Microtubules are conserved cytoskeletal elements that form by the polymerization of alpha- and beta-tubulin heterodimers. The formation of polymerization-competent tubulin heterodimers requires that alpha-tubulin and beta-tubulin be properly folded. Specific cofactors are required for the folding of alpha- and beta-tubulin in vitro and homologs of these cofactors have been found in many organisms, including S. cerevisiae. In S. cerevisiae, ALF1 is a non-essential gene that is homologous to mammalian cofactor B 1, 2. In vitro, cofactor B acts in the post-chaperonin folding of alpha-tubulin 1. Consistent with in vitro studies, Alf1p genetically acts upstream of Pac2p/cofactor E 1, 2. ALF1 genetically interacts with the other tubulin cofactors, and is essential in combination with specific alpha-tubulin mutants 1, 2. alf1 null mutants are super-sensitive to benomyl, a microtubule depolymerizing drug 2. Alf1p interacts with alpha-tubulin in the yeast two-hybrid and immunoprecipitation assays 2. Alf1p and cofactor B both contain a single CLIP-170 domain, which is found in several microtubule-associated proteins and is required for the Alf1p-alpha-tubulin interaction 2. Alf1p binds to a face of alpha-tubulin distinct of that of beta-tubulin binding 2. Alf1p-GFP localizes to cytoplasmic microtubules, suggesting that Alf1p may play an additional role in microtubule maintenance 2.