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Short communication

Targeted disruption of *maebl* in *Plasmodium falciparum*

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Malaria parasites have a complex life cycle that requires invasion of several different cell types in both vertebrate and mosquito hosts. In *Plasmodium falciparum*, the merozoite must attach to and invade a new erythrocyte in order to continue parasite development in the blood of an infected host. Merozoite entry into erythrocytes is a multi-step process requiring merozoite adhesion, re-orientation, junction formation and invasion [1]. Each step is thought to be mediated by the coordinated interactions of numerous specific merozoite ligands and erythrocyte surface receptors [2]. A number of mediators involved in the invasion process are positioned on the merozoite surface and in the organelles of the apical complex. However, the functions of molecules involved in this interaction are still poorly characterized.

Understanding the complex process of *P. falciparum* merozoite invasion requires identification and characterization of numerous potential parasite ligands and their potential interactions. MAEBL is a paralogue of the duffy binding-like erythrocyte binding protein (DBL-EBP) family, but has a chimerical structure and shares similarity with AMA1 [3]. M1 and M2 are tandem cysteine-rich regions with similarity to AMA1 and are present in the N-terminal portion of the MAEBL ectodomain. MAEBL was identified in *P. yoelii* and *P. falciparum* blood-stage parasites as a minor membrane protein with erythrocyte binding activity expressed in the apical organelles and on the surface of invasive merozoites [3–6]. Expressed abundantly in sporozoites, MAEBL appears to be important for sporozoite invasion into the mosquito salivary glands and in establishing exoerythrocytic schizonts [6–12]. It has been reported that sera from infected individuals living in a malaria endemic region of western Kenya recognized M2 recombinant antigen and had the ability to inhibit M2-erythrocyte binding [6]. However, whether MAEBL is essential or even has a significant role for erythrocytic stage growth is unclear.

To evaluate MAEBL expression in *P. falciparum* erythrocyte stages and its potential role in merozoite invasion of erythrocytes, *maebl* disrupted parasite lines were created and examined for possible importance of MAEBL in erythrocytic invasion pathways (Fig. 1A). Homologous integration into the *maebl* locus was carried out by using the plasmid pHH1/Δ*maebl* containing the selection marker hDHFR and targeting sequence that disrupted *maebl* coding sequence (CDS) after the second ligand domain (M2). A cloned parasite line of the *P. falciparum* W2mef isolate was transfected with this construct and two independent clones, B7 and D8, were obtained through intermittent selection with WR99210. In order to confirm that pHH1/hDHFRΔ*maebl* had integrated through single crossover homologous recombination into the *maebl* locus, genomic DNA from parental wild-type W2mef and W2mef Δ*maebl* clones were analyzed by Southern blot hybridization probed with *hDHFR* and *maebl* M2 CDS (Fig. 1B). Integration of multiple copies of the whole plasmid into the *maebl* locus was observed in B7 and D8 clones.

RT-PCR analysis in parental and Δ*maebl* clones indicated that *maebl* continued to be actively transcribed down stream to the disruption site (Fig. 1C). In order to determine if nor-

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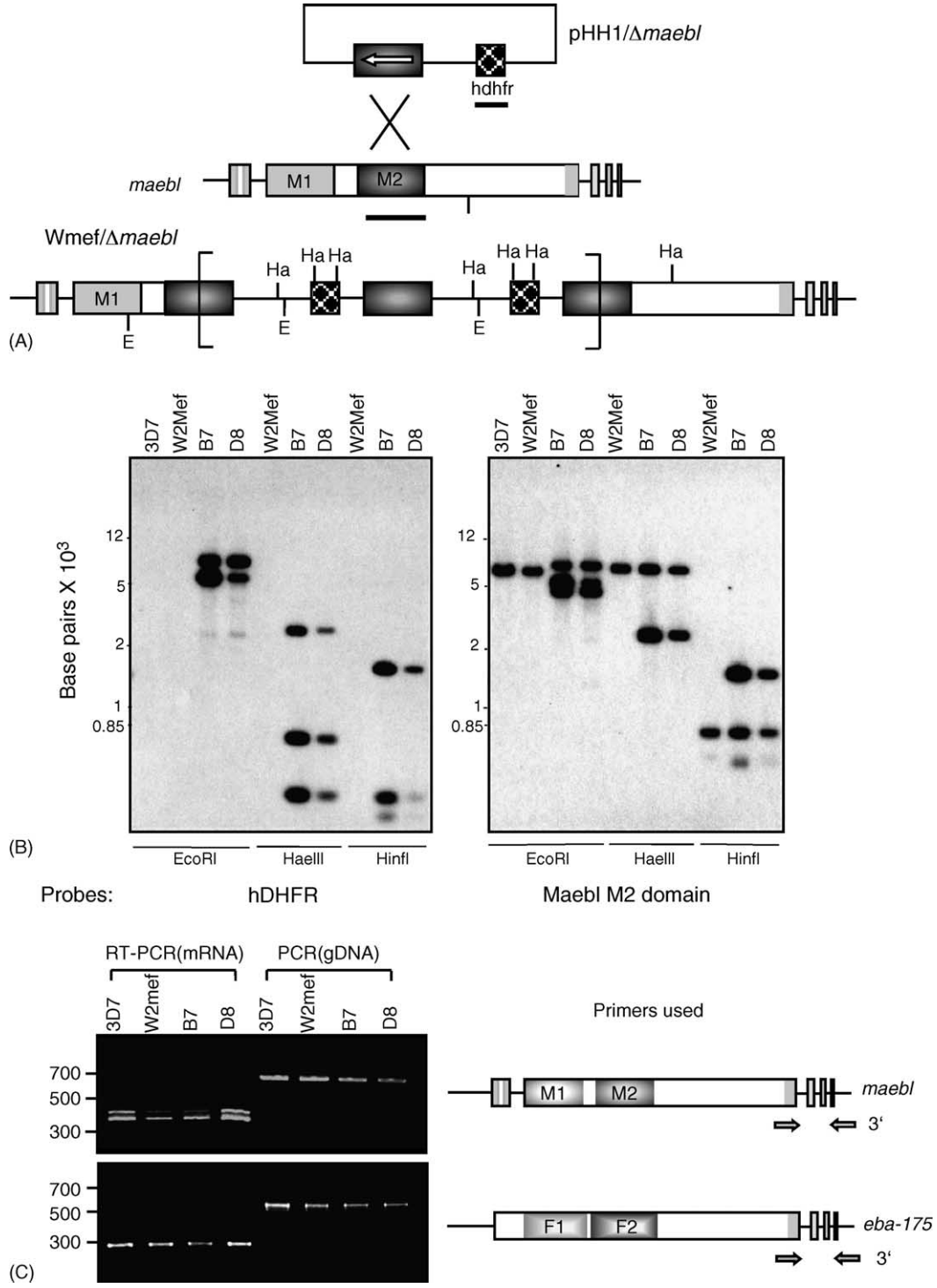

Fig. 1. (A) The region of *maebl* encoding the M2 domain was cloned into the *Xho*I site of plasmid pHH1/hDHFR and introduced into *P. falciparum* W2mef parasites by electroporation as described previously [21]. Parasites were cultivated using standard techniques in the presence of 10 nM WR99210 in order to select for parasites carrying the plasmid and resulted in two independent *maebl* knockout clones designated B7 and D8. *Eco*RI(E) and *Hae*III (Ha) restriction sites used in mapping the plasmid integration events are shown. Solid bars indicate the probes from *maebl* M2 region and hDHFR used for Southern blot analysis in B. (B) Southern blot hybridizations were performed using standard protocols to confirm integration in the *maebl* locus [5]. Two micrograms genomic DNA from *P. falciparum* 3D7 (reference clone), W2mef and Δ*maebl* clones was digested with *Eco*RI, *Hae*III or *Hin*fI. The restricted fragments were separated by 0.8% agarose gel, transferred to nylon membranes and hybridized with ^{32}P-labeled probes of *hDHFR* and *maebl* M2 domain. Clones B7 and D8 showed disruption of the endogenous *maebl* locus. (C) Products from PCR and RT-PCR reaction in *P. falciparum* clones (3D7 and W2mef) and W2mefΔ*maebl* clones (B7 and D8) were analyzed by agarose gel electrophoresis. Approximate locations of each primer pair are shown as arrows.