Effects of Mosquito Genes on *Plasmodium* Development

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**Materials and Methods**

**Mosquito rearing and** *P. berghei* infections. *A. gambiae* strain G3 rearing was performed as previously described (*S1*, *S2*). Four days post dsRNA-injection, mosquitoes were fed on CD1 mice infected with the *P. berghei* *PbCTRPp.GFP* transgenic line (*S3*), for 30 min at 21°C. Infected mosquitoes were kept at 21°C until dissection. Dissected midguts were fixed in 4% para-formaldehyde for 30-60 min and fluorescent parasites were visualized with a fluorescence microscope.

**RNA isolation, quantitative RT-PCR and semiquantitative RT-PCR.** Total RNA was isolated using the TRIzol Reagent (Invitrogen) according to the supplier’s instructions, and contaminant genomic DNA was removed by DNase I treatment. For quantitative RT-PCR, cDNA was synthesized from total RNA (2-3 µg) using the moloney murine leukemia virus reverse transcriptase and oligo(dT)12-18 as described by the supplier (Life Technologies, Inc.). Quantitative RT-PCR was performed using the SYBR Green PCR Master Mix kit (Applied Biosystems) according to the manufacturer’s instructions and the ABI Prism 7000 Sequence Detection System. The primer pairs used in these reactions were: *LRIM1*-1914F and *LRIM1*-1983R; *CTL4*-1 and *CTL4*-2; *CTLMA2*-1 and *CTLMA2*-2; *S7*-1 and *S7*-2 (primer sequences are found below). *LRIM1* and *CTLMA2* KO efficiency was monitored by quantitative RT-PCR using the *LRIM1*-1914F and *LRIM1*-1983R and *CTLMA2*-3 and *CTLMA2*-4, respectively. Relative gene expression values were calculated using the Comparative C<sub>T</sub> Method after checking for the efficiency of target amplification as described in the ABI Prism 7700 Sequence Detection System User Bulletin #2. The S7 ribosomal protein gene was used as internal reference. For semiquantitative RT-PCR, cDNA synthesis was primed using oligo(dT)25 magnetic beads as described (*S4*). *CTL4*-3 and *CTL4*-4 primers were used to amplify a 473-bp fragment according to the following program (45 s at 95°C; 60 s at 50°C, 60 s at 72°C) for 25 cycles. The S7 internal control (*S5*) was amplified for 20 cycles using the following program (45 s at 95°C; 60s at 59°C; 60 s at 72°C). Analysis of the PCR-amplified products was carried out as described (*S6*).

**Primer sequences**

*LRIM1*-1914F; 5’-CATCCCGCATTGGGGATATGT-3’
*LRIM1*-1983R; 5’-CTTCTTTGAGCCGTGCATTTTC-3’
*CTL4*-1; 5’-AAGACTGACACGATCGCAGAAA-3’
*CTL4*-2; 5’-CCTGTCCGGGCGATCAAACTA-3’
*CTL4*-3; 5’-GTTAGCAGCATTGGGATTACCCTCG-3’
*CTL4*-4; 5’-GAAGTCGCAACCCAGCTCATTGTAG-3’
*CTLMA2*-1; 5’-GAGGTGCTGAACGAACGA-3’
*CTLMA2*-2; 5’-GAGGTGCTGAACGACGA-3’
*CTLMA2*-3; 5’-CACAGTGGTTGTGTTGACACTA-3’
*CTLMA2*-4; 5’-CATGGGTTTTGTTGAAGATATCATC-3’
CTLMA2-2; 5'-CTCGTCATCCGAGTCGGATT-3'
S7-1; 5'-GTGCAGCTGGAGGAGAAGA-3'
S7-1; 5'-ATCGGTGTGGCCAGAATGC-3'

Fig. S1

**Fig. S1.** A schematic model of LRIM1 and CTL (CTL4 and CTLMA2) protein action during *Plasmodium* development in the mosquito midgut. During or soon after invasion across the midgut epithelium (four downward oriented arrows), three out of four invading ookinets are eliminated, partly through the antagonistic action of LRIM1 (upward oriented bold arrows). However, CTL4 and to a lesser extent CTLMA2 protect the remaining ookinets from the melanization response (slanted black bars); melanization also requires LRIM1 activity (horizontal arrow). In addition, CTL4 has a minor antagonistic effect favoring ookinet killing (dashed arrow).

**References and Notes**