The Identification of Candidate NBA1 Interacting Proteins

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G protein coupled receptors (GPCR's) are the largest family of membrane bound receptors. They are characterized by seven transmembrane helices. NBA1, a generic gene identifier, will be used to reference a certain GPCR which cannot be disclosed. NBA1 is a poorly studied orphan GPCR. A mutation in the intracellular domain of NBA1 is associated with a heritable form of novel behavioral abnormality. NBA1 is a group C GPCR, and as such is likely to interact with other protein subunits as is typically the case for members of this protein family. NBA1 contains a protein-protein interaction domain, suggesting that it dimerizes to function properly. In order to discover the function of NBA1 through the identity of interacting proteins, a yeast two hybrid (Y2H) screen was conducted to detect protein-protein interactions using the intracellular portion of NBA1 as the bait protein. A few proteins of interest from the screen were YWHAB, MLLT4, LPL, and APP. These candidate interactions were further verified through a co-immunoprecipitation approach. Optimization experiments were conducted to determine the best lysis buffer and antibodies for a co-immunoprecipitation. YWHAB, GOLGA8A, GOLGA8B, VCP and MLLT4 were successfully pulled down with NBA1, indicating protein interactions. In conclusion, identifying partners for NBA1 provides a direction for future research of NBA1 function based on the function of the interacting protein.

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