IMC1g knockdowns reveal malaria blood-stage alveolin functions

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Abstract

Plasmodium alveolins are cytoskeletal proteins with important roles in cell shape, tensile strength, and motility of mosquito-stage ookinetes and sporozoites. Two recent studies by Cepeda Diaz *et al.* and Liu *et al.* employ inducible knockdown of the essential blood stage-expressed alveolin IMC1g to identify new roles in merozoite intracellular survival, schizogonic cell division, and male gametogenesis.

Keywords: alveolin; inducible knockdown; subpellicular network; inner membrane complex; motility

It is 20 years ago that a novel family of cytoskeletal proteins of *Plasmodium* and related apicomplexan parasites was discovered and first reported [1]. The proteins in question, now collectively called alveolins, are putative intermediate filament proteins that form the main building blocks of the subpellicular network (SPN), a cortical cytoskeletal structure that supports the inner membrane complex (IMC) in apicomplexan zoites (**Figure 1**). Alveolins are structurally defined by possessing domains composed of short valine- and proline-rich tandem repeat sequences with a consensus 12 amino acid periodicity [2]. In the genus *Plasmodium*, up to 15 conserved and syntenic alveolin family members have been identified [2,3] which display differential expression among the three zoites stages: merozoite, ookinete and sporozoite [1,4-6]. The first alveolin study in 2004, and similar alveolin gene knockout studies that followed, identified key roles for several alveolins in facilitating cell shape, tensile strength, motility and infectivity of mosquito stage ookinetes and sporozoites [1,4,5,7,8]. However, the roles of blood stage-expressed alveolins in parasite development and infectivity have remained less well understood, due to their refractoriness to gene disruption [6]. Here, we highlight two recent studies that have employed inducible systems for reducing expression of the essential blood stage-expressed alveolin IMC1g to try and determine its contributions to intraerythrocytic parasite development [9,10].

The first of these studies by Cepeda Diaz and colleagues [9] was carried out with the human malaria parasite species *P. falciparum.* Using inducible *pfimc1g* gene excision, as well as inducible knockdown via prevention of *pfimc1g* mRNA translation, the authors achieved highly efficient depletion of PfIMC1g expression. In both approaches, PfIMC1g-depletion resulted in a total inhibition of blood stage parasite replication. The study goes on to identify two components of this developmental block. First, the authors show that PfIMC1g depletion led to a schizogonic segmentation defect, manifested by a small proportion of daughter cells possessing abnormal size or lacking a nucleus. This resulted in an estimated 30% reduction in the number of viable merozoites produced. Nonetheless, the majority of PfIMC1g-deficient merozoites were capable of egress and subsequent invasion of naive red blood cells (RBCs) *in vitro*. The second component to the block in asexual blood stage parasite replication is a catastrophic event suffered by the PfIMC1g-deficient merozoites shortly

after internalisation, which results in parasite death and ultimately clearance of infected RBCs.

Further examinations using ultrastructure expansion microscopy and transmission electron microscopy (TEM) revealed that PfIMC1g-depleted merozoites by and large possess a normal cell structure, including a tubulin cytoskeleton, apical polar ring, basal body and secretory organelles, consistent with their ability to egress and invade RBCs. However, microscopy also provided evidence for an increased deformability of the PfIMC1g-deficient merozoites, such as increased post-fixation roundness observed in TEM, and distorted cell shapes in blood smears on microscope slides. The latter observations are consistent with a loss of tensile strength. The authors therefore propose that the main phenotype in PfIMC1g-deficient merozoites is caused by a loss of cytoskeletal integrity/elasticity. This causes irreparable damage to the cells during RBC invasion, leading to death of the internalised parasite before the amoeboid ring stage is established.

The second study on IMC1g by Liu and colleagues [10] was carried out with the rodent malaria parasite *P. berghei*. This study used a dihydrofolate reductase destabilising domain fused to the target PbIMC1g protein for inducible knockdown, which achieved up to 75% reductions of PbIMC1g levels within 2h of withdrawal of the stabilizing compound trimethroprim (TMP). Knockdown of PbIMC1g expression did not lead to measurable effects on merozoite egress, invasion or intraerythrocytic parasite development upon infection of mice with cultured schizonts. Nonetheless, closer examination of cultured schizonts in the absence of TMP showed that they formed significantly fewer merozoites, and subsequent TEM of these schizonts revealed incomplete cytokinesis and IMC formation consistent with a role of PbIMC1g in cytoskeletal integrity.

The Liu *et al.* study [10] also used inducible knockdown of PbIMC1g to investigate its contribution to the development of sexual and sporogonic stages in the mosquito. While the production of blood stage male and female gametocytes was unaffected in PbIMC1g knockdown parasites, male gamete formation *in vitro* was adversely affected as determined by a small, but significant, reduction in exflagellation. This observation was accompanied at an ultrastructural level (TEM) by an increased number of male

gametes that possessed abnormal axonemes. Also of note was the clear effect of PbIMc1g knockdown on ookinete motility in Matrigel, with speeds significantly reduced.

The IMC1g studies described in *P. falciparum* [9] and *P. berghei* [10], respectively, reveal several similarities, for example in demonstrating that IMC1g is targeted to the IMC and physically interacts and co-localises with the blood stage-expressed alveolin IMC1c. Both studies furthermore identify a minor role for IMC1g in schizogonic segmentation. However, there are also clear differences between the outcomes from the two studies, most notably the catastrophic event that happens shortly after merozoite invasion in *P. falciparum,* but not in *P. berghei*, upon IMC1g depletion. Whilst some of these observed differences could reflect species-specific differences, the apparent differences in IMC1g knockdown efficacies reported between *P. falciparum* and *P. berghei* makes it difficult to directly compare the two studies: knockdown phenotypes in *P. berghei* could be less severe, because a substantial amount of functional PbIMC1g is still being expressed. A more robust inducible knockdown system in *P. berghei* would be required to resolve this issue.

Overall, the two studies identify roles for IMC1g that are compatible with those reported of other alveolins expressed solely in the mosquito stages (e.g. IMC1a, IMC1b, IMC1h [1,4,5]), including contributions to tensile strength, cytoskeletal integrity and zoite locomotion. A possible exception is cell shape, which was not obviously affected by IMC1g depletion especially in merozoites [9,10]. This could reflect the distinct repertoires of expressed alveolins [2], or the rounder shape of the merozoite compared to the elongated ookinete and sporozoite. The described role of PbIMC1g in male gametogenesis and axoneme formation [10] is arguably the most unexpected find, which warrants more thorough investigation.

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Declaration of interests

The authors declare no competing interests.

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Figure 1. Example cortical structure of a *Plasmodium* zoite and alveolin subcellular location in the SPN. (A) Transmission electron micrograph of a *P. berghei* sporozoite cross-section. Scale bar = 0.1μm. (B) Cartoon of the cortical zoite structure (shown boxed in panel (A)) displaying the plasma membrane (PM), inner membrane complex (IMC), subpellicular network (SPN) and subpellicular microtubules (SPMTs). (C) Confocal fluorescence image of a sporulated *P. berghei* oocyst expressing the SPN-resident alveolin IMC1c, tagged with the red fluorescent protein mCherry. Scale bar = 20μm. (D) Higer magnification image of the boxed region in panel (C), showing cross-sectioned sporozoites displaying cortical localisation of IMC1c::mCherry.