ABSTRACT

To invade host erythrocytes, the malaria parasite, *Plasmodium falciparum,* produces invasion proteins, which are translocated through the secretory pathway by an N-terminal signal peptide. Additional motifs allow the proteins to be transported to the secretory organelles, the rhoptries and micronemes. Proteins of the Duffy Binding-Like Erythrocyte Binding Protein (DBL-EBP) family possess a C-terminal cysteine-rich domain, proposed to be the motif necessary for targeting. To investigate this hypothesis, mini-gene constructs were created from two DBL-EBPs, MAEBL and JESEBL by overlap extension PCR, and used to transfect 3D7 *P. falciparum* parasites. It was anticipated that only chimaeric proteins containing the domain would be correctly localized to the secretory organelles, while those without the domain would remain within the cytoplasm. Two constructs were produced for MAEBL, containing the signal peptide, transmembrane domain and cytoplasmic tail, with the C-terminal cysteine-rich domain either present, M1 (CCys), or absent, M2 (ΔCCys). These were cloned into the pARL-GFP plasmid that had been modified by replacing the *crt* promoter with the *ama-1* promoter for stage-specific expression. A third construct contained the JESEBL signal peptide and C-terminal cysteine-rich domain, J (CCys), which was cloned into the pARL-mCherry/*ama-1* promoter plasmid. Successfully transfected parasites were visible 25-30 days post-transfection and the presence of the episomal plasmids was verified by PCR. Microscopic analysis of live parasites revealed diffuse fluorescence in the cytoplasmic regions for control transfectants carrying the empty pARL2-GFP and pARL-mCherry vectors, as expected. The chimaeric M2 (ΔCCys) protein showed a similar pattern to the control GFP, however, chimaeric M1 (CCys) was localized to the parasite apex, indicating secretory organelle localization. These results showed that the C-terminal cysteine-rich domain is sufficient and necessary for apical trafficking of MAEBL. The chimaeric J (CCys) protein was localized throughout the cytoplasmic and apical regions, indicating partial trafficking of the protein to the secretory organelles. This was further confirmed by co-localization studies whereby EBA-175 was used as a micronemal marker. The JESEBL C-terminal cysteine-rich domain is therefore sufficient but not completely adequate for correct protein trafficking. Based on these data and other published studies, two hypothetical models are proposed for the transport of MAEBL and JESEBL to the apical organelles.