Trends in Parasitology

CellPress

Review

# Three Rosetting in *Plasmodium falciparum*

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**The intracellular malaria parasites extensively modify host erythrocytes to allow nutrient uptake, ensure homeostasis, and evade the host's immune response. To achieve this, the parasite exports several proteins to the erythrocyte surface. In *Plasmodium falciparum,* the parasite responsible for the most severe form of human malaria, three major variant surface antigen families – PfEMP1, STEVOR, and RIFIN – have been implicated in contributing to immune evasion, parasite sequestration, and parasite-mediated rosetting of uninfected erythrocytes. Sequestration and rosetting have been linked to parasite-mediated pathology, making the variant surface antigens of *P. falciparum* major virulence factors. Here we review our current understanding of rosetting mechanism, recent findings of STEVOR, RIFIN-mediated rosetting, and their implication on the severity and pathology of the disease.**

## Trends

Rosetting, the binding of uninfected RBCs to a parasite-infected RBC (iRBC), has been directly linked to the severity of clinical disease.

Three parasite protein families, PfEMP1, STEVOR, and RIFIN, mediate rosetting in *Plasmodium falciparum*.

Sequential timing of surface expression of PfEMP1, RIFIN, and STEVOR on iRBC suggests that the parasite has developed three different rosette formation mechanisms, implicating a critical function for parasite survival.

PfEMP1 mediates rosetting through CR1, heparan sulfate, and blood group A antigen on RBCs. STEVOR mediates rosetting through glycophorin C and, RIFIN mediates rosetting predominantly to blood group A antigen as well as glycophorin A on the surface of the RBC.

## Variant Surface Antigens of *Plasmodium* Parasites: A Role in Rosetting

*Plasmodium* parasites have a complex life cycle involving a mosquito vector and a mammalian host. The parasite transitions through various stages of growth within the host body, infecting the liver as a sporozoite and undergoing schizogony and gametocytogenesis within the erythrocyte [1]. Disease symptoms in the host occur during the asexual replication of the parasite in the erythrocyte due to the release of inflammatory cytokines, anemia caused by the destruction of erythrocytes, and obstruction of blood flow caused by parasite sequestration [2,3]. During its complex developmental cycle the parasite extensively modifies the surface of the infected red blood cell (iRBC). These surface modifications are directly linked to the ability of the trophozoite and schizont stages to sequester within the deep tissues of the host by binding to endothelial cell receptors and are a significant contributor to the morbidity caused by *P. falciparum.* As the parasite matures intracellularly, it exports several proteins that are important for its survival and sequestration. Three major variant surface antigen (VSA) families have been identified in *P. falciparum*: *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) encoded by the *var* genes [4], subtelomeric variant open reading frame (STEVOR) encoded by the *stevor* genes, and repetitive interspersed repeats (RIFIN) encoded by the *rif* genes [5]. Of these three families, PfEMP1 has been most extensively studied and has been shown to mediate both antigenic variation as well as adhesion of the iRBC [6]. Another striking feature of iRBCs is their ability to bind to uninfected red blood cells (RBCs) forming rosettes. The biological role of rosetting is not clear though some studies have linked it to parasite virulence [7–9]. PfEMP1 has been shown to mediate the binding to a variety of host cell receptors, including **CD36**, intercellular adhesion molecule 1 (**ICAM1)** and chrondroitin sulfate A (**CSA)** (see Glossary). The binding of PfEMP1 to complement receptor 1 (**CR1**) on the RBC has been shown to lead to rosetting [10]. Recent studies have shown that, in addition to PfEMP1, both STEVOR and RIFIN are able to mediate rosetting by binding to glycophorin C and blood group A antigen, respectively [11,12] (Figure 1). In addition, cumulative evidence from different studies show that the transcription and timing of surface expression of PfEMP1, RIFIN, and STEVOR from

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laboratory-cultured parasite is sequential though RIFIN and PfEMP1 appear on the erythrocyte surface at the same time [13–17] and reviewed in [18,19] (Figure 1A). This suggests that the parasite has developed three different mechanisms that mediate rosette formation suggesting a critical function for parasite survival.

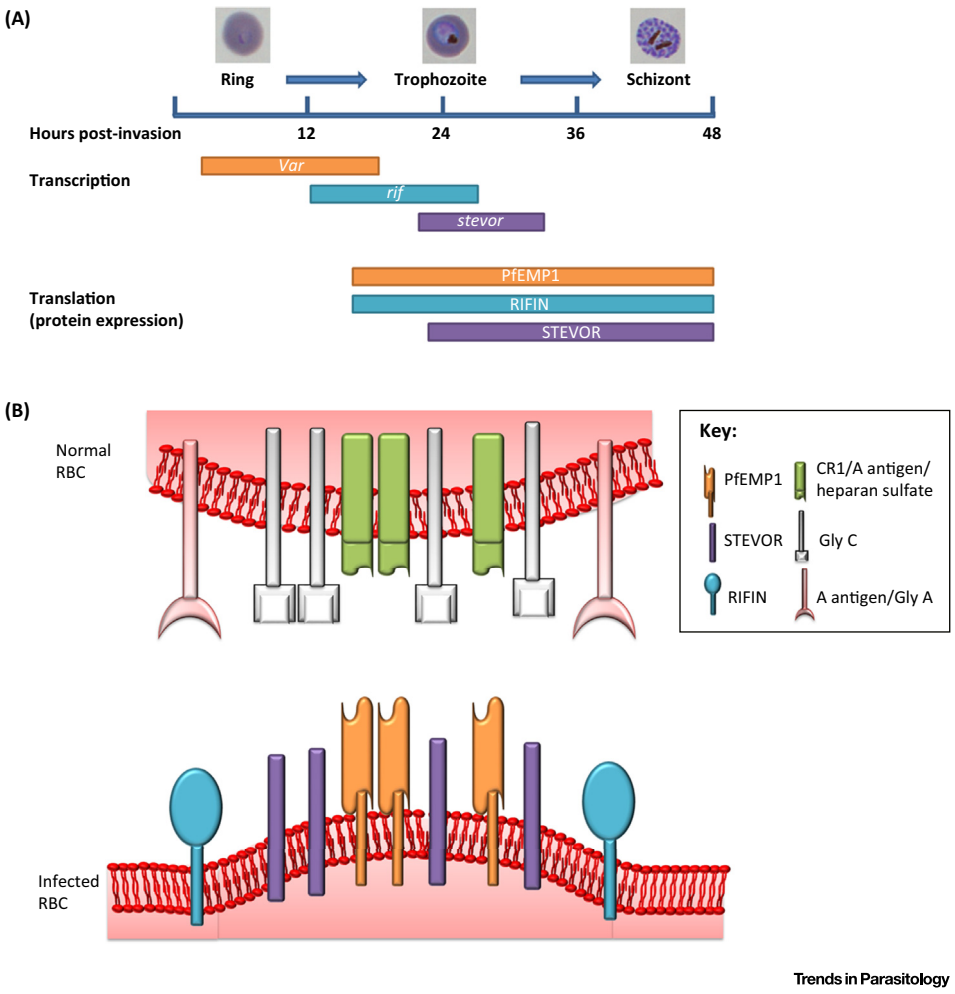


Figure 1. The Three Major Multigene Families in *Plasmodium falciparum*. (A) Schematic expression profile of *var*, *rif*, and *stevor* multigene families during asexual developmental stage of the laboratory-adapted cultured parasite. Transcription of the *var* gene starts early in the ring stage parasite followed by the *rif* and *stevor* in the late ring/early trophozoite stage. Despite differences in the timing of transcription, both PfEMP1 and RIFIN proteins appear on the infected RBC (iRBC) surface during late ring/early trophozoite parasite stages, while STEVOR appears on the surface later during the late trophozoite/schizont stage. At the late schizont parasite developmental stage, all three of these variant antigens are present on the parasite surface. (B) Rosetting phenotype of PfEMP1, RIFIN, and STEVOR. These variant antigens expressed on the surface of the iRBC mediate rosetting to uninfected RBC using different receptors expressed on the host cell surface. PfEMP1, located at the knobs, mediates rosetting via binding to CR1, heparan sulfate, and blood A antigen on the RBC surface. Variations in the DBL1α domain of PfEMP1 involve interaction with different receptors, resulting in different degrees of rosetting and disease severity. STEVOR appears to cluster at the base of the knobs and mediates rosetting via binding to glycophorin C (Gly C) receptor. RIFIN-mediated rosetting forms giant rosettes via binding to the blood group A antigen on RBC, and forms smaller rosettes via binding to glycophorin A (Gly A) receptor. RIFIN-mediated rosettes also appear to protect PfEMP1 from antibody recognition [83]. In all, the parasite has developed three different mechanisms that mediate rosette formations, suggesting a critical function for parasite survival. Abbreviations: PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1; RIFIN, *repetitive interspersed repeats*; STEVOR, *subtelomeric variable open reading frame*; iRBC, infected red blood cell; DBL, *Duffy* binding ligand; CR1, Complement receptor 1.

## Glossary

**CD36:** cluster of differentiation 36 is an integral membrane protein found on the surface of many cell types such as blood cells, platelets, spleen cells,and some skin endothelial cells. CD36 binds to a broad range of ligands which can be of proteinaceous and lipidic nature, such as collagen, thrombospondin, lipoprotein, and phospholipids, as well as red blood cells parasitized with *Plasmodium falciparum.*

**CR1:** complement receptor type 1 (CR1) encoded by the CR1 gene belongs to the regulators of complement activation family. It is a monomeric single-pass type 1 transmembrane glycoprotein expressed on the blood cells, leukocytes, and mircoglia. CR1 is a receptor for complement components C3b/C4b which helps in the regulation and activation of the complement immune system. CR1 is also responsible for *Plasmodium falciparum* rosetting.

**CSA:** chondroitin sulfate A (CSA) is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating *N*-acetylgalactosamine and D-glucuronic acid and sulfate residues in equimolar quantities where carbon 4 of the *N*-acetylgalactosamines is sulfated. It is usually attached to proteins as part of a proteoglycan and found in skin, cornea, cartilage, and umbilical cord. CSA is found to be the receptor for sequestration of *Plasmodium falciparum* in the placenta.

**Heparan sulfate (HS):** a linear polysaccharide that occurs as a proteoglycan to which two or three HS chains are attached closely. This glycoprotein is found at the cell surface and in the extracellular matrix where HS binds a large number of protein ligands to regulate a range of biological activities. HS also serves as cellular receptor for various viruses and pathogens.

**ICAM-1:** intercellular adhesion molecule-1 (ICAM-1), also known as CD54 (**C**luster of **D**ifferentiation 54), is a glycoprotein typically expressed on the surface of endothelial cells and leukocytes. It has a single transmembrane domain with an N-terminal extracellular domain and a C-terminal cytoplasmic tail.

**Thrombospondin:** multimeric multidomain glycoprotein found at cell surfaces and in the extracellular