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Molecular mechanism of erythrocytic invasion by the merozoite stage of *Plasmodium falciparum*

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ABSTRACT

Malaria is an ancient disease that continues to cause enormous human morbidity and mortality mainly due to the invasion of the erythrocyte by the merozoite stage of the parasite (*Plasmodium falciparum*), as the parasite spends much of its life intracellularly. Therefore, the aim of this article is to review the level of knowledge on the mechanism of erythrocytic invasion by the merozoite of *P. falciparum*. The invasion process is a coordinated series of protein/protein interactions, protease cleavage events, intracellular signals, organelle release, and engagement of actin–myosin motor. The invasion is achieved through either sialic acid (SA)-dependent pathway, which uses erythrocyte-binding antigen 140 (EBA-140), EBA-175, EBA-181, and PfRH1, or SA independent pathway, which uses PfRH2 and PfRH4. The process of successful invasion is completed in the following steps: merozoite activation, merozoite binding, reorientation and deformation, junction formation, and parasite entry. Merozoite activation involves egresses of the merozoite from the erythrocyte, where exoneme secretes the first protein called subtilisin-like protease-1 (PfSUB-1) into the parasitophorous vacuole, which ensures primary proteolysis of serine-repeat antigen which in turn acts on the parasitophorous vacuole and red blood cells to initiate host cell rupture. Dipeptidyl-peptidase 3 also contributes to the egress by regulating the maturation of PfSUB-1. The initial interaction between the merozoite and the erythrocyte is due to random and weak collisions and presumptively involves, in the first instance, a reversible interaction between proteins on the merozoite surface and the host erythrocyte. Here, the ligands bind to specific receptors on the merozoite, where EBA-175 binds to glycophorin A, EBA-140 binds to glycophorin C, erythrocyte-binding-like-1 protein binds to glycophorin B, PfRh4 binds to complement receptor-1, and PfRh5 binds to basigin (CD147) on the erythrocyte. This is followed by permanent positioning of the apical organelles of the merozoite on to the red blood cells (reorientation) and release of adhesive proteins (adhesins), protease, and membrane-altering agent, specifically subtilisin-like protease-2 (PfSUB-2) and apical membrane antigen-1 (AMA1). Junction formation is an intimate circumferential contact with the host cell called tight junction and it is formed under the influence of AMA1 and rhoptry neck. Finally, the merozoite penetrates the erythrocyte bilayer with the help of the actin–myosin motor, TRAP, and aldolase.

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1. Introduction

Malaria is a life-threatening, parasitic disease and is considered as a complex and overwhelming public health problem. The disease is an ancient disease that continues to cause enormous human morbidity and mortality. Human malaria occurs after the bite of an infected female Anopheles mosquito carrying the *Plasmodium* parasites (sporozoites); these parasites invade the liver, and subsequently, merozoites invade the red blood cells (erythrocyte), giving rise to periodic shivering, pyrexia, and sweating with enlargement of the spleen. This may be followed by severe anemia and, in some cases of malignant tertian malaria, with local blocking of capillaries in individual organs (Francis et al., 2014). The disease emerges mostly in tropical and subtropical regions around the world. Five *Plasmodium* species infect humans: *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. *Plasmodium falciparum* is the most pathogenic and most frequently associated with mortality (Ararat-Sarria et al., 2019). Pregnant women and children below the ages of five are most vulnerable to the disease due to low immunity.

The *Plasmodium* species is a delicate unicellular organism that cannot survive in free form for more than a couple of minutes in the bloodstream. So, for much of its life in the human host it only lives intracellularly, with only brief direct exposure of the "naked" parasite to the immune system (Moxon et al., 2011). Therefore, the parasites shuttle between replicative and motile life-cycle stages, with the motile forms or "zoites" (sporozoites or merozoites) highly adapted to the invasion of host cells. During the stages of blood infection, the merozoite targets and invades the red blood cells (RBCs) rapidly (<30 seconds) (Koch et al., 2017). The erythrocyte is the most preferred way of attack for *P. falciparum*, simply because it constitutes the largest population of blood cells and contains high concentrations of iron-rich hemoglobin. Merozoites invasion of red blood cells is an essential step in parasite replication and it is responsible for all clinical manifestations of the disease and is the leading cause of mortality.

Therefore, the invasive forms of the malaria parasites are all members of the phylum Apicomplexa characterized by a unique set of organelles, like the rhoptries, micronemes, dense granules, and to some extent exonemes, that play a crucial role in the invasion process (Iyer et al., 2007). The invasion process consists of multiple molecular events during which the proteins of the RBC membrane and merozoite-coat are engaged in specific receptor–ligand interactions to form unique invasion pathways (Baldwin et al., 2015). In other words, it requires a coordinated series of protein/protein interactions, protease cleavage events, intracellular signals, organelle release, and engagement of actin–myosin motor. The complete mechanism of erythrocytic invasion by merozoite is completed in the following steps: merozoite activation, initial merozoite binding, reorientation and erythrocyte deformation, junction formation, and parasite entry.

2. Materials and Methods

2.1. Literature search

In order to obtain reliable and authentic research and published articles on the topic, the following databases were visited: PubMed, Web of Knowledge, EMBASE, Web of Science, Scopus, Google scholar, World Health Organization's WHOLIS, and Medline.

2.2. Search terms used

Search terms that were directly or indirectly linked to the mechanism of erythrocytic invasion by the merozoite of *P. falciparum* were used in order to generate relevant research articles. The following search terms were specifically used: merozoite invasion, merozoite surface protein (MSP), erythrocyte binding, apical membrane antigen, subtisilin-1 and 2, activation process of merozoite, proteolytic processing, secondary processing, and protein involved in merozoite invasion. All research articles generated were carefully and critically analyzed and scrutinized, after which the articles were grouped and classified based on the various headings and subheadings of the title of the reviewed topic.

3. Activation of the Merozoite

The initial stage of erythrocytic invasion by the merozoite involves the activation or priming of the merozoite prior to the invasion process. The initial priming process begins with an egress either from the hepatocyte or erythrocyte (Cowman et al., 2012). The egress of *P. falciparum* merozoites from RBCs requires breaching of multiple barriers, including the parasitophorous vacuole membrane, the host cytoskeleton, and the host plasma membrane. Parasite egress is synchronized with the completion of it replicative cycle

when the next generation of invasive parasites have developed fully (Singh and Chitnis, 2017). Multiple protein processing events occur to initiate the egress of mature merozoite from the schizont. This preparatory stage ensures that all ligands necessary for the erythrocytic invasion are well processed and ready to mediate the invasion process (Beeson et al., 2016).

Therefore, subpopulations and a subset of the secretary organelles, called exoneme, secrete the first erythrocyte protein called subtilisin-like protease-1(PfSUB-1) into the parasitophorous vacuole (Alan et al., 2016; Beeson et al., 2016). The main function of this protein (PfSUB 1) is to ensure the primary proteolysis of serine-repeat antigen, which in turn acts on the parasitophorous vacuole and red blood cells to initiate host cell rupture. The second protein is dipeptidyl peptidase-3, which contributes to the egress by regulating the maturation of PfSUB-1 (Dhangadamajhi et al., 2010). PfSUB-1 is also responsible for extensive proteolytic processing (Cleavage) of major MSPs, including MSP1, which is processed from the high molecular mass protein (MW 180 kDa) to $MSP1_{83}$, $MSP1_{30}$, $MSP1_{38}$, and the C-terminal glycosylphosphatidylinositol (GPI)-anchored $MSP1_{42}$ fragments (Cowman et al., 2012; Marshall et al., 1997; Moss et al., 2011; Soares et al.,1997). The primary processing coincides with the merozoite maturation, while the secondary proteolytic processing coincides with merozoite invasion. In the secondary proteolysis, $MSP1_{42}$ is cleaved or processed into MSP_{33} and the glycosylphosphatidylinositol-anchored C-terminal MSP_{19} (Kauth et al., 2006).

This is the only protein that is carried into the host cell; the others are shed with the other protein complex (Fleck et al., 2003; Thái et al., 2018) as shown in Figure 2.

The main function of secondary proteolytic processing is to expose the epidermal growth factor-like modules which strengthen the interactions between the merozoite and the erythrocyte. The exact role or function of these cleavages and/or processing is not well known, but it is very essential for erythrocytic invasion. Figure 1 shows the processes of activation.

4. Erythrocyte Invasion Process

Erythrocyte invasion is a complex molecular process which involves the interaction between host (erythrocyte) and parasitic molecular proteins (Fleck et al., 2003).The major proteins that are usually found at the merozoite surface and also play a very vital role during invasion are MSP-1, MSP-2, MSP-3, MSP-4, MSP-5, MSP-6, MSP-7, MSP-8, MSP-9, and MSP-10 (Claire et al., 2009; Jäschke et al., 2017; Kauth et al., 2006). MSP-1 is a large molecular mass that weighs about 200 kDa, and it is usually synthesized during the late trophozoites and schizont stages (Jennings et al., 1998).

Successful invasion of human erythrocytes by *P. falciparum* merozoites requires infection of the host and parasitic survival. The early stages of invasion are mediated via MSPs that interact with human erythrocytes (Lin et al., 2016). Merozoite entry into erythrocytes is driven by complex recognition events between parasitic proteins and sugar moieties on the host RBCs. Erythrocyte invasion by *P. falciparum* merozoite is achieved through two distinct pathways: SA-dependent and SA independent pathways (Goel et al., 2003). *P. falciparum* heavily depend on

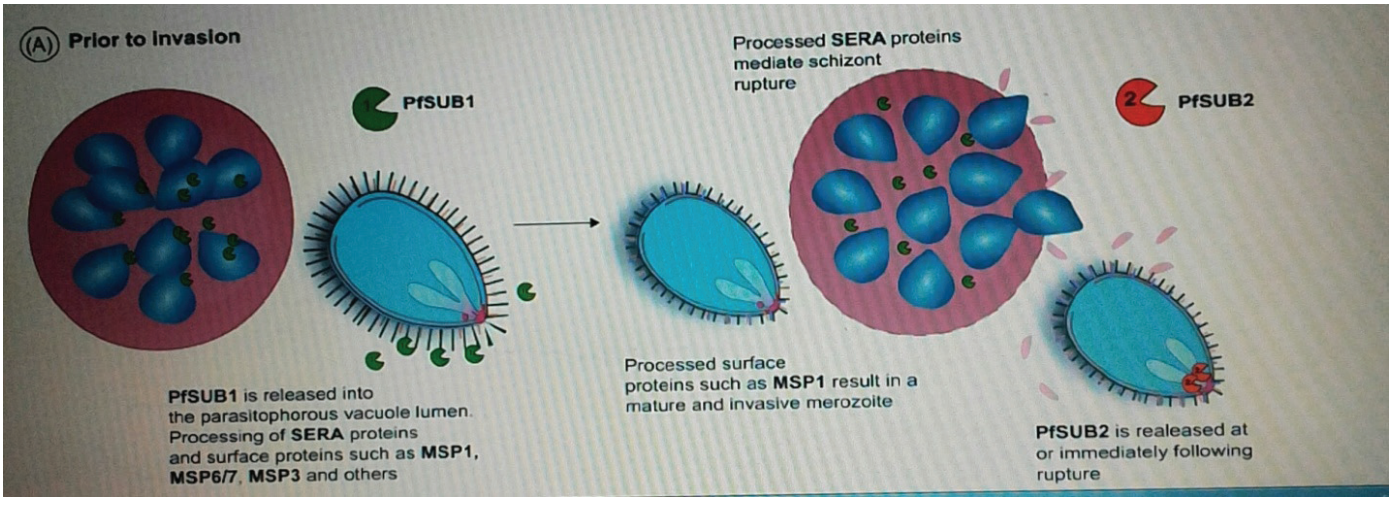

Figure 1. Activation process of the merozoite.

the SA residue located on the erythrocyte, i.e., EBA-140, EBA-181, EBA-175, and PfRH1 are involved in SA-dependent invasion, but in the absence of this, *P. falciparum* use the SA-independent pathway for the invasion (Chandramohanadas et al., 2014). So, PfRH2 and PfRH4 are important proteins in SA-independent invasion pathways, as shown in Figure 3.

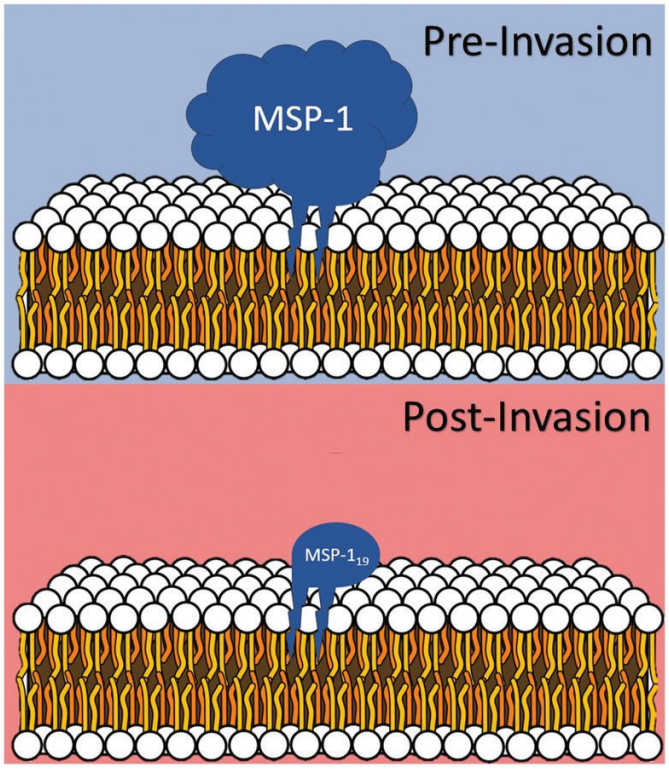

Figure 2. Primary and Secondary Proteolytic Processing during the erythrocyte invasion by the merozoite.

3.1. Initial merozoites binding

The initial interaction between the merozoite and the erythrocyte is due to a random and weak collision and presumptively involves, in the first instance, a reversible interaction between proteins on the merozoite surface and the host erythrocyte. Several MSPs and AMAs have been described (Chandramohanadas et al., 2014). The AMA1 is held in the micronemes of merozoites inside the erythrocytes (Subhash et al., 2007). The most abundant MSP is the GPI-anchored MSP1 (Cowman et al., 2012; Lin et al., 2016). This protein is uniformly distributed on the merozoite and also antibodies against MSP-1 inhibit erythrocyte invasion; apart from that, MSP-1 does not bind to band three. For these reasons, it has now been established that MSP-1 is the mainstay for the successful invasion of the erythrocytes by the merozoites.

Therefore, the primary merozoite invasion is mediated by GPI-anchored MSPs; however, specific receptor–ligand interactions remain elusive. The subsequent binding of merozoites mainly depends on two major protein families, these are EBA and reticulocyte binding-like homologous (PfRH) protein families (Badiane et al., 2013). Different members of these ligands (adhesins) bind to specific receptors on the merozoite, for example, EBA-175 binds to glycophorin A, EBA-140 binds to glycophorin C, while erythrocyte-binding-like-1 protein (EBL-1) binds to glycophorin B, and PfRh4 binds to complement receptor-1,

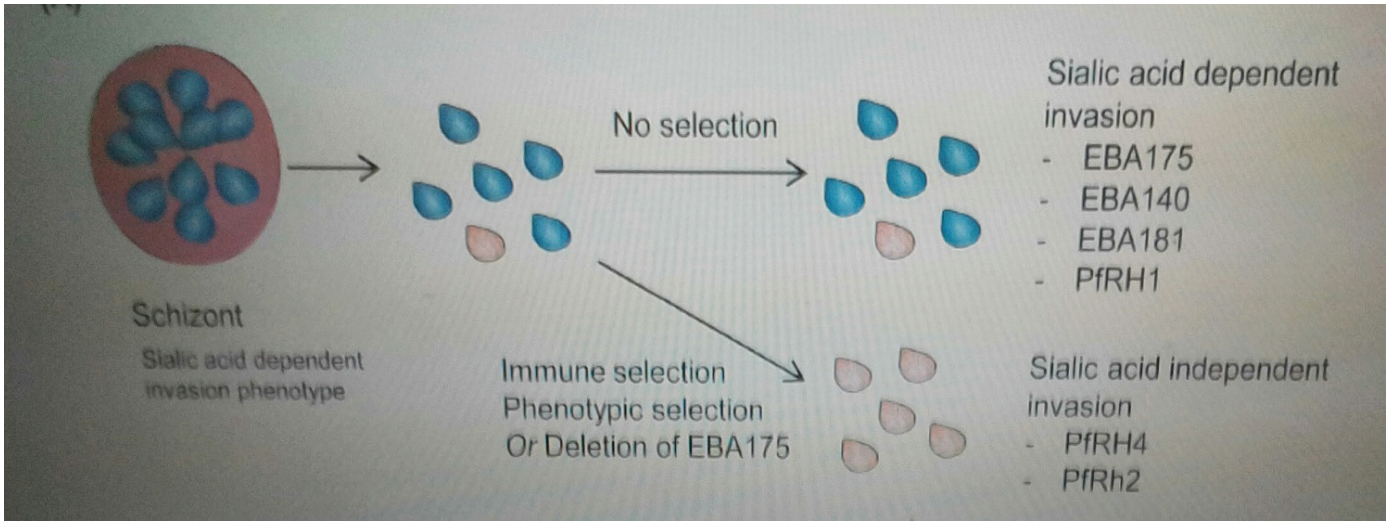

Figure 3. Proteins associated with SA-dependent and independent pathways during the invasion process.

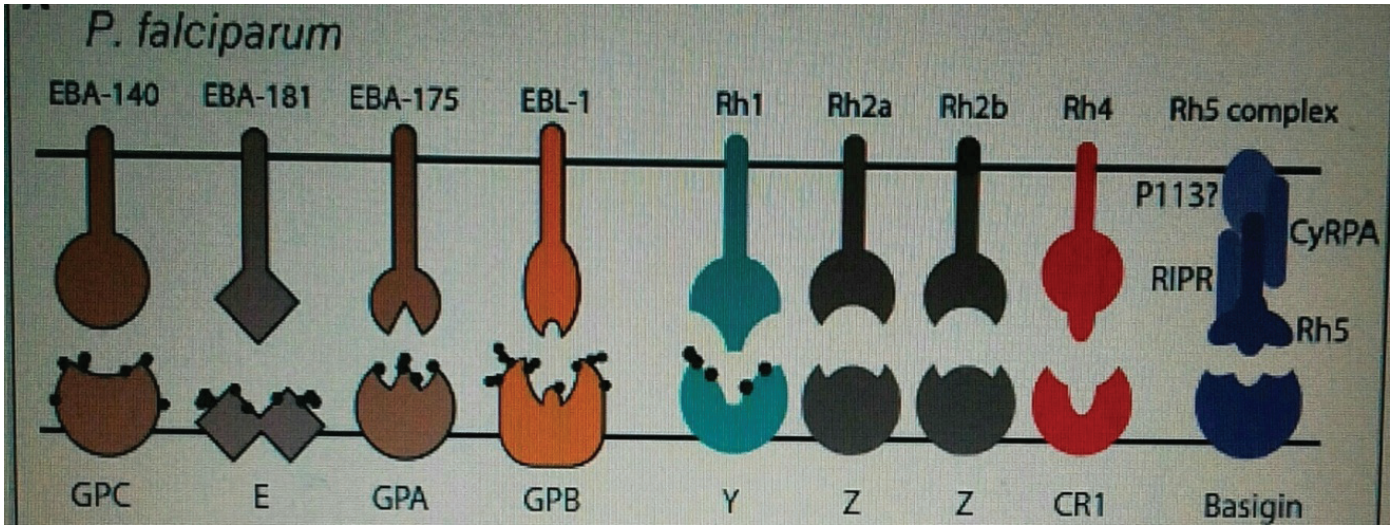

Figure 4. Receptor–ligands interaction during erythrocytic invasion by the merozoite.

while PfRh5 binds to basigin (CD147) on the erythrocyte (Cecila et al., 2012). Figure 3 shows *P. falciparum* ligand–receptor interaction during merozoite invasion of RBCs.

3.2. Reorientation and erythrocyte deformation

The initial weak attachment of the merozoite to the erythrocyte is followed by permanent attachment, and then the reorientation and erythrocyte deformation. Reorientation is a permanent positioning of the apical organelles of the merozoite on the RBCs (Dvorin et al., 2010). Merozoite reorientation coincides with a transient erythrocyte deformation. Like the initial attachment, where the apical organelles secretes some proteins for this attachment, so also during orientation and erythrocyte deformation, rhoptries and micronemes secrete some adhesive proteins (adhesins), protease and membrane-altering agents (Bannister and Sherma, 2009), specifically PfSUB-2, AMA1, cleaved $MSP1_{43}$, PfROM1, PfROM4, etc. (Beeson et al., 2016). These merozoite proteins are responsible for the formation of the parasitophorous vacuole, removal of the merozoite coat, and some adhesins, which allow parasite entry and junction formation.

3.3. Junction formation

During merozoite invasion, the merozoites of *P. falciparum* form an intimate circumferential contact with the host-cell called tight junction or moving junction. Tight Junction is a molecular aperture in the RBCs through which the parasite passes enroute to infection. The junction, acting as the key organizing nexus for invasion, is thought to correspond to a key molecular interaction between two classes of parasitic proteins: the rhoptry neck proteins (RONs), which are embedded in or under the target erythrocyte membrane (although parasite derived) and the microneme protein AMA1, which is present on the merozoite surface at the time of invasion (Riglar et al., 2016). AMA1 and RON protein are the proteins responsible for this tight junction formation (Giovannini et al., 2011). Immediately after successful reorientation, several merozoite proteins, especially those located on the micronemes and rhoptries, mediate high affinity attachments by the establishment of a high tight junction, and this in turn initiates the release of some merozoite proteins from the rhoptry (bulb) which also play a significant role in the formation of parasitophorous vacuole (Dreyer et al., 2012).

3.4. Parasite entry

Immediately after merozoite attachment and reorientation, the merozoite penetrate into the erythrocyte bilayer with the help of actin–myosin motor, proteins of the thrombospondin-related anonymous protein family and aldolase, creates a parasitophorous vacuole to seal itself from the host-cell cytoplasm, thus creating a hospitable environment for its development within the RBCs.

Figure 5 shows the erythrocytic invasion process by the merozoite.

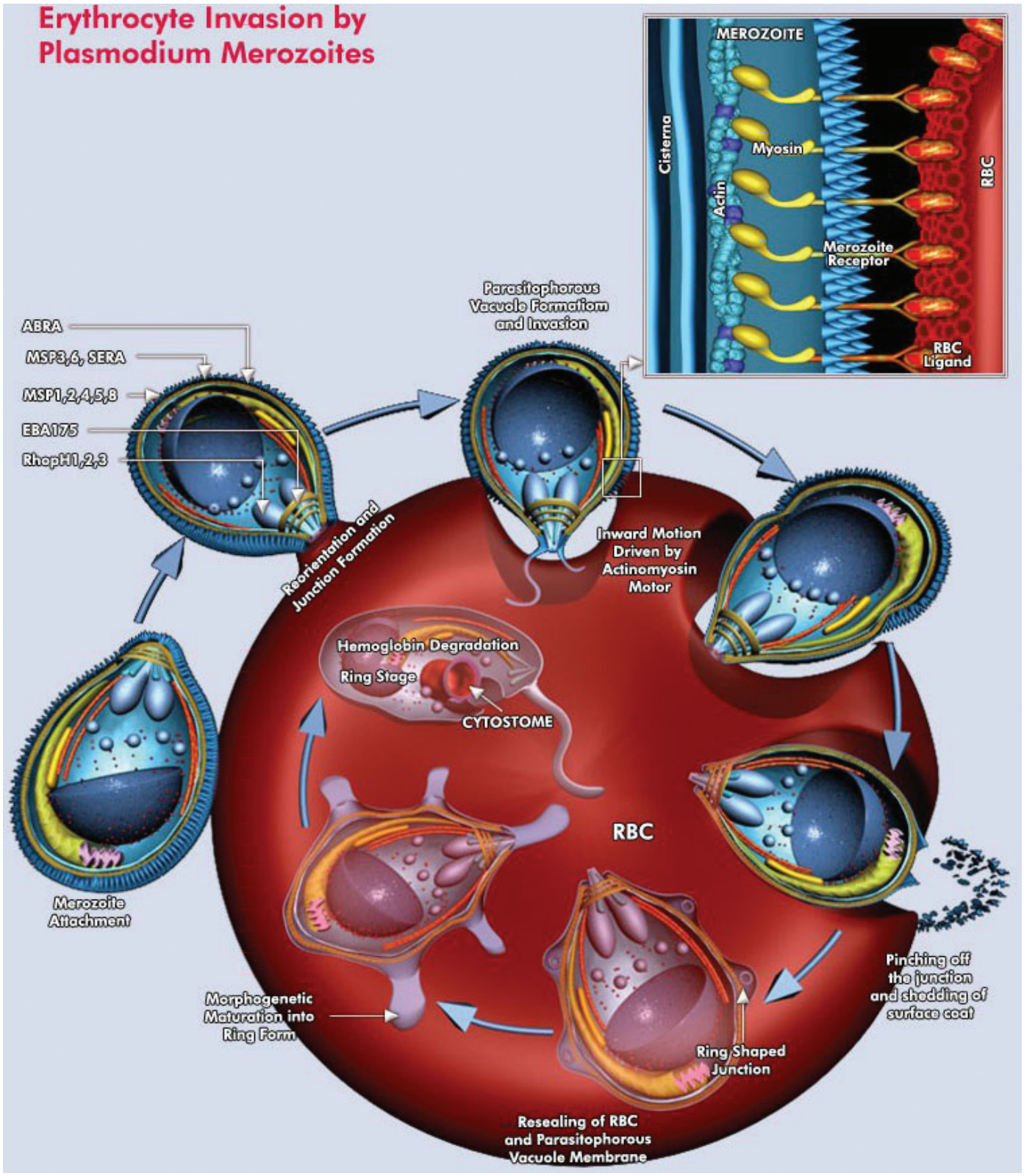

Figure 5. Steps involved in the erythrocyte invasion by the merozoites.

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