Protein-protein Interaction Important for Cytokinesis

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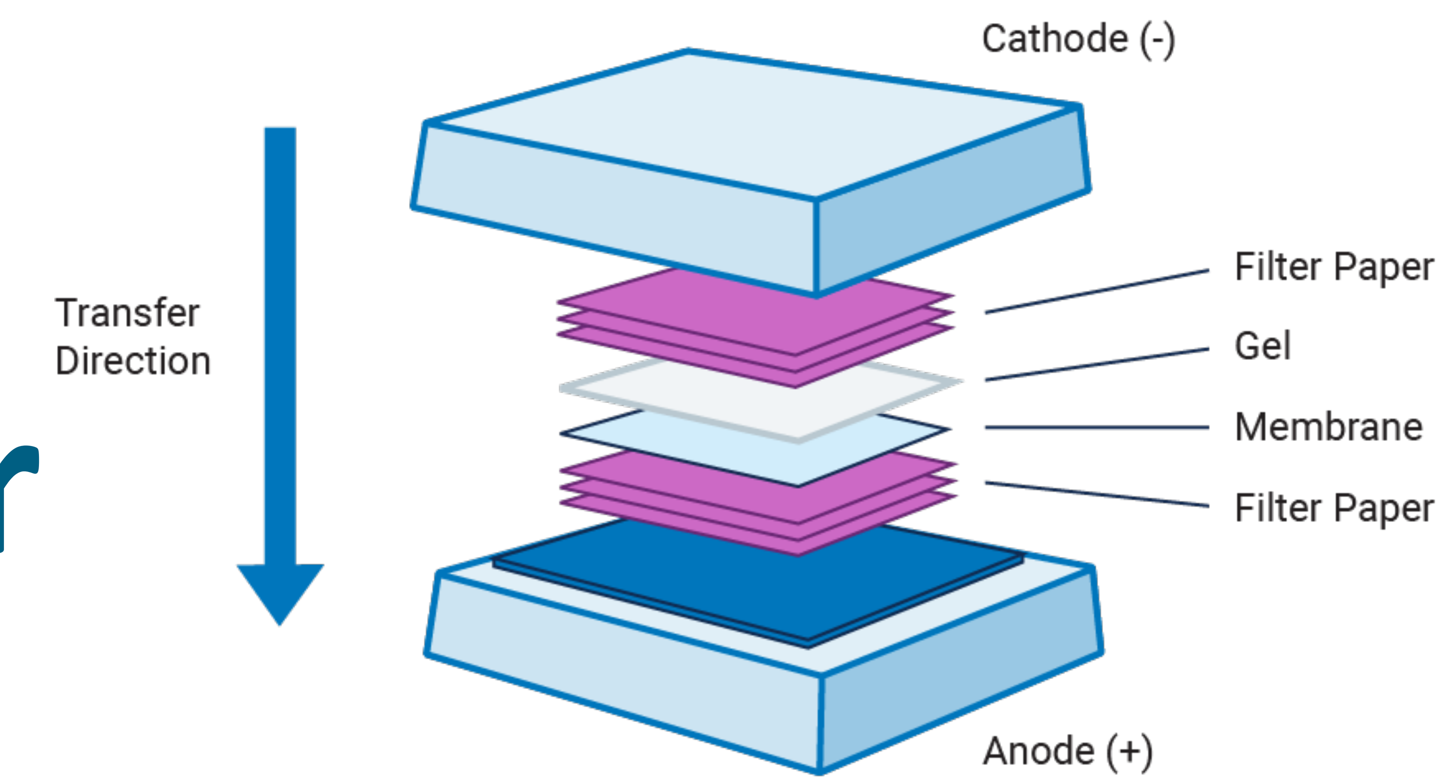


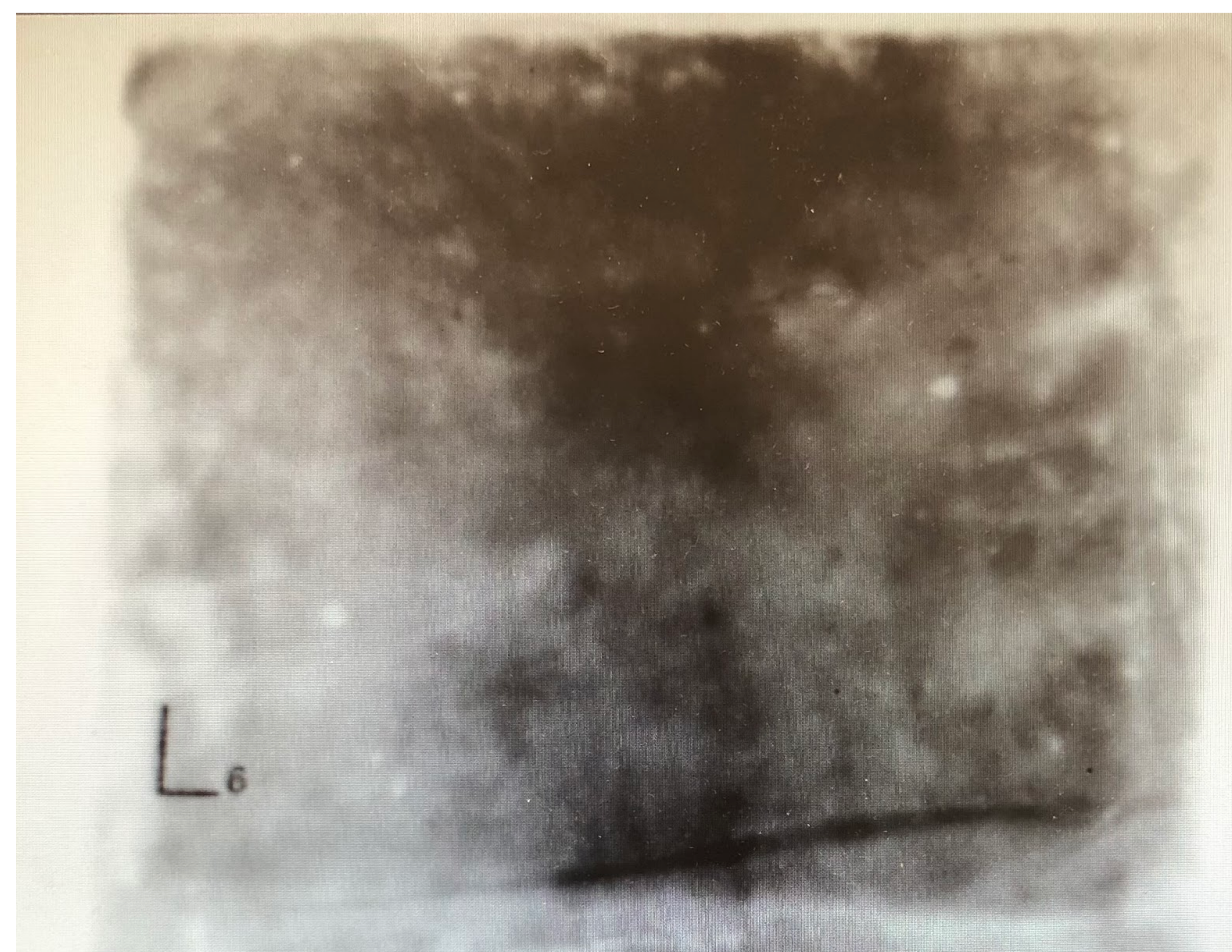
ABSTRACT

In budding yeast cells, the gene Iqg1 is a gene necessary for proper actin ring formation and contraction. We have shown that Iqg1 interacts with the yeast formin proteins. This project will determine which domains of Iqg1 mediate the protein-protein interactions, which is important to understand the function of the binding between Iqg1 and formins in cytokinesis. We will use a GST pull down assay to study the binding of Iqg1 to the yeast formins Bni1 and Bnr1. We will compare binding of formins to full length Iqg1 to the ability of formins to bind deletion mutants of Iqg1. The expected outcome is that using the assay, we will identify which region or regions of the Iqg1 protein are required for binding to the formins. This area of research is important for new therapeutic targets in cancer treatment.

BACKGROUND

Cytokinesis is the final step in cell division and divides the cytoplasm between two daughter cells. Iqg1 is an actin filament binding protein that enhances actin ring formation. It is an IQGAP family member required for assembly and contraction of the actomyosin ring. The functions of IQGAPs can be corrupted during oncogenesis and are usurped by microbial pathogens. Therefore, IQGAPs can be seen as possibilities for therapeutic agents. Because the human homologs of Iqg1 have been implicated in cancer, learning about the role of Iqg1 may lead to development of new therapeutic targets. The goal of this project is to figure out which part of the Iqg1 gene binds to yeast formins Bni1 and Bnr1.

RESULTS



METHODS

- To purify GST-Bnr1, we began by purifying soluble GST-fused proteins from E. coli. GST-Bnr1 was purified from the bacterial cells and then tested on an SDS-PAGE gel.
- GST pulldown was performed using strains KSY 133, KSY 419, and KSY 462. Extracts were mixed with protein beads before performing experiment on 7.5% SDS-PAGE gel.
- Western blotting is the procedure we use after our gels have finished running and transferring. To transfer the complete gel, we start by removing the stacking portion of the completed gel. The gel is then stacked between sponge, filter paper, nitrocellulose, transfer paper, and another sponge before being transferred.
- Transfers are imaged in the chemidoc imaging system using the chemi hi-sensitivity program.

SUMMARY

There were bands that appeared on the image towards the bottom of the gel, but they were not in the correct location that Iqg1 should be in. Because of this, we could not draw a conclusion to our original hypothesis. Despite this, trouble shooting and testing continues as we plan to make new extracts and protein beads before mixing new samples and performing another GST pulldown assay.