Identification of QkI-6 Binding Partners

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Introduction

The neocortex is the largest portion of the cerebral cortex, which deals with conscious mental functions within the mammalian brain. The neocortex contains four primary areas that process specific types of information (motor output and auditory, somatosensory, and visual input).[1] These areas are formed during embryonic development through a process called neocortical arealization which is mediated in part by graded expression of the transcription factor Empty Spiracles 2 (Emx2). The concentration of Emx2 in neural progenitor cells along the anterior (low)-posterior (high) axis determines the functional identity of the neurons born (Figure 1).[2]

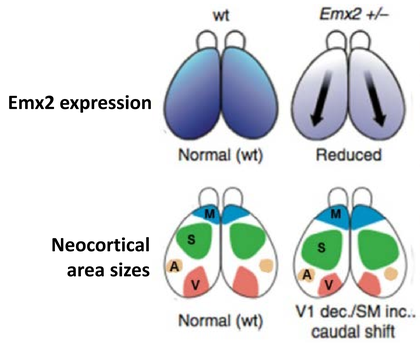


Figure 1. Emx2 levels determine the sizes of the neocortical areas. (top) Emx2 expression levels in embyonic brains from normal (wt) and in genetically-altered mice ($Emx2^{+/-}$). (bottom) These Emx2 levels regulate the size of the neocortical areas: reducing Emx2 levels increases the size of the motor and somatosensory areas at the expense of the visual area.

Although it is clear that the gradient of Emx2 influences the sizes of the areas of the neocortex, little is known about *how*. In order to gain an understanding into *how* Emx2 functions in this regard, the Kroll lab conducted a yeast two-hybrid screen to identify proteins that interact with Emx2. One of the proteins identified by this screen is called QuakingI, which exists in at least three forms in mice and humans (QkI-5, QkI-6, and QkI-7).[3] Since these are mRNA-binding proteins that are thought to function in a very different way from Emx2, we chose to investigate these proteins in more depth. Of the QkI proteins, QkI-6 was selected for further analysis using a second yeast two-hybrid screen. This screen provided approximately 3200 yeast colonies each containing plasmids encoding potential QkI-6 binding partners. The proteins encoded by these plasmid needed to be isolated from yeast, rescued in *E. coli*, and then sequenced in order to determine their identities.

Methods and Materials

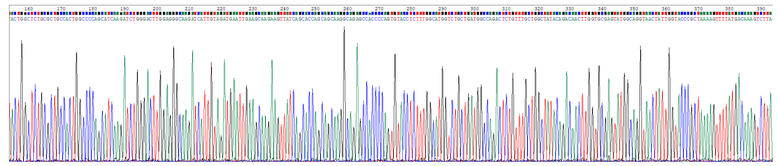
Isolation of bait plasmids from Y2H Gold Yeast One yeast colony per sample was incubated in 0.5 mL SD/-Leu liquid media at 30 °C and 230 rpm overnight. The majority of the supernatant was removed, the pellets resuspended with buffer containing lyticase, and al incubated at 37 °C and 230 rpm for one hour. Samples were lysed by vortexing with 10 µL 20 % SDS and glass beads, briefly freezing at -20 °C, then thawed in a 42 °C water bath and vortexed. The volume of the tube was brought to 200µL with TE buffer pH 7. Next, 200µL phenol:chloroform isoamyl alcohol (25:24:1) was added, vortexed, and centrifuged at 15000 rpm for 10 minutes. The supernatant was removed and the plasmid DNA precipitated with 8 µL of 10 M ammonium acetate and 500 µL of 95% ethanol at -70°C for an hour. The samples were centrifuged for 10 minutes at 15000 rpm and the plasmid DNA resuspended in 20 µL distilled H_2O.

Transformation One-half (10 µL) of the isolated plasmids were combined with 100 µL of chemically-competent DH5-α *E. coli*. The samples were gently mixed, placed on ice for 30 minutes, heat shocked in a 42°C water bath for 45 seconds, then placed on ice again for 2 minutes. 1 mL LB broth was added and the samples were incubated at 37 °C with shaking at 250 rpm to allow for recovery. The cells were then plated on LB + Amp plates and incubated overnight at 37°C.

Plasmid miniprep One colony from each plate was placed in a 5 mL of LB and Amp, and incubated overnight at 37 °C and 300 rpm shaking. About 1.5 µL of the solution was poured into a microcentrifuge tube and given a quick spin in the centrifuge, and the supernatant aspirated. Next, 200 µL P1 was added to resuspend the cells and digest contaminating RNA. Then, 200 µL P2 was added and mixed to lyse the cells, followed by addition of 200 µL of P3 buffer to neutralize the sample. The samples were incubated on ice for 5 min, centrifuged at 15000 rpm for 15 min, and the supernatant transferred to a new microcentrifuge tube. The plasmid DNA was precipitated by the addition of 900 µL of 2-propanol. The solution was centrifuged at 15000 rpm for 20 min and the supernatant aspirated, and the pellets were left to dry for a few minutes. The pellets were resuspended in 22 µL distilled H_2O. The DNA samples were then sent for sequencing and the DNA sequence analyzed using BLAST searches.

Results

The yeast two-hybrid screen using QkI-6 as the bait protein yielded approximately 3200 colonies containing potential binding partners. Many of the plasmids from these colonies have been isolated, transformed, prepped, and sequenced (example shown below).



Approximately 10-20% of the of the clones encode potentially interesting proteins. Examples include CacyBP (Calcylin Binding Protein), Rack1 (Receptor for Activated C Kinase 1), and Actin Beta (Table 1).

Clone #	NCBI accession # (protein)	Protein name	Part of protein retrieved (total length)
6-28	NP_03396.1	Calcyclin binding protein (Cacybp)	40-119 (229)
6-53	NP_001239330.1	Transducin-like enhancer of split (Tle2)	580-732 (732)
6-73	XP_021067101.1	Receptor for activated kinase 1 (Rack1)	186-271 (271)
6-71	NP_001300852.1	β actin (Actb)	116-253 (253)
6-78	NP_001300852.1	β actin (Actb)	105-253 (253)

Table 1. CacyBP is involved in cell division and other cellular functions[4]; Actin Beta in neural development and gene expression[5]; and Rack1 is a ribosomal scaffold protein.[6]

Discussion and Future Work

The yeast two-hybrid screen using QkI-6 as bait produced about 3200 colonies containing plasmids encoding potential binding partners. The majority of these plasmids have been processed and sequenced. Proteins of potential interest will be cloned (the screens usually only retrieve fragments of the protein sequences), so that the full-length proteins can be confirmed (or rejected) as true binding partners for QkI-6. This will be done by using a yeast two-hybrid assays and GST pull-down assays. The true binding partners will then be tested for their abilities to bind to Emx2 and other Emx2-binding partners. These results will allow the Kroll lab to begin probing more deeply into the question of how Emx2 regulates the process of neocortical arealization during embryonic neocortical brain development in mammals.

References and Acknowledgements

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