Episomal expression of interaction domain that is derived from autophagy-related proteins specifically inhibits autophagosome formation by antagonizing their native interaction in vivo.

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Protein-protein interactions play vital roles in every biological event executed in, on, and between cells. Although large-scale interactome-analyses that were conducted on various model organisms have uncovered a number of novel interactions, in turn, daunting numbers of interactions of which biological functions are unknown are remained. In the post-interactome analysis, therefore, it is clear that development of an efficient method that allows functional analysis on individual interactions is the key.

I found an episomally expressed interaction domain effectively inhibits the interaction between its native proteins. It suggests that a protein fragment that is responsible for the interaction can be used as a molecular probe to elucidate the biological function hidden behind the targeted interaction.

Atg12-Atg5.Atg16 (ATGs) are subunits of a yeast's protein complex that plays a crucial role in autophagy. So far, I have shown that an episomal expression of the protein domain derived from the intra-complex interactions specifically inhibits autophagosome formation, thus leading to autophagy impairment, while no influence on the host growth. In this year, I performed biochemical analyses to clarify this inhibition mechanism. To determine the interacting ability of the interaction domains *in vivo*, I prepared the cMyc-tagged domains and His6-tagged interaction partners and allowed them to be co-expressed in a recombinant yeast. As a result, cMyc-tagged domain was co-purified with its His-tagged partner, suggesting their *in vivo* interaction. Then I examined the changes in subunit composition of the complex upon the domain over-expression. I purified the complex from Atg16-TAP strain of which autophagy was impaired by the domain overexpression. The purified complex contained the episomally expressed domain. The results strongly suggest that dysfunction of autophagy upon the domain overexpression was caused by the physical interference with the inter-subunit interaction. The applicability of the domain-based interaction-targeting is exemplified in this study.