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Short communication

Census, molecular characterization and developmental expression of Leucine-Rich-Repeat proteins in *Plasmodium falciparum*☆

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Abstract

The capacity of Leucine-Rich Repeat or LRR proteins to interact with many ligands enables them to contribute to important cellular functions ranging from the regulation of the cell cycle to protein trafficking and signal transduction. In *Plasmodium falciparum*, little is known about the expression of these LRR proteins. Here, we identified the PfLRR genes and determined their transcriptional expression during the intraerythrocytic parasite life cycle. Exhaustive analysis of the *P. falciparum* genome revealed 14 potential genes encoding LRR-containing proteins, designated from PfLRR1 to PfLRR14. Molecular cloning and sequencing of the corresponding cDNA indicated that all PfLRRs contain 4–10 LRR motifs. Real-time quantitative PCR revealed that most of genes are highly expressed in late intraerythrocytic stages, including late trophozoites and schizonts. The ability of *P. falciparum* to express LRR-containing proteins will enable further investigations into the parasite interactome and create opportunities for discovering candidate drug targets.

Keywords: *Plasmodium falciparum*; Leucine-Rich Repeat (LRR); Ligand–protein interactions; Genomes

Protein–protein interactions through Leucine-Rich Repeats (LRR) are widely utilized in cellular communications from viruses to mammalian cells. So far, more than 2000 LRR-containing proteins have been found, expressed not only as soluble molecules but also as transmembrane proteins and described as participating in a variety of biological important processes [1]. The LRR motifs consist of 20–29 amino acid residues and contain a conserved 11-residue region with the consensus sequence LXXLXLXXN/CXL (X = any amino acid) [2]. Valine, isoleucine and phenylalanine have also been identified in the positions occupied by leucine [1]. The 3D structural analysis of several LRR proteins indicate the presence of a horseshoe-shaped protein structure within a single domain formed by a minimum of four LRR motifs, each with a conserved β-sheet on the inner side and more divergent and predominantly α-helical fragments on the outer side [3–5].

Recent structure-function studies showed that the tandem arrays of LRR constitute an interface in host–pathogen binding [6,7]. In many species, LRR proteins are well known to play important roles in innate immunity as pathogen recognition factors, such as NACHT-LRR proteins, Toll-Like Receptors (TLR) and plant R genes [7–9]. In humans, the TLRs (TLR1 to TLR10) that have been described to contain a series of LRR motifs interact with Pathogen Associated Molecular Patterns (PAMPs) from bacteria, viruses and fungi [7]. They have been shown to function according to a bipartite process, in which the LRRs dictate ligand binding and the cytoplasmic domain facilitates signal transduction. Interestingly, more recent studies using genetic approaches showed that silencing of two LRR proteins in *Anopheles gambiae*, *Anopheles plasmodium*-responsive Leucine-Rich Repeat 1 (APL1) and Leucine-Rich Repeat immune gene (LRIM1) con-

☆ *Note:* The nucleotide sequences reported in this paper have been submitted to the GenBank™ Data Bank with accession numbers: AY898265 for PfLRR1, AY898266 for PfLRR2, AY898267 for PfLRR3, AY898268 for PfLRR4.1, AY898269 for PfLRR4.2, AY898270 for PfLRR4.3, AY898271 for PfLRR5, DQ017480 for PfLRR6, AY898272 for PfLRR7, DQ017481 for PfLRR8, DQ017482 for PfLRR9, AY898273 for PfLRR10, DQ017483 for PfLRR11, DQ017484 for PfLRR12, DQ017485 for PfLRR13, EF570415 for PfLRR14.1 and AY898274 for PfLRR14.2.

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tributed to a significant increase in *Plasmodium berghei* oocytes numbers, indicating that these proteins mediate protection from *Plasmodium* infection [10,11]. In the case of plants, the LRR proteins react with pathogens and induce local programmed cell death to control their propagation [12–14].

LRR-containing proteins expressed by pathogens also play a role in interactions with the host, notably in the host invasion phase. For instance, the *Leishmania* parasite interacts with and invades macrophages through a ligand that includes LRR motifs, the parasite surface antigen 2 [15]. In a recent study in *Plasmodium falciparum*, we described the characterization of a potential *PfPP1* (*P. falciparum* protein phosphatase type 1) regulatory binding protein belonging to the Leucine-Rich-Repeat protein family designated PfLRR1 (*P. falciparum* Leucine-Rich Repeat 1) [16]. In the related protein from *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*, sds22, the LRR regions play an essential role in its association with and consequently activation of, the protein phosphatase type 1 involved in yeast cell division [17]. The gene product of *PfLRR1* is capable of binding to PfPP1, of inhibiting its activity, and of overriding a G2/M cell-cycle checkpoint in *Xenopus* oocytes. In the parasite, *PfLRR1* mRNA and protein are highly expressed in young trophozoites and schizonts, respectively, with lower levels detected in ring stage parasites. In contrast, *PfPP1* gene expression appears to be constitutive, suggesting that PfPP1 activity is regulated by interactors, such as PfLRR1. Affinity-pull down studies reveal that both PfLRR1 and PfPP1 are present in the cytoplasm and nucleus, although their molecular interaction takes place only in the nuclear compartment (unpublished results). These data suggest that the PfLRR1 protein may play a critical role in the regulation of the *P. falciparum* cell cycle through modulation of phosphatase activity.

The wealth of information available on the crucial role of LRR proteins in cellular functions have emphasized the importance of identifying LRR-containing proteins in *P. falciparum* that could serve as a starting point for future fundamental research and the identification of possible therapeutic targets. Hence, we initiated a search for the presence of LRR encoding genes in the *P. falciparum* genome by screening the predicted orfs present in the public database, PlasmoDB (http://www.plasmodb.org), using as queries the LRR motif [ILV]XX[ILV]X[ILV]XX[NC]X[ILV], the regions containing many LRRs present in human TLRs (AAC34137, AAC34133, AAC34134, AAC34135, AAC34136, NP_619542, NP_619633) or the sds22 LRR proteins described in *S. pombe* and *S. cerevisiae* (AAA35342 and NP_012728, respectively). Analysis of the current *P. falciparum* database allowed the identification of a large number of orfs (open reading frames) which contain single LRR motifs and 14 orfs with repeated motifs that could be potentially assigned to the LRR protein family (Table 1). The retrieved sequences were designated as PfLRR proteins based on the presence of at least four consecutive LRR signature motifs, four motifs being the minimum number so far described for an LRR protein to interact with a ligand [1].

As a first step towards the molecular identification of LRR proteins in *P. falciparum,* forward and reverse primers derived

Table 1
Identification, molecular characterization and relative expression of genes encoding Leucine-Rich-Repeat proteins in the *Plasmodium falciparum* genome

Designated names (gene identifier number)	Genbank accession number	Size (pb)	Number of LRR motifs	Similarity with known domains	Relative expression of PfLRR
PfLRR1 (PF10_0320)	AY898265	741	10	–	Young trophozoites
PfLRR2 (PFE0455w)	AY898266	1017	6	–	Late trophozoites
PfLRR3 (PF14_0496)	AY898267	1017	9	–	Rings, young trophozoites, late trophozoites
PfLRR4.1 (MAL13P1.238)	AY898268	771	6	–	
PfLRR4.2	AY898269	570	6	–	Late trophozoites
PfLRR4.3	AY898270	537	6	–	
PfLRR5 (PF14_0305)	AY898271	891	8	–	Late trophozoites
PfLRR6 (PFF0595c)	DQ017480	5595	7	TFII A (transcription factor IIA)	Late trophozoites
PfLRR7 (MAL8P1.46)	AY898272	588	4	–	Late trophozoites, schizonts
PfLRR8 (PFI1470c)	DQ017481	1020	5	–	Schizonts
PfLRR9 (PFI0330c)	DQ017482	1467	6	–	Young and late trophozoites
PfLRR10 (PF11-0476)	AY898273	954	6	DNA topoisomerase IV	Late trophozoites
PfLRR11 (PF11_0243)	DQ017483	2445	7	DNA gyrase B	Late trophozoites
PfLRR12 (PFL2380c)	DQ017484	2592	10	–	Late trophozoites
PfLRR13 (PFL1360c)	DQ017485	1344	4	FTH (F-box associated domain)	Late trophozoites
PfLRR14.1 (PF14_0651)	EF570415	750	4		Late trophozoites
PfLRR14.2	AY898274	887	Stop codon (87–90)	–	Late trophozoites

PfLRR genes are designated from *PfLRR1* to *PfLRR14* and their accession numbers (Genbank) are listed. Oligonucleotides were designed to contain the start and stop codons of the open reading frames detected *in silico*. PCR assays were performed on cDNA obtained by RT on total RNA from asynchronous culture pretreated with DNase (Ambion). The sizes of PCR products are listed. The PCR products were cloned in TOPO TA vector (Invitrogen) and three to five positive clones were sequenced in both orientations. The number of LRR motifs and the presence of known domains were determined using the software http://scansite.mit.edu. For PfLRR6, 10, 11 and 13, the similarities observed were 40% with TFIIA (spanning 317a.a., accession number PF03153), 75% with DNA topoisomerase IV (spaning 25 a.a., accession number PF00521), 43% with DNA gyrase B (spanning 128 a.a., accession number PF01119) and 52% with FTH (spanning 129 a.a., accession number PF01827), respectively. The stage specific expression of *PfLRR* genes are indicated in the right column (for experimental details and data see Fig. 2).