Chapter Seven

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Erythrocyte invasion by merozoites is a crucial step in the propagation of blood stage malaria parasites. Recent studies have identified and characterized the motor protein complex utilized by the parasite to mediate this process (Baum et al., 2006; Jones et al., 2006; Green et al., 2006) The complex referred to as the glideosome comprises four proteins PfMyoA, PfMTIP, PfGAP45 and PfGAP50. Although these proteins are known to be essential for invasion and parasite viability, it is not clear what signaling mechanisms regulate their action in the parasite. PfPKB, the PKB homologue in Plasmodium is expressed late in the parasite life cycle, localizes at the apical tip of merozoites and has been implicated in invasion (Kumar et al., 2004; Vaid et al., 2006; Vaid et al., 2008). PfCDPK1, a calcium dependent kinase has also been implicated in erythrocyte invasion and has been shown to target PfGAP45 and PfMTIP which are part of the glideosome complex (Green et al., 2008; Kato et al. 2008). The major goal of this thesis was to understand how these kinases contribute to the process of erythrocyte invasion by merozoites.

Attempts to decipher the role played by PfPKB and PfCDPK1 in erythrocyte invasion led to the following findings, which provide novel insights into the function and regulation of these kinases.

1) PfPKB was found to be essential for the survival of the asexual blood stages of the parasite.
2) PfPKB co-localized with PfGAP45, a component of the glideosome complex. It also interacted directly with PfGAP45 and the glideosome complex
3) PfGAP45 was phosphorylated by PfPKB in vitro and in the parasite at S103.
4) PfCDPK1 phosphorylated PfGAP45 in vitro at S89, S103 and S149. Phosphorylation of PfGAP45 at the three identified sites was confirmed in the parasite.
5) PfGAP45 phosphorylation at S103 and S149 was regulated by Ca\(^{2+}\) release mediated by PfPLC. This correlated well with the observations that the two kinases that phosphorylate PfGAP45, PfPKB (Vaid et al., 2008) and PfCDPK1 are controlled by the PLC-Ca\(^{2+}\) pathway in the parasite.

6) Phosphorylation of PfGAP45 at S103 and S149 was developmentally regulated in the parasite which correlated well with the expression profiles of the candidate kinases that target these sites.

7) Activation of PfPKB and PfCDPK1, as well as the subsequent phosphorylation of PfGAP45 at S103 and S149 may be negatively regulated by sustained cAMP levels in the parasite via the action of PfPKA.

8) Phosphorylation at S89, S103 and S149 did not affect the sub cellular localization of PfGAP45 or the association of the glideosome complex.

PfPKB has been identified as a target of Ca\(^{2+}/CaM\) in the parasite (Vaid et al., 2006). Binding of Ca\(^{2+}/CaM\) to the N terminal regulatory region of PfPKB results in its activation. The following evidence suggests that PfCDPK1 can crosstalk with PfPKB and may be able to regulate its activity by an alternate mechanism.

1) PfCDPK1 phosphorylated ΔPfPKB (constitutively active form of PfPKB lacking the N terminal regulatory region), and activated it in vitro by targeting multiple sites that are distinct from the previously identified autophosphorylation sites, S271 and S442.

2) PfCDPK1 was able to activate PfPKB independent of Ca\(^{2+}/CaM\) in vitro.