IDENTIFICATION OF PIST PDZ DOMAIN INTERACTORS

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Glutamate receptors are proteins that mediate most excitatory communication between neurons in the brain. One type of glutamate receptor, alpha-amino-3-hydroxy-5-methylisoxazolepropionate receptor (AMPAR), is moved to and from the synapse to control the strength of synaptic transmission. This movement is critical for the synaptic changes that underlie some forms of learning and memory. Abnormal targeting of AMPARs can cause epilepsy and learning disorders. Proteins interacting specifically with TC10 (PIST) are important for AMPAR targeting synapses; it acts as a chaperone for the AMPAR and an AMPAR-binding protein, stargazin. PIST contains several important domains, regions that are conserved between many proteins, and are known to have specific functions. For PIST, these include two coiled-coil domains, a leucine zipper, a PDZ domain (named after several proteins that contain this domain, *P*SD-95, *D*iscs Large, and Zona Occludens), and an acid cluster. PIST interacts with several proteins through its coiled-coil region, and its PDZ domain is known to bind to and traffic many transmembrane proteins outside the brain. PIST binds to stargazin/AMPAR at amino acid residues separate from its named domains. However, experiments in our lab suggest that, although the PIST-PDZ domain does not bind stargazin/AMPA receptors, it is also critical for the synaptic targeting of these proteins. Because the PIST-PDZ domain is important for AMPAR synaptic targeting, we sought to identify novel proteins that interact with the PIST-PDZ domain. Using an assay of protein-protein interaction, the yeast two-hybrid system, we screened a cDNA library of brain proteins using the PIST-PDZ domain and obtained 100 possible interacting clones. By reintroducing these clones into yeast containing the PIST-PDZ domain, we sought to confirm specific interaction with PIST. We performed restriction digest and DNA sequencing of interacting clones to identify the interacting proteins. By identifying PIST-interacting proteins, we wanted to increase knowledge on the mechanism by which PIST serves as a chaperone for stargazin/AMPAR in their delivery to the synapse.

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RESEARCH AIMS AND HYPOTHESIS

Our goal was to determine how and to what the PIST-PDZ domain binds in the brain. Each protein comprises a series of amino acids and each amino acid is translated from codons. Each codon represents three DNA sequence pairs so that proteins that are found can be DNA sequenced to determine the genetic identity of the proteins. In a yeast two-hybrid experiment, a prey and a bait vector are used to determine interactions. Our bait vectors were PIST proteins and our prey vectors were unknown proteins. If they interacted, then the yeast that they are put into grows on nutrient-deficient plates. Only positive interactions trigger growth in yeast. If they don't interact, then no yeast grows on the plates. Our goal was to find out what interacts with the PIST protein and to send it for DNA sequencing.

METHODS AND MATERIALS

The first step was to screen PIST against a library of unknown rat brain DNA. A directed interaction was performed with PIST and the unknown in yeast. The yeast used, AH109, does not have the ability to make leucine, tryptophan, histidine, and adenine. The plasmid that PIST is in, PGBKT7, has the ability to make tryptophan and the rat brain library is constructed in PGADT7 which has the ability to make leucine. One hundred colonies were then selected and restreaked with a pipette tip onto –LWHA plates (nutrient plates that do not contain leucine, trptophan, histidine, and adenine.) After five days of growth in the 37° incubator, the plates were restreaked again and an Xgal assay was performed on the original plates. The bluest colonies of yeast were selected to grow in –L solution to insolate the rat brain DNA since it is inserted in PGADT7 and can grow in –L. The solution is then miniprepped for DNA and transformed into bacteria with chemical competent cells. Colonies that grew were analyzed for homogeneity and the DNA was checked for specific or non-specific interactions by reinserting PIST and unknown DNA back into AH109 for a yeast two-hybrid directed interaction. Specific interactions were then DNA sequenced. (Figure 1)

Clone 301 is an *E. coli* plasmid that contains the PIST-PDZ domain (281–455). Clone 513 was made by cutting 301 with R1/Bam at insertion sites and inserting it into a new vector GBKT7. Clone 513 was then transformed into the yeast AH109 and a library screen was done against 6uL of rat brain DNA. The screen was plated on 20 –LW plates in the 30°C incubator for five days. Many yeast colonies grew on the 20 plates and 100 of the colonies were selected and restreaked on new –LWHA plates. Filter papers were cut and an Xgal assay was performed to test for blueness on the restreaked plates and put into the 30°C incubator for five days. All 100 of the colonies were miniprepped for DNA. The blueness from the Xgal assay were ranked from two to zero, two being the most blue. Twenty of the bluest colonies were picked and transformed into bacteria. Subsequently, these were plated on LB AMP plates overnight. Two colonies were picked from each of those plates and put into 3ml of LB AMP. Only 3:1, 9:1, 9:2, and 16:1 grew in LB AMP. They were miniprepped and cut with R1/Bam for one

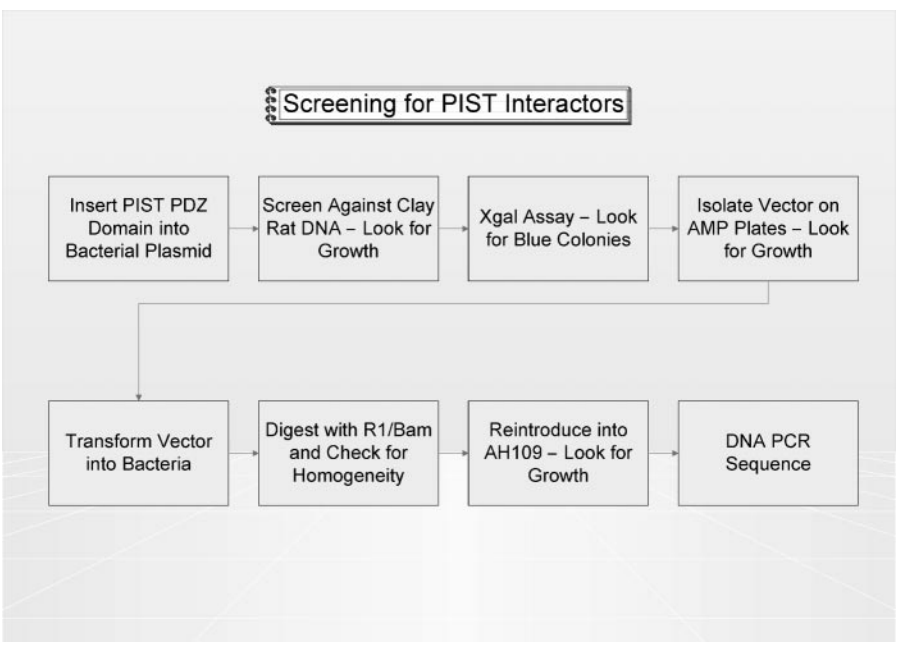

Fig 1. Process for screening for PIST indicators

hour. The DNA was run on an agarose gel during electrophoresis. Only 3:1 and 9:2 showed up on the gel after viewing under UV light.

PRELIMINARY FINDINGS

Thirteen strands of DNA of interest were found and inserted back into AH109 with PIST and a yeast two-hybrid directed interaction was performed. Out of the 13, three showed specific interactions with PIST and were sent to be PCR sequenced. Unknown 23-2 was discovered to be a DNA ligase protein (low interest). Unknown 25-1 was revealed to be a PDZ-binding protein (medium interest). And finally unknown 27-1 was discovered to be AMPA receptor binding protein (ABP), a scaffolding protein that binds to PIST (very high interest). ABP is a cytoskeletal protein that directs intracellular trafficking and also is enriched in the post synaptic density (PSD). ABP has proposed roles during long-term potentiation and long-term depression in the delivery and anchorage of AMPA receptors at synapses. The amount of ABP directly affects the excitatory synaptic strength. ABP is expressed in pyramidal cells and interneurons in cortex and hippocampus and also in neurons of the cerebellum and spinal cord. It is found at the cytoplasmic face of the postsynaptic membrane of excitatory synapses, where it is juxtaposed to synaptic AMPA receptors and also associated with membranes of vesicles in the cytoplasm.

The function of ABP bonded to PIST is still unknown but it is worthy of future research, which could include determining which part of ABP interacts with PIST and if ABP interacts with other scaffolding proteins. In addition, future research could answer the question about how the ABP–PIST interactions affect brain and neuron activity.