

Characterization of Proteins that Interact with Alpha Subunits of Actin Capping Protein

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Introduction

Actin plays a key role in many cellular functions including muscle contraction, cytokinesis, cell motility, size and shape. Actin is regulated by a variety of accessory proteins including actin capping protein (CP). CP is a heterodimer composed of an alpha (α) and beta (β) subunit.

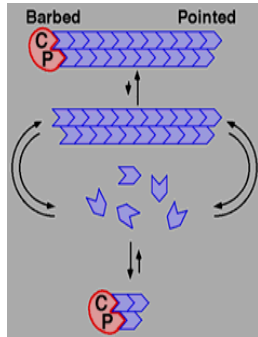


Figure 1. Actin Capping Protein (CP) provides regulation on the barbed ends of the actin filaments.

Background

Lower organisms have one isoform of both the α and β subunit. In contrast, in higher organisms, three α subunit isoforms have been identified.

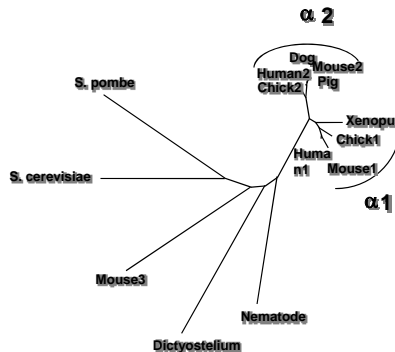


Figure 2. Phylogenetic Tree for CP α . Vertebrates have two conserved alpha isoforms, which suggests that the isoforms have different functions.

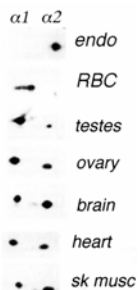


Figure 3. 2D Immunoblots of murine tissues. CP α isoforms are expressed in different levels in murine tissues suggesting divergent function.

Hypothesis

We hypothesize that the α isoforms have unique cellular and biochemical roles in tissue and cells, and therefore interact with different cellular proteins.

Experimental Design

A yeast two hybrid screen was employed using a murine embryonic cDNA library as prey, and either $\alpha 1$ or $\alpha 2$ as bait to identify protein interactions between $\alpha 1$ and $\alpha 2$ and other structural or regulatory proteins. The $\alpha 1$ screen identified five interacting clones and the $\alpha 2$ screen identified seven interacting clones. Sequence analysis confirmed the identity of four of the $\alpha 2$ clones as actin, myosin heavy chain 3, serine proteinase inhibitor, and a novel gene on Musculus chromosome 1. The purpose of my research is to characterize the remaining clones.

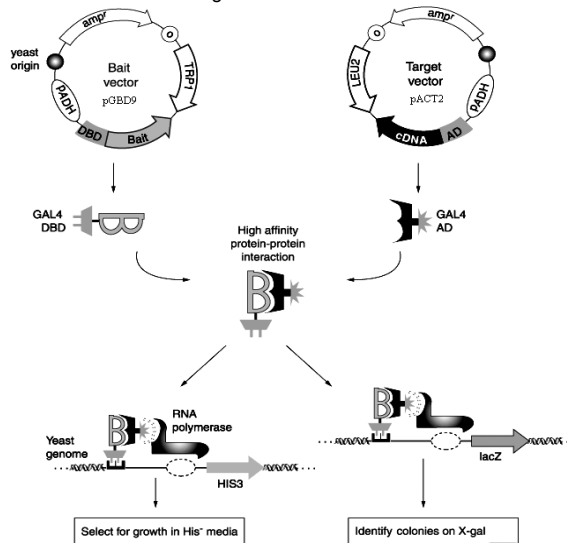


Figure 4. Interaction of two hybrid proteins in the two-hybrid system brings together the two functional domains (bait and prey) of the yeast transcription activator and results in transcription of the reporter gene.

Methodology

- 1) To isolate plasmid DNA, yeast cultures were grown in a selective drop out media overnight at 250 RPM. Cultures were then centrifuged at 13,000 rpm for 5 minutes. Lyticase was added and cells were incubated at 37 ° Celsius for 1 hour. 20% SDS was also added.
- 2) Samples were frozen at -20 ° Celsius and thawed.
- 3) The Qiagen miniprep protocol was followed according to manufacturer's guidelines.
- 4) 30 μ l of eluted DNA was used to transform chemically competent KC8 cells. Cells were plated on LB/Ampicillin and colonies were isolated.
- 5) DNA was quantified using absorbance at A260.
- 6) Insert DNA was amplified using plasmid specific primers via PCR and samples were analyzed using Agarose Gel Electrophoresis.

Results

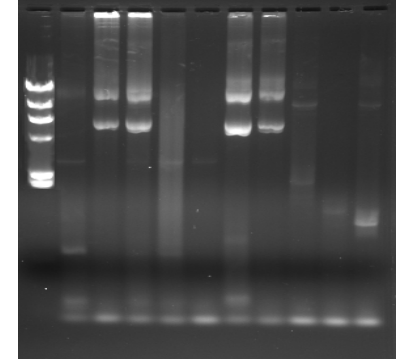


Figure 5. Quantitative Agarose Gel Electrophoresis of Insert DNA. Associated clone samples were loaded in lanes 2-11 (order shown below).

Lane	Clone/Sample	PCR Product
1	Hind III/Lambda	
2	A6	15kb, 3.4kb, 439bp, 142 bp
3	F1	14kb, 7.2kb
4	C1	13.7kb, 6.8kb, 3.4kb, 142 bp
5	F10	3.4kb, 439bp, 142bp
6	D3	3.4kb, 142bp
7	37/6	13.5kb, 6.3kb, 563bp, 142bp
8	33/4	13.7kb, 6.7kb
9	11/2	11.7kb, 2.2kb
10	1/3	1.06kb
11	46/3	18.6kb, 9.7kb, 816bp

Figure 6. PCR Product size for corresponding clones was determined from the HindIII standard curve from the Gel.

Conclusion

1. Chemically competent cells were prepared, transformed and plated on selective media.
2. We have isolated plasmid DNA from interacting clones identified in a yeast two hybrid screen and determined the plasmid size.
3. DNA was quantified and inserts were characterized via PCR.
4. Alteration of protein-protein interactions is known to contribute to many diseases. The manipulation of protein-protein interactions that contribute to a disease may be used as a potential therapeutic strategy.

Future Studies

1. Sequence clones of interest.
2. Analyze sequence information.
3. Test interaction via co-immunoprecipitation or *in vitro* "pull down" assay.

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