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Angelique DuCharme
Minnesota State University, Mankato

Charity Zabel
Minnesota State University, Mankato

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Characterization of Proteins Interacting with the Alpha Subunit of Actin Capping Protein

Angelique DuCharme and Charity Zabel *(Department of Biological Sciences)*
Faculty Mentor Dr. Marilyn Hart *(Department of Biological Sciences)*

Abstract

Actin, a filamentous component of all cells, contributes to cell shape, cell motility and force transmission. Actin assembly and dynamics are regulated by a diverse array of regulatory proteins, including actin capping protein (CP). CP is a heterodimeric protein composed of two subunits, alpha (α) and beta (β). Three isoforms of each subunit exist in eukaryotes. The β isoforms have been shown to have distinct functions *in vivo.* The functions of the specific α isoforms have yet to be determined. Overall, the amino acid sequence of the α isoforms are highly conserved, sharing approximately 90% sequence identity. The region of divergence is also highly conserved among higher organisms, suggesting that the alpha isoforms have distinct functions *in vivo*. We hypothesize that the alpha isoforms perform different functions in cells/tissues. The purpose of this research is to investigate the function of the alpha proteins of CP by identifying proteins that interact with the α1 and α2 subunits. In a previous yeast two hybrid genetic screen, potential interacting proteins were identified. Two methods were utilized to purify the DNA from the yeast. The first method, the small-scale preparation of yeast DNA, did not give a high yield of DNA. The second method, the rapid isolation of yeast DNA, not only produced a high yield of DNA, but also transformed into *E. coli*. Future research is required to complete the characterization of the proteins that interact with the alpha subunits and determination of the subunits' distinct functions.

Introduction

Actin, a filamentous component of all eukaryotic cells, contributes to cell shape, cell motility and force transmission[i]. Actin assembly and dynamics are regulated by a diverse array of regulatory proteins, including actin capping protein (CP). CP is a heterodimeric protein, composed of two subunits, alpha (α) and beta (β). In lower organisms, there is one α and one β CP form. On the other hand, in higher organisms, three forms of α (α1, α2, α3) and three forms of β (β1, β2, β3) have been identified[ii]. Whereas the α3 and β3 subunits have only been found in male germ cells, the α1 and α2 subunits are expressed in somatic cells[iii]. In mouse heart tissue, both β forms are expressed but have distinct functions[iv]. In contrast, the functions of the α subunits have yet to be determined. We hypothesize that the α subunit isoforms also have distinct functions *in vivo* and interact with distinct proteins. In previous studies, a yeast two-hybrid screen was performed to identify proteins that interact with CPα1 and CPα2. Briefly, plasmids containing the complete coding region of either CPα1 or CPα2 and a murine embryonic cDNA library were co-transformed into yeast. Interaction of CPα1 or CPα2 resulted in the expression of a reporter gene, in this case beta-galactosidase. Putatively interacting clones were collected and frozen for future extraction and characterization[v]. See Figure 1.

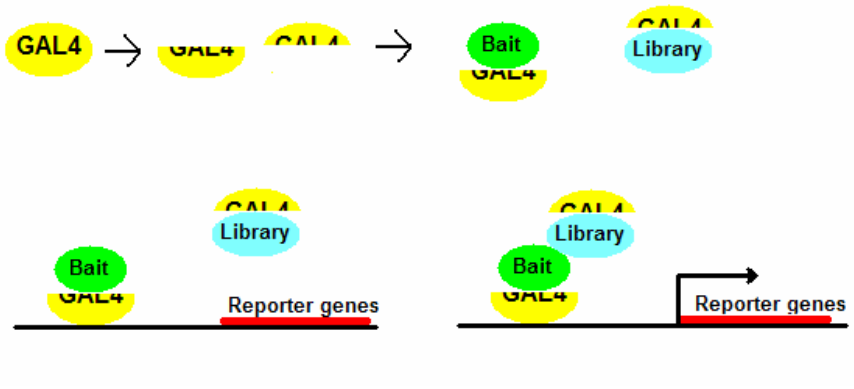

Figure 1—Yeast two-hybrid screen: The cDNA library and the alpha subunit were bound to separate plasmids, each with a portion of the Gal4 promoter. Upon interaction, the Gal4 promoted the expression of the reporter gene, beta-galactosidase. Figure taken from http://en.wikipedia.org/wiki/Image:Two-hybrid_system.PNG.

Purpose

The purpose of this research was to characterize the potentially interacting proteins, identified from the previous yeast two hybrid screen. Plasmid DNA will be extracted from yeast and transformed into *E. coli*. Transformation of *E. coli* will allow the plasmids to be separated as only one plasmid will enter the cell. Using this strategy, the plasmid containing the interacting protein can be isolated and further investigated. By understanding the role of CP, we will gain knowledge that may be applied to cell structure and function and ultimately, human disease.

Methods

Small-scale Preparation of Yeast DNA

This procedure was taken from Sambrook and Russel. "Small scale Preparation of Yeast DNA." Molecular Cloning: A Laboratory Manual. Chapter 4. Protocol 12. Volume 1. 2001.

- Suspend Yeast in Sorbitol buffer
- Add 100T Zymolase. This lyses the cell wall.
- Centrifuge and add yeast resuspension buffer
- Add 10% SDS and 5M Potassium Acetate
- Centrifuge and transfer supernatant, which should only contain DNA, RNA, and nucleic acids.
- Add isopropanol to precipitate nucleic acids
- Centrifuge and resuspend the purified DNA in TE (pH 8.0) with RNase to destroy the RNA
- Add 3M Sodium Acetate and isopropanol
- Centrifuge and resuspend the plasmid DNA in TE (pH 7.4)

Rapid Isolation of Yeast DNA

This procedure was taken from Sambrook and Russel. "Rapid Isolation of Yeast DNA." Molecular Cloning: A Laboratory Manual. Chapter 6. Protocol 7. Volume 1. 2001.

- Suspend pellet in STES

- Add acid-washed beads, TE (pH 7.4), and phenol:chloroform, followed by vortexing. This lyses the cell.
- Centrifuge and transfer supernatant, which contains the DNA, RNA, nucleic acids, and some other water soluble compounds, while the cell fragments remain in the phenol:chloroform and non-soluble layers.
- Perform Ethanol precipitation—selectively precipitates DNA and RNA
- Centrifuge, remove supernatant, and rinse with 70% ethanol
- Centrifuge and resuspend in TE (pH 7.4) with RNase

Transformation into *E. coli*

This procedure was taken from Sambrook and Russel. "Preparation and Transformation of Competent *E. coli* using CaCl2." Molecular Cloning: A Laboratory Manual. Chapter 1. Protocol 25. Volume 1. 2001.

Preparation of Cells

- Grow KC8 *E. coli* cells in LB broth with Kanamycin antibiotic to prevent contamination of other bacteria.
- Centrifuge cells, decant supernatant, and resuspend with 0.1M CaCl2
- Centrifuge cells, decant supernatant, and resuspend with 0.1M CaCl2
- Incubate cells for 24 hours at 4°C

Transformation

- Combine competent cells and DNA, and follow by gentle mixing
- Cool on ice. This allows time for the DNA to enter the *E. coli* cells.
- Heat shock at 42°C, then chill in ice bath.
- Add LB broth and incubate the cells at 37°C. This recovery period allows the cells to utilize the plasmid and recover from the heat shock.
- Plate the *E. coli* onto LB plates with Ampicillin antibiotic and onto M9 minimal media plates, lacking Leucine and containing the Ampicillin antibiotic, and then incubate plates at 37°C for 20 hours.

Results

Table 1—Quantitative Comparison of the DNA Isolation Techniques

	Small-scale Preparation		*Rapid Isolation*	
Clone number	Absorbance	Concentration (ng/µl)	Absorbance	Concentration (ng/µl)
1/2	0.003	7.50	0.023	230
1/4	0.019	31.67	0.018	90
2/2	0.005	5.00	0.013	325
2/3	0.002	10.00	0.021	525

4/1	0.009	11.25	0.025	250

Table 1—Quantitative Comparison of the DNA Isolation Techniques: This table contains a sample of the absorbance readings at 260nm and calculated concentrations of the DNA isolated using both extraction methods. Comparison of the two protocols revealed that each had definite advantages and disadvantages. The small scale preparation method had very involved methods and low yield of DNA but effectively removed RNA from the solution. Although the rapid isolation technique did not remove the RNA from the final solution, the plasmid DNA concentration was much higher, resulting in successful transformation into *E. coli*.

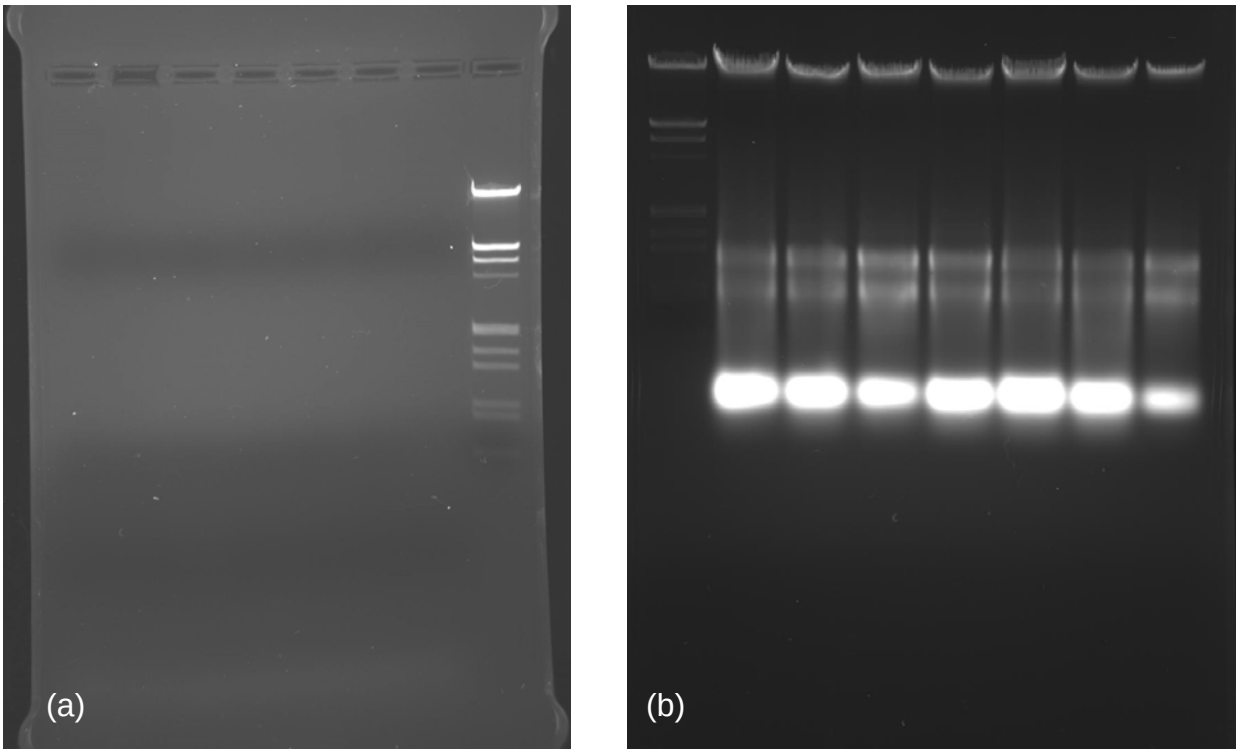

Figure 2—Agarose electrophoresis gels: Agarose Electrophoresis gels were run to visualize the amount of DNA present from the purification. Figure 2(a) has sample DNA from the small-scale preparation, while Figure 2(b) displays a gel with DNA samples obtained from the rapid isolation technique. The only bands present in Figure 2(a) were from the standard DNA, indicating that the extracted DNA concentrations were so low that they were unable to be detected by ethidium bromide visualization. On the other hand, the results in Figure 2(b) validated the effectiveness of the rapid isolation technique, as there were visible bands present on the gel.

The initial transformation of the DNA from the rapid isolation technique yielded colonies on LB Ampicillin plates. There was no growth on the M9 minimal media plates, which should have selected for colonies containing the plasmid of interest.

Conclusions

A low yield of DNA was obtained using the small-scale preparation technique; however, the rapid isolation technique led to the extraction of higher concentrations of DNA for each clone. DNA was extracted successfully from all 160 clones. A portion of the clones' DNA were effectively transformed into *E. coli* on LB ampicillin plates.

Future Research

Further investigation into the M9 minimal media plates will be needed to determine why there was no growth. Plasmid DNA will then be transformed and the plasmid of interest selected using the improved M9 minimal media. The DNA will then need to be amplified with PCR and analyzed by sequence analysis at the University of Minnesota Sequencing Center.

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Authors' Biographies:

Angelique DuCharme graduated from New Prague High School in 2001, and immediately enrolled in classes at Minnesota State University, Mankato. Having received her Nursing Assistant's licensure in 2000, she continued to work throughout college in her hometown nursing home and at Immanuel St. Joseph's hospital in Mankato. She worked for two years in Dr. Marilyn Hart's research lab, first as an assistant to a graduate student, then on this project. She has actively participated in Pre-med Club, serving as Treasurer during the 2004-2005 school year, and in Chi Alpha Christian Fellowship, leading a small group discussion each week. She has been an active member of the New Market, Elko, and Webster Lions Club for 3 years, volunteering her time in many of the events they have each year. In May of 2006,

Angelique graduated Summa Cum Laude from Minnesota State University's College of Science, Engineering, and Technology with a Bachelors of Science in Biology, with an emphasis in Human Biology. She will be getting married in June and moving to the northern metro area while her husband pursues his degree. Angelique will be working, shadowing medical professionals, and volunteering. She will also be applying to medical school to pursue a career in psychiatry.

Charity Faith Zabel is a 2001 graduate of Waconia High School in Waconia, Minnesota. Charity then journeyed to Minnesota State University, Mankato (MSU) to further her education. During the summer of 2001, Charity had received her Nursing Assistant Certification, and actively worked as a nursing assistant in a variety of settings throughout her college career, including a nursing home, an assisted living facility, and a hospital. In addition to classes, Charity devoted her time to Campus Crusade for Christ, serving as a vocalist on the worship team and as a small group leader for a number of years, and to the Pre-med Club. Charity's fifth year led her back to the dorms, where she held the Anatomy of Nursing Learning Community Coordinator position, which gave her a chance to use all the knowledge and wisdom that she'd gained to make a difference in the lives of freshmen prenursing students. This spring Charity graduated Summa Cum Laude from the MSU's College of Science, Engineering, and Technology with a Bachelor of Science in Biology with a Human Biology emphasis. Charity is getting married at the end of June, and the couple will reside in Waconia, Minnesota for a year or two. During that time, Charity plans on spending plenty of time volunteering, job shadowing Physician's Assistants and Medical Doctors, and working as a Nursing Assistant. Within a year or two, Charity hopes to get accepted to Physician's Assistant School or Medical School, in order to continue her journey in the medical field.

Faculty mentor's biography:
Dr. Marilyn Hart is a professor of biology at Minnesota State University, Mankato. She received her Ph.D. in Cellular and Molecular Biology from the St. Louis University School of Medicine. Dr. Hart is also chair of the Undergraduate Research Conference.

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