Abstract

Mitochondria are targeted by various intracellular parasites due to their central roles in multiple cellular pathways, including immune responses. During infection, the parasite *Toxoplasma gondii* associates with host mitochondria and induces the shedding of their outer mitochondrial membrane. This process depends on the interaction between the parasite effector protein TgMAF1 and the host mitochondrial protein TOM70. Not all TgMAF1 isoforms bind TOM70; Only TgMAF1b, unlike TgMAF1a, does interact with TOM70. However, the molecular basis for TgMAF1b binding to TOM70, and the selective targeting of TOM70 over other mitochondrial proteins, has remained unclear. Here we show that TgMAF1 mimics a TOM70 interacting chaperone by using its C-terminus to bind the clamp domain of TOM70. We found that, over the course of evolution, TgMAF1b diverged from TgMAF1a at its C-terminus to mimic the chaperone HSP90. A small number of amino acid substitutions at the C-terminus enable TgMAF1b to imitate the EEVD motif of HSP90, allowing it to bind the TPR structural motif of TOM70. Moreover, we discovered that this interaction drives the formation of HMA and SPOT structures and prevents MAVS from binding to TOM70. Our findings reveal how parasites exploit host pathways through molecular mimicry to disable immune responses. We anticipate that our results will contribute to a deeper understanding of how effector proteins target and modulate host functions. This insight may also help uncover fundamental principles of host defense mechanisms and offer future opportunities for drug discovery. For instance, structural motifs exploited by *Toxoplasma gondii* could serve as templates for the development of novel therapeutic strategies.