SUMMARY OF Ph.D. DISSERTATION

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Title

Construction of screening systems of protein-protein interactions using *in vitro* virus and a screening and characterization of Jun-associated proteins

Abstract

Although yeast two-hybrid assay and biochemical methods combined with mass spectrometry have been successfully employed for the analyses of protein-protein interactions in the field of proteomics, these methods encounter various difficulties arising from the usage of living cells, including inability to analyze toxic proteins and restriction of testable interaction conditions. Totally *in vitro* display technologies such as ribosome display and mRNA display are expected to circumvent these difficulties. In this study, we developed an mRNA display technique, named *in vitro* virus (IVV), and applied to screening for interactions of a basic leucine zipper domain of Jun protein in a mouse brain cDNA library. By performing iterative affinity selection and sequence analyses, we selected 16 novel Jun-associated protein candidates in addition to four known interactors. By means of real-time PCR and pull-down assay, ten of the 16 newly discovered candidates were confirmed to be direct interactors with Jun *in vitro*. Furthermore, interaction of six of the ten proteins with Jun was observed in cultured cells by means of co-immunoprecipitation and observation of subcellular localization. These results demonstrate that this *in vitro* display technology is effective for discovery of novel protein-protein interactions and can contribute to the comprehensive mapping of protein-protein interactions. Furthermore, we discussed about the improvement of the IVV selection technique toward more comprehensive and high-through put screening of protein-protein interactions.