

P010 Roles of ALG-2 in the calcium-dependent interaction between endosomal sorting regulators Alix and TSG101
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ALG-2 interacts with various proteins, including Alix and TSG101 that are known to associate each other. In this study, we examined the possibility that an ALG-2 homodimer functions as a bridging factor that links both Alix and TSG101 and helps Alix-TSG101 interaction. To investigate the interaction of Alix with ALG-2 and TSG101 in mammalian cells, we performed pulldown assays using HEK293T cells expressing either Strep-tagged wild type Alix (Alix^{WT}), TSG101 binding site deletion mutant (Alix^{ΔPSAP}) or ALG-2 binding site deletion mutant (Alix^{ΔABS}). The cleared cell lysates were incubated with Strep-tactin beads and the proteins bound to the beads (pulldown products) were analysed by Western blotting. TSG101 was pulled down with Alix^{WT} and Alix^{ΔPSAP} in a Ca²⁺-dependent manner. Both ALG-2 and TSG101 were not detected in the pulldown products of Strep-Alix^{ΔABS}. FLAG-CEP55, but not TSG101, was pulled down by Alix^{WT} in the absence of Ca²⁺. Furthermore, in the ALG-2 depleted cells, the interaction between TSG101 and Alix^{WT} was not detected even in the presence of Ca²⁺. These results suggest that ALG-2 functions as a Ca²⁺-dependent adaptor protein and stabilizes the Alix-TSG101 complex.