IMMOBILIZATION OF THE TYPE XIV MYOSIN IN *TOXOPLASMA* MEMBRANES IS CHOLESTEROL-DEPENDENT

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Toxoplasma gondii is an intracellular protozoan parasite of animal cells that can cause severe disease or death in fetuses and immunocompromised individuals. Movement of the parasite within the infected animal as well as actual host cell penetration is believed to be facilitated by the interaction of F-actin, associated with a cell surface adhesin of the parasite, and myosin-A, a type XIV myosin. Myosin-A is found in a complex, called the glideosome, with three additional subunits: a myosin light chain (MLC1) and two accessory proteins, GAP45 and GAP50. The latter is an integral membrane glycoprotein that is responsible for anchoring the motor complex in the inner membrane complex (IMC), a membrane system underlying the parasite plasma membrane. In order for net movement to be produced by this motility system it is critical that the glideosome is firmly anchored within the plane of the IMC. Using FRAP analysis of YFP-tagged GAP50 we demonstrate here that this is indeed the case. While YFP-tagged cytoplasmic proteins or proteins associated with the IMC through acylation demonstrate a rapid recovery after photobleaching, we could not detect recovery of photobleached GAP50-YFP, suggesting that this protein is immobilized within the plane of the MC membrane. This immobilization is not due to the interaction of GAP50 with other IMC proteins as judged by failure to detect these by chemical cross-linking or co-immunoprecipitation. The use of different detergent extraction approaches reveals that the glideosome, as well as the whole IMC membrane, is solubilized by Triton X-100 in a temperature-dependent fashion. We find that the IMC contains a significant proportion of cholesterol and that association of the glideosome is sensitive to depletion of cholesterol. These findings suggest that the glideosome is found in a cholesterol-rich membrane fraction that may constitute a large portion of the IMC.