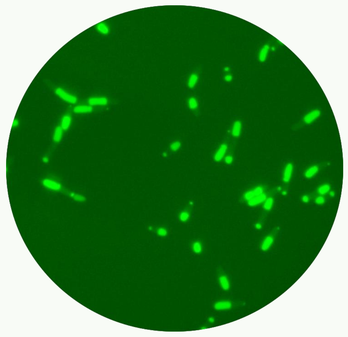

Developing an assay for Polar Organizing Protein Z (PopZ) mutant screening

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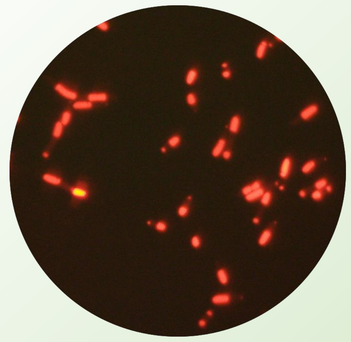


Abstract

The long-standing model that protein structure is critical to function has been expanded to include intrinsically disordered proteins (IDPs). IDPs facilitate several biological functions and have significance to human health. IDPs lack stable structures and exist more as conformational ensembles making studying IDP structure more challenging. The mechanisms of polar organization in bacteria are being used to study IDP structure experimentally. Recent efforts have exploited the mechanisms of polar organization with the IDP PopZ in *E. coli* to identify several PopZ protein binding partners. Four recombinant *E. coli* strains with PopZ and known protein binding partner plasmids were used to develop an assay. This assay was based on protocols for the purification of PopZ and many binding partners. Liquid cultures for the four strains were inoculated, diluted, and induced with IPTG and arabinose. Slides were then prepared for fluorescent microscopy. PopZ was labeled with mCherry. The binding partners and control protein were labeled with GFP. PopZ and mutant PopZ exhibited polar localization. GFP and mCherry polar localization resulted from binding between PopZ and the ChpT and ParB strains. Diffused GFP was observed for both DivK and the mutant PopZ with ParB strains. These results verified the effectiveness and accuracy of the assay. Detailed protocols were written for PopZ mutant screening, planned for the summer of 2017 at UW-Casper. Future work will include modifications to the concentration of IPTG and arabinose to assure the appropriate levels of protein expression during screening.

Introduction

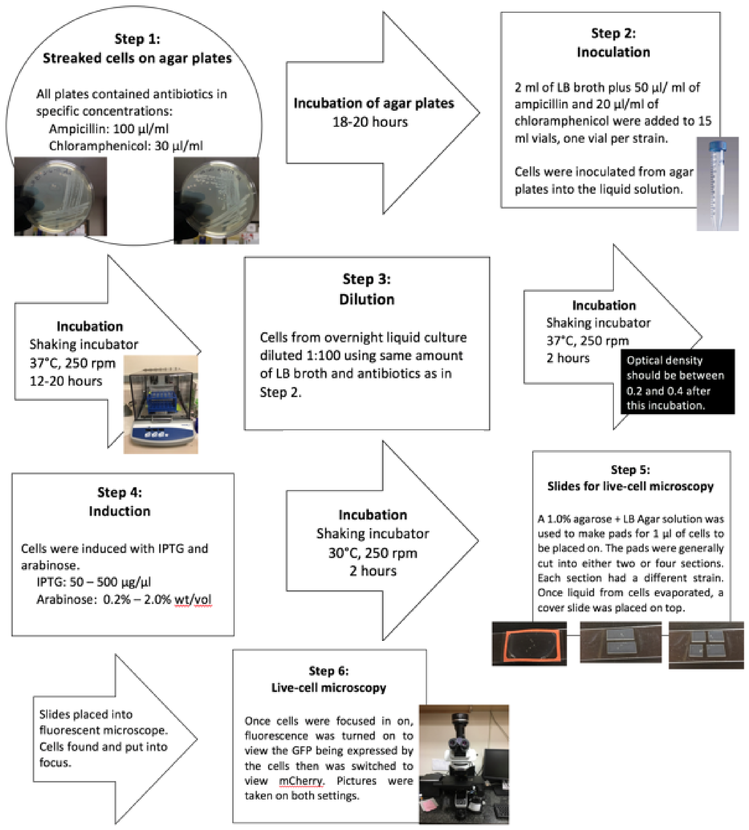
Ordered proteins require a folding pattern that forms a structure that is specific to the function it performs. Intrinsically disordered proteins (IDPs) have regions of amino acid sequences that do not have a definite folding pattern, but are still able to function similarly to an ordered protein (1). When an IDP encounters a binding partner, the disordered region will fold specifically to bind with that partner. IDPs can have numerous binding partners and cellular functions (2). When mutated, some IDPs have been discovered to be associated with certain diseases, including cancer (2). One example is human protein, p53 (3). A connection between p53 function and cancer has been identified with about half of all human cancers having mutations in p53 (4). Studying IDPs structure-function relationship could have many potential benefits to human health. Polar organizing protein Z (PopZ) is an IDP found in *Alphaproteobacterium Caulobacter crescentus* (3). PopZ is required for polar localization of several proteins (3). Polar localization is important in rod-shaped bacterial cells, for example, in constructing structures such as flagella (5).

The assay developed for this project is designed to experimentally confirm potential positive and negative PopZ binding partners. In these experiments, recombinant *Escherichia coli* (*E. coli*) was used to express the recombinant proteins which are fused with GFP and mCherry fluorescent markers (Table 1). Visualization of proteins in the cell verified whether the PopZ protein was interacting with the specified binding partner. The purpose of these efforts is to finalize an assay to be used with PopZ mutants.

Strain	Plasmid Vectors	Expected Microscopy Results
155 *Positive Control*	mCherry/PopZ + GFP/ChpT	Polar co-localized PopZ (red) and ChpT (green)
JH81 *Positive Control*	mCherry/PopZ + GFP/ParB	Polar co-localized PopZ (red) and ParB (green)
68 *Negative Control*	mCherry/PopZ + GFP/DivK (Control, negative binding partner)	Polar localized PopZ (red), dispersed DivK (green)
JH160 *Negative Control*	mCherry/PopZ mutant*+ GFP/ParB *PopZ has three mutations on the N-terminal region that keep it from interacting with ParB. ParB was shown to be a PopZ binding partner in prior research (3).	Polar localized PopZ (red), dispersed ParB (green)
Note: Arabinose induces for binding partner expression and IPTG induces for PopZ expression.		

Table 1 Summary of the four recombinant strains used in the development of the assay. The *E. coli* strain BL 21 was used to develop the above 4 recombinant strains

Materials and Methods



Results

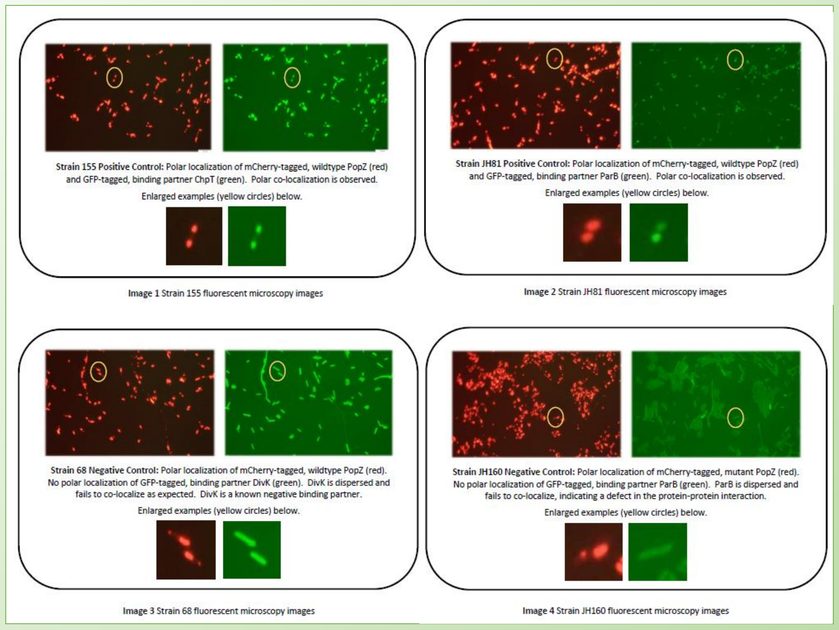
Fluorescent microscopy observations offered support to verify the effectiveness and accuracy of this assay. As expected, wildtype PopZ and mutant PopZ exhibited polar localization in all strains. Wildtype PopZ exhibited polar localization, and the GFP-tagged binding partners, ChpT and ParB, co-localized with the polar PopZ foci (Images 1 and 2). DivK is a known negative protein binding partner with wildtype PopZ. While the wildtype PopZ exhibited polar localization, the GFP-tagged DivK did not co-localize with the polar PopZ foci and was observed to be dispersed throughout the cell (Image 3). A mutant form of PopZ was investigated in this experiment. It localized to the cell poles, but the ParB binding partner failed to co-localize, indicating a defect in protein-protein interaction (Image 4).

All strains were observed to express proteins at adequate levels for verification of the assay. The protein expression induction was performed at 0.2% wt/vol arabinose and 100µg/µl of IPTG.

References

1. Oldfield, C.J., and Dunker, A.K. (2014) Intrinsically disordered proteins and intrinsically disordered protein regions. *Reviews in Advance*. 83(25): 1-32.
2. Babu, M.M., van der Lee, R., de Groot, N.S., Gsponer, J. (2011) Intrinsically disordered proteins: regulation and disease. *Current Opinion in Structural Biology*. 21(3):432-440.
3. Holmes, J.A. Follett, S.E., Wang, H., Meadows, C.P., Varga, K., and Bowman, G.R. (2016) *Caulobacter* PopZ forms and intrinsically disordered hub in organizing bacterial cell poles. *Proceedings of the National Academy of Sciences*. 113(44): 90-95.
4. Alberts, B., Bray, D., Hopkin, K., Johnson, A.D., Lewis, J., Raff, M., Roberts, K., Walter, P. (2014) Essential Cell Biology. *Garland Science Taylor & Francis Group, LLC*. (4): 615.
5. Bowman, G.R., Lyuksyutova, A.I., and Shapiro, L. (2011) Bacterial polarity. *Current Opinion in Cell Biology*. 23(1): 71-77.

Results



Discussion and Future Plans

The observed behavior and interaction of PopZ and binding partner proteins were as expected, based on previous experiments. These results served to verify the effectiveness and accuracy of this assay. This assay is planned to be used to screen for co-localization with new strains that will include mutant PopZ and many binding partners. Consistent, accurate results from the developed assay are critical for the next phase of the project.

In these experiments, inducers are used to initiate the process of transcription (4). The inducer concentration can be adjusted to increase or decrease gene expression. Using too much IPTG causes excessive PopZ expression. Excessive expression of PopZ can negatively affect the *E. coli*, in essence making it sick. It can also reduce the expression of the protein binding partner. This can distort the microscopy results and analysis of the interaction between PopZ and binding partners. Further efforts may include modifications to the concentration of the IPTG and arabinose inducers. Previous research has suggested a range from 50µg/µl to 500µg/µl final concentration at induction of IPTG (3). Observations from initial results suggested that the amount of IPTG should be adjusted to a lower concentration. The level of arabinose is not as critical to *E. coli* health if it falls into the upper range of the recommended induction range, 0.02%- 2.0% wt/vol. Our results suggested that the arabinose concentration may need to be increased from the current 0.2% concentration for the 68, JH81, and JH160 strains. The current 0.2% concentration for the 155 strain was shown to be adequate for expression levels.

In the next phase of the project, PopZ mutants will be created and screened for binding with normally positive binding partners. It is hypothesized that certain segments in the amino acid sequence of PopZ are responsible for its binding interactions. Removing these sequences or mutating them will result in PopZ failing to interact with a normally binding partner. These experiments will support efforts to further understand the structure-function relationship of IDPs.

Acknowledgments: This project was supported in part by grants from the National Center for Research Resources (P20RR016474) and the National Institute of General Medical Sciences (P20GM103432) from the National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.