Egress of malaria parasites from the infected blood cell visualised by electron tomography, fluorescence microscopy and X-ray tomography

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Egress from infected erythrocytes is an important stage in the malaria parasite lifecycle, essential for continuing rounds of infection. In the blood stage of malaria infection a single parasite invades a blood cell and divides to form up to 32 daughter cells, while digesting the cell contents. The parasites are contained within a parasitophorous vacuole inside the blood cell. Egress occurs via sequential rupture of the vacuole and erythrocyte membranes. Although a number of malarial proteases such as SUB1 and the SERA family play a role in regulating and mediating egress, the precise mechanisms are not fully understood. Treatment of late stage infected cells with compound 1, a selective inhibitor of SUB1 discharge, results in accumulation of mature parasites unable to egress. Treatment with the broad-spectrum cysteine protease inhibitor E64 stalls egress partway through, resulting in clusters of parasites contained within the red cell membrane. We have used a combination of electron tomography, electron energy loss spectroscopy, fluorescence microscopy and X-ray tomography of compound 1 and E64 treated late stage infected cells to study membrane disruption in egress. We have demonstrated evidence of mixing of the contents of the red blood cell and vacuole in the presence of compound 1 while the vacuole membrane remains intact. This indicates localised disruption to the vacuole membrane prior to rupture, upstream of SUB1 and SERA action. With E64 treatment the vacuole membrane is ruptured to form extensive, multi-layered vesicles. The results illustrate multiple steps in egress, starting with permeabilisation of the vacuole membrane immediately preceding its breakdown into swirl-like fragments, followed by breakdown of the erythrocyte membrane.

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