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ROM4-mediated intramembrane proteolysis of AMA1 triggers *Toxoplasma* to switch from an invasive to a replicative mode

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Apicomplexan parasites invade host cells and immediately initiate a program of cell division. The extracellular parasite possesses secretory organelles called micronemes which discharge several type I transmembrane proteins onto the surface to participate in motility and invasion. These proteins are shed by intramembrane proteolytic cleavage, a process that is associated with invasion but is otherwise poorly understood. We will report the functional analysis of the *Toxoplasma gondii* rhomboid protease 4 (ROM4), a parasite surface intramembrane serine protease. Conditional over-expression of a catalytically inactive form of this protease (ddROM4$_{S\text{-}A}$) using the ddFKBP system led unexpectedly to a profound block in parasite replication at a late stage of the cell cycle (left graph). This effect, probably due to sequestration of a key substrate (scheme), was reversible and was not linked to invasion. Among known rhomboid substrates, apical membrane antigen 1 (AMA1) plays a key role at invasion. Transgenic expression of the cleaved cytoplasmic tail of *Toxoplasma* or *Plasmodium* AMA1 (right graph) but not of *Toxoplasma* micronemal protein 2 (MIC2) functionally reversed the phenotype induced by ddROM4$_{S\text{-}A}$. These results reveal a novel function for AMA1 in parasite replication, distinct from its function in invasion, mediated through cleavage by ROM4. This study establishes a new concept in apicomplexan biology in which proteins involved in invasion are concomitantly implicated in a checkpoint that signals the parasite to switch from an invasive to a replicative mode.

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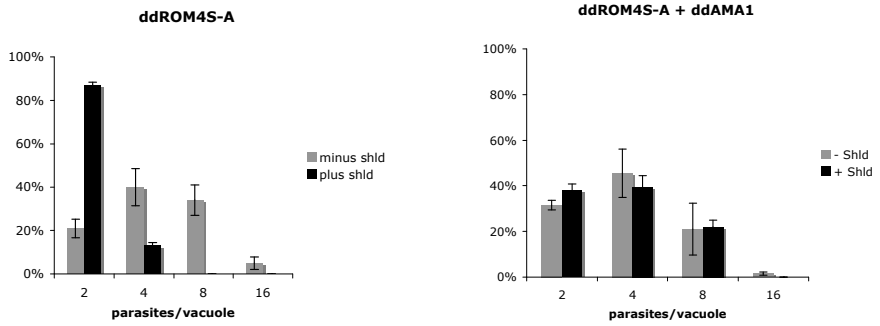

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