Role conserved c-terminal region plays in trafficking of ETRAMP 10.3, an asexual intraerythrocytic PVM protein

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Abstract

The ability of *Plasmodium falciparum* to remodel the host's cells drastically increases the virulence of this malaria parasite. One such source of remodelling is the formation of a parasitophorous vacuole (PV), which creates a controlled and hospitable environment within the host's cells. Certain parasite proteins that traffic to the parasitophorous vacuole membrane (PVM) have been found to be essential for the parasite's progression through its life-cycle. Some of these proteins share a conserved sequence in the C-terminus, which is thought to be a secretory signal motif. One such protein is ETRAMP 10.3, which is a protein up-regulated during the red blood cell (RBC) stage of the life-cycle and has been found to be essential for the intraerythrocytic asexual growth stage. Removal of this conserved sequence in another PVM protein, Pfs16, inhibits the proper trafficking of the protein to the PVM. It would be hypothesized that removal of the sequence of ETRAMP 10.3 would cause similar problems and possibly prevent the proliferation of the parasite in the blood. This proposal suggests the creation of two GFP (green fluorescent protein) tagged, transformed parasite lines and the use of epifluorescent microscopy to determine protein movement throughout parasite development with and without the conserved sequence.

Background

Each year millions of people are infected with the most severe malaria parasite, *Plasmodium falciparum*, and despite decades of vaccine research, an effective vaccine remains elusive. *Falciparum's* virulence is in part due to the parasite's ability to remodel its host's cells. An example of the parasite's ability to remodel is seen in the formation of a parasitophorous vacuole (PV), as the parasite invades both hepatocyte and erythrocyte cells (Cowman *et al*. 2006). The PV is surrounded by a membrane (PVM) which creates a controlled and hospitable environment for the parasite within the host cell (Cowman *et al*. 2006). Much of the PVM is created from the host membrane material; however, additional molecules are secreted from the parasite to the PVM (Cowman *et al*. 2006). Some *Falciparum* proteins, known as PVM proteins, are found to traffic to the PVM, including EXP-1 (PF11_0224), members of the ETRAMP gene family, and Pfs16. Studies have found some of these PVM proteins necessary for continuation of the parasite life-cycle. This includes the rodent malaria protein UIS4, which is essential to parasite proliferation in the liver stage of P. *berghei* and P. *yoelii* (Mueller *et al*. 2005). More recently, Mackeller *et al*. (2010) found evidence that ETRAMP 10.3 (PF10_0164), a protein up-regulated in the RBC stage during the transition from ring to trophozoite stage (Spielmann *et al*. 2002), is vital for intraerythrocytic asexual growth stage.

In a recent a study of PVM protein trafficking, Eksi and Williams (2011) provided evidence for a secretory signal motif for PVM proteins located in the proteins' C-terminus. The conservation of this domain was seen in members of the ETRAMP family, EXP-1, and Pfs16. They found that removal of this motif in Pfs16 inhibited the proper trafficking of Pfs16 to the PVM. ETRAMP 10.3 is known to possess this conserved sequence, but it is unknown whether the deletion of this sequence will also prevent movement of ETRAMP 10.3 to the PVM. Because it is essential for parasite proliferation in the RBC stage, the trafficking of ETRAMP 10.3 is of special interest (Mackeller *et al*. 2010). This study will use DNA constructs to determine the role of this motif in ETRAMP 10.3 trafficking. Previously, Mackeller *et al*. determined that ETRAMP 10.3 localizes to the PVM during the trophozoite intraerythrocytic stage and then progresses into the host erythrocyte, suggesting association with Maurer's Clefts (2010). If this motif serves as a PVM secretion signal for ETRAMP 10.3, we would hypothesize the removal of this motif would prevent localization to the PVM during the trophozoite stage and subsequent export into the host erythrocyte.

Specific Aims

1.To use the *Falciparum* transformation plasmid, pDH.TgA.22, to construct two *in vitro* transformed parasite lines tagged with GFP, pDH.TgA.22.10.3.FL.GFP and pDH.TGA.22.10.3.NM.GFP, to express the full length ETRAMP 10.3 protein sequence and the ETRAMP 10.3 sequence excluding the C-terminal motif, respectively.
2.To use epifluorescent microscopy to track the movement of the two ETRAMP 10.3 constructs (FL and NM) throughout the three stages of intraerythrocytic parasite development: ring stage, trophozoite stage, and schizont stage.

Experimental Protocol

Reporter Constructs
Two GFP reporter constructs will be created to determine the role of a secretory motif in trafficking of ETRAMP 10.3. These constructs will then be utilized to create two transformed parasite lines, pDH.TgA.22.10.3.FL and pDH.TGA.22.10.3.NM.

Eski and Williamson (2011) showed that ETRAMP 10.3 and Pfs16 show some conservation of amino acid sequence in the C-terminus. In ETRAMP 10.3, this motif is between amino acid 64 and 105 and overlaps with the transmembrane region of the C-terminus (53-75aa). This is consistent with the overlap of motif and transmembrane region seen in Pfs16. Due to this overlap, we will include the entire transmembrane sequence with our conserved sequence (53-105aa) in our proposed signal sequence.

The GFP reporter constructs will be created following the methods outlined in Eksi and Williamson (2011). The first construct (FL), which will serve as a control, will contain the full length ETRAMP 10.3 amino acid sequence, and the second (NM) will include the entire amino acid sequence minus the signal sequence (Figure 1). First, the 5'- Flanking Region (FR; Eksi *et al*. 2008) containing the promoter and the exact sequence of the N-terminus will be determined using sequencing. Then, the *Falciparum* transformation plasmid pDH.TgA.22, which confers resistance to pyrimethamine, will be used to create these transformed parasite lines (Wu *et al*. 1996). Four sequence

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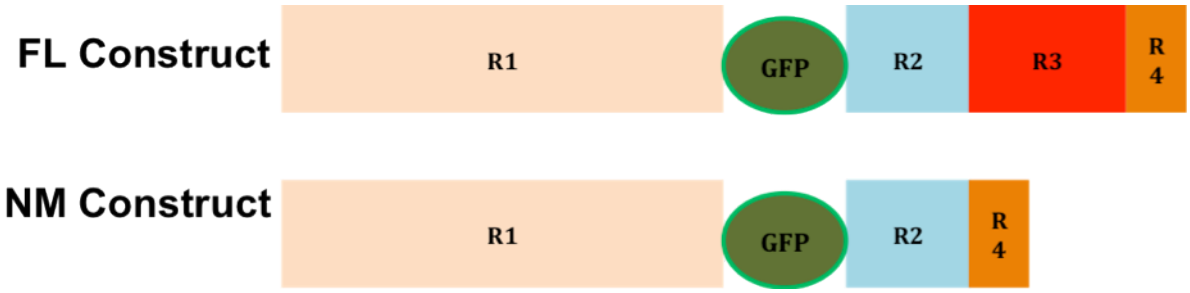

Figure 1: The two GFP reporter constructs, FL and NM, that will be produced. R1 is the 5'-FR and N-terminus, R2 is the core amino acid region between the N- and C-termini, R3 is the overlapped region containing the transmembrane and secretory signal sequences, and R4 is the remaining sequence of the C-terminus.

regions will be digested with specific restriction enzymes, amplified via PCR, and ligated into the pDH.TgA.22 plasmid at the corresponding restriction site. The regions are as follows: (1) the 5'- FR and the N-terminus, (2) the core amino acid region between the N- and C-termini, (3) the overlapped region containing the transmembrane and predicted secretory signal sequences, and (4) the remainder of the C-terminal region. Each region will be amplified using primers specific to that sequence.

Region 1 will be inserted between the SacII and XbaI sites of pDH.TgA.22. Next, the coding region of GFP, amplified using primers GFP.1.s and GFP.720.93 (VanWye and Haldar, 1997), will be inserted between the XbaI and BamHI sites, just 3' of the FR-N sequence. For the FL construct, regions 2, 3, and 4 will be amplified together and inserted 3' to the GFP sequence. In the NM construct, region 2 and 4 will be amplified separately and will be inserted consecutively 3' to the GFP sequence.

Automatic DNA sequencing will be used to confirm reporter construct sequences. Then 100µg of pDH.TgA.22.10.3.FL or pDH.TGA.22.10.3.NM will be used to transform *Falciparum*3D7 parasites infecting RBCs (5ml culture of 5 to 6% rings), and 1 ng/ml pyrimethamine will used to select for transformed parasites (Eski *et al*. 2002).

Falciparum parasites

Transformed *Falciparum*3D7 parasites will be maintained in cultured by using 0.5% AlbuMAX (Invitrogen Groningen, Switzerland) in place of human serum (Trager and Jensen, 1978; Dorn *et al*. 1995). *In vivo* parasites are generally all synchronized to the same stage of development in the blood. To maintain this synchrony, *in vitro* transformed parasites will be treated with 5% sorbitol as done by Lambros and Vanderberg (1979).

In vivo epifluorescence

In vitro parasites of both pDH.TgA.22.10.3.FL and pDH.TGA.22.10.3.NM transformed lines will be harvested at each of the three stages of asexual intraerythrocytic development: ring stage, trophozoite stage, and schizont stage. Each line will then be directly assayed at each stage of development using epifluorescence microscopy using an Axiovert 200 microscope and Axiovision 4.3 software.

Expected Results

The pDH.TgA.22.10.3.FL line parasites should express ETRAMP 10.3, similar to the expression seen by Mackeller *et al*. (2010). This full length construct contains the entire ETRAMP 10.3 gene and serves as a control to visualize the correct trafficking of ETRAMP 10.3 throughout asexual development in erythrocytes. If ETRAMP 10.3 is not expressed similarly in this transformed line, it may mean the GFP reporter construct was not formed properly. This potential for improper production of GFP reporter constructs is a potential pitfall of this study. If the constructs do not form well, we will not be able to compare trafficking between the two constructs. If the motif is a PVM secretory signal, as predicted, the NM ETRAMP 10.3 in the pDH.TgA.22.10.3.NM line should not localize to the PVM in the trochozoite stage. Instead, ETRAMP 10.3 should remain within the parasite, possibly remaining in a secretory organelle, as seen in trafficking of Pfs16 lacking the motif (Eski and Williamson, 2011). If it is unable to be secreted to the PVM, we would not expect ETRAMP 10.3 expression in the schizont stage.

Conclusion

ETRAMP 10.3 is an important PVM protein to study because it has been shown to be essential in proliferation of asexual intraerythrocytic parasites (Mackeller *et al*. 2010). If this motif enables proper trafficking of ETRAMP 10.3 to the PVM, it may have an effect on the development of the trophozoites into schizonts. Prevention of this developmental pathway could possibly prevent rupturing of the RBC and proliferation of RBC merozoites, which may lead to an attenuated vaccine candidate.

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