Molecular Protozoology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan*
[d] *Department of Tropical Medicine & Parasitology, Dokkyo Medical University School of Medicine, Mibu, Shimotsuga, Tochigi 321-0293, Japan*
[e] *School of Life Sciences, Arizona State University, P.O. Box 874501, Tempe, AZ 85287, USA*
[f] *Laboratory of Malariology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan*
[g] *The Institute of Biological Sciences, University of Tsukuba, Tsukuba 305-8572, Japan*

Received 6 February 2007; revised 23 January 2008; accepted 29 January 2008
Available online 6 February 2008

Abstract

Unlike other eukaryotes, malaria parasites in the genus *Plasmodium* have structurally and functionally different paralogous copies of the cytosolic (cyto-) SSU rRNA (18S rRNA) gene that are expressed at different developmental stages. In *P. falciparum*, *P. vivax*, and *P. berghei*, A-type cyto-SSU rRNA is expressed in asexual stage, while S-type in sporozoite stage. A third type (O-type) has been described in *P. vivax*. It is expressed only in oocyst stage in the mosquito. Recently, it has been shown that the maintenance of heterogeneous cyto-SSU rRNAs in *Plasmodium* can be modeled as a birth-and-death process under strong purifying selection [Rooney, A.P., 2004. Mechanisms underlying the evolution and maintenance of functionally heterogeneous 18S rRNA genes in Apicomplexans. Mol. Biol. Evol. 21, 1704–1711]. In this study, we performed detailed phylogenetic analyses of *Plasmodium* cyto-SSU rRNAs with special emphasis on the evolution of multi-copy genes in simian *Plasmodium* species. We sequenced paralogous copies of the cyto-SSU rRNA genes from an African simian *Plasmodium* species, *P. gonderi*, and Asian simian *Plasmodium* species, *P. fragile*, *P. coatneyi*, *P. inui*, *P. hylobati*, *P. fieldi*, *P. simiovale*, and *P. cynomolgi*. Interestingly, all Asian simian *Plasmodium* species have a single S-type-like gene and several A-type-like genes. Alignment analysis demonstrated for the first time that an approximately 50-residue insertion in the V7 variable region near the stem 43 is shared exclusively by the S-type-like sequences of the Asian simian *Plasmodium* species and the S- and O-type sequences of *P. vivax*. We comprehensively analyzed all cyto-SSU rRNA sequences of the genus *Plasmodium* currently available in the database. Phylogenetic analyses of all publicly available cyto-SSU rRNA sequences for the genus *Plasmodium* clearly demonstrated that gene duplication events giving rise to A- and S-type-like sequences took place independently at least three times in the *Plasmodium* evolution, supporting the hypothesis that these genes evolve according to a birth-and-death model.

Keywords: *Plasmodium*; SSU rRNA; Molecular phylogeny; Simian malaria parasites; Multi-copy genes

1. Introduction

The genus *Plasmodium* comprises unicellular malaria parasites that infect various vertebrate hosts including primates, rodents, reptiles, and birds. Five *Plasmodium* species, *P. falciparum*, *P. vivax*, *P. malariae*, *P. knowlesi*, and *P. ovale*, are known as human parasites. To better understand the evolution of the genus *Plasmodium* and to iden-

☆ Sequences: The sequences reported in this paper have been submitted to GenBank, EMBL, and DDBJ databases under Accession Nos., AB265789–AB265791 and AB287269–AB287290.

* Corresponding author. Address: The Institute of Biological Sciences, University of Tsukuba, Tsukuba 305-8572, Japan. Fax: +81 29 853 6614.
E-mail address: hashi@biol.tsukuba.ac.jp (T. Hashimoto).

1055-7903/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2008.01.031

tify the closest relative of each human *Plasmodium* species, investigations on molecular phylogeny are essential.

Plasmodium phylogeny is still controversial. Optimal trees reconstructed using different genes are not always consistent with each other. Previous attempts for phylogenetic reconstruction of the genus *Plasmodium* have used several molecular markers such as cytosolic small subunit rRNA (cyto-SSU rRNA or 18S rRNA) (e.g. Qari et al., 1996; Escalante et al., 1997; Leclerc et al., 2004; Rooney, 2004), mitochondrial cytochrome B (CytB) (Escalante et al., 1998; Perkins and Schall, 2002), apicoplast caseinolytic protease (ClpC) (Rathore et al., 2001), circumsporozoite protein (CSP) (McCutchan et al., 1996; Escalante et al., 1995; Vargas-Serrato et al., 2003), and merozoite surface antigen 9 (MSP9) (Vargas-Serrato et al., 2003). There are two phylogenetic analyses that simultaneously consider more than one gene (Escalante et al., 2005; Perkins et al., 2007). Until recently, based on these phylogenetic analyses, the monophyly of simian (non-hominoid) *Plasmodium* species and *P. vivax* (referred to as 'simian *Plasmodium* + *P. vivax* clade' in this report) and the monophyly of rodent *Plasmodium* species ('rodent *Plasmodium* clade') have been demonstrated.

Among the molecular markers described above, cyto-SSU rRNA has been examined most frequently and many trees have been proposed (e.g. Qari et al., 1996; Escalante et al., 1997; Leclerc et al., 2004; Rooney, 2004). Since cyto-SSU rRNA is the most common marker generally applied in the field of eukaryotic phylogeny, and with a wealth of sequence data reported from various *Plasmodium* species, phylogenetic inference of the *Plasmodium* tree based on the cyto-SSU rRNA sequences is always important and indispensable, especially when it entails the analysis and positioning of a new species. There are, however, inherent problems in using cyto-SSU rRNA genes in reconstructing *Plasmodium* phylogeny. Unlike most of the eukaryotic organisms analyzed so far, the *Plasmodium* species have divergent, paralogous copies of cyto-SSU rRNA gene. For example, in *P. falciparum*, sequence difference among the multi-copy genes amounts to as high as 11% (McCutchan et al., 1988). Different genes are expressed at various stages of the parasite life cycle. The presence of asexual- (A-) and sporozoite- (S-) types has been reported in *P. falciparum*, *P. berghei*, *P. vivax*, and several other species (Rogers et al., 1998; van Spaendonk et al., 2001). An additional oocyst- (O-) type has also been reported in *P. vivax* (Li et al., 1997). These gene duplication events could potentially complicate inference of *Plasmodium* phylogeny, because an inferred tree always reflects the history of gene duplication and speciation events. However, this issue has not been confronted in previous attempts to infer a species tree, with most reports largely focusing only on the A-type-like genes (Qari et al., 1996; Leclerc et al., 2004).

Recently, the underlying mechanism maintaining different copies of cyto-SSU rRNAs in apicomplexan parasites, the genus *Plasmodium* and the genus *Cryptosporidium*, has been investigated (Rooney, 2004). In the simian *Plasmodium* + *P. vivax* clade of the *Plasmodium* tree, different SSU rRNA genes were found to form three clades in a between-species clustering manner, which was explained by a birth-and-death model under strong purifying selection. In this model, new genes are created by gene duplication and some duplicate genes stay in the genome for a long time, while others are inactivated or deleted from the genome. The model is in contrast to the concerted evolution model, in which all member genes of a family evolve as a unit in concert (for review see Nei and Rooney, 2005). Since eukaryotic rRNA genes were generally considered to evolve in a concerted manner, the finding that cyto-SSU rRNAs of the genus *Plasmodium* evolve in the birth-and-death manner was a novel insight. However, even though Rooney (2004) used all *Plasmodium* cyto-SSU rRNA sequences available at that time, the *Plasmodium* species originating from simian taxa were limited in number. In this case, we wondered whether additional patterns not detected in Rooney (2004) might be revealed through further analysis of the cyto-SSU rRNA phylogeny when genes from additional species are considered, especially in the simian *Plasmodium* + *P. vivax* clade.

In this work, we cloned and sequenced the cyto-SSU rRNA genes from eight simian *Plasmodium* species (*P. gonderi*, *P. fragile*, *P. coatneyi*, *P. inui*, *P. hylobati*, *P. fieldi*, *P. simiovale*, and *P. cynomolgi*). Natural host and geographic distribution of these species are summarized in Table 1, together with those of *P. knowlesi* and *P. vivax*. *P. gonderi* is found in African monkeys while other species are found in Asian monkeys mainly in *Macaca* spp. *P. hylobati* is considered to have switched its host from macaques to gibbons (Mu et al., 2005). The human parasite, *P. vivax*, has been shown to be closely related to these simian parasites, but its closest relative has not been clearly established (Escalante et al., 2005). Close relationships between *P. inui* and *P. hylobati*, between *P. coatneyi* and *P. knowlesi*, and between *P. simiovale* and *P. fieldi* and the earliest branching status of *P. gonderi* in the simian *Plasmodium* tree have been suggested by several different phylogenetic markers (Perkins and Schall, 2002; Vargas-Serrato et al., 2003; Leclerc et al., 2004; Escalante et al., 2005; Tanabe et al., 2007). However, the positions of other species and the branching order among the simian *Plasmodium* + *P. vivax* clade, except for *P. gonderi*, remains to be determined.

Here, we report the sequences of A- and S-type-like cyto-SSU rRNA genes of the above eight simian *Plasmodium* species. Detailed alignment analysis together with exploration of the secondary structure model of the *P. vivax* A-type cyto-SSU rRNA demonstrated an approximately 50-residue insertion in the V7 variable region near the stem 43 is shared exclusively by the S-type-like sequences of the Asian simian *Plasmodium* species and the S- and O-type sequences of *P. vivax*. Phylogenetic analyses including all sequences from the human, simian, rodent, and avian *Plasmodium* species demonstrated that