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Yeast Bxi1p/Ybh3p is a pH-sensitive calcium channel in Escherichia coli.

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ABSTRACT

Yeast Bax inhibitor-1 (BXI1/YBH3) encodes a protein that belongs to the Bax Inhibitor (TMBIM) family of proteins, which has been linked to different tumor types in human patients. The crystal structure of a prokaryotic member of the family, BsYetJ, has revealed that the Bxi1 proteins are pH sensitive calcium leaks. Our laboratory has shown that Bxi1p is localized to the yeast ER and vacuole and our genetic studies suggest that the protein is a channel that controls the efflux of calcium from the ER. We have also over expressed Bxi1p in E.coli and have used a fura-2 based calcium assay to show that the protein facilitates the influx of extracellular calcium into the cell. Further studies have suggested that the influx of calcium can be altered by the pH of the extracellular environment, and that Bxi1p functions as a generalized cation channel. We have initiated a screen to identify small molecule blockers for the channel in the hopes of identifying drugs that would kill cancer cells that are addicted to Bxi1p. Preliminary results suggest that Gadolinium is a potential molecular inhibitor of yBxi1p. [Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

INTRODUCTION

The Bax inhibitor-1 (BXI1) gene, a member of the Bcl-2 family, was first discovered by its ability to inhibit Bax-induced program cell death in the budding yeast Saccharomyces cerevisiae [1]. This gene was found to be conserved in a wide range of fungi, plant, and animal species [3].This conservation as well as its link to serveral kinds of cancers when over expressed have spoken to the importance of the gene and has led to its continued study. It is known that BI-1 plays a role in the regulation of endoplasmic reticulum (ER) stress [4].

We are working to determine the function of BXI1 in calcium transport into the cell. By using E.coli transformed with YBXI1p we can assess the transport of calcium directly into the cell using fura-2. Our data suggests that bxi1 is a pH sensitive calcium leak, that is inhibited by acidic pH and is enhanced by slightly basic pH. Data also suggests that the calcium leak is inhibited by the Gadolinium cation.

FIGURE 1: The crystal structure of the Bax Inhibitor family of proteins reveals that they are pH-sensitive calcium leaks.

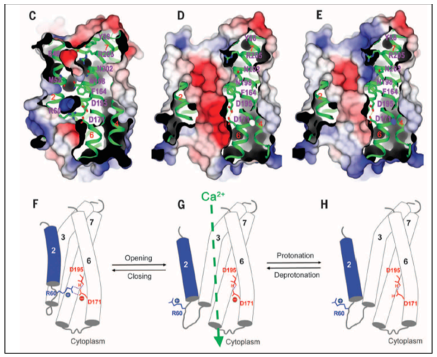

FIGURE 2: yBxi1p-GFP can be overexpressed in *E. Coli.*

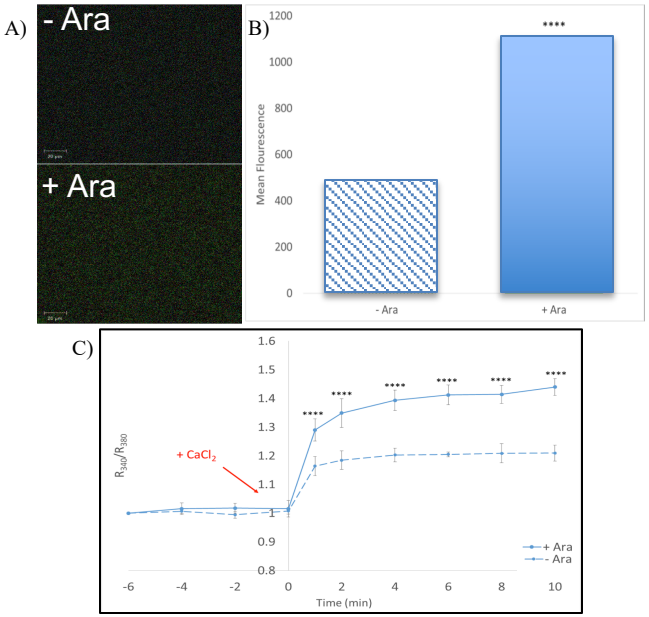

FIGURE 2: A) *E. coli* cells transformed with a plasmid expressing BXI1-GFP were incubated with arabinose to express Bxi1p-GFP. Cells incubated in arabinose showed GFP fluorescence. B) Flow cytometry readings of GFP fluorescence after 6 hours. C) Bxi1p-GFP *E. coli* expressing cells were cultured for six hours, with and without arabinose. They were then collected, washed, and incubated with Fura-2 for two hours. 250 µl of cells were aliquoted into a 96-well plate. A Cytation3 Cell Imaging Multi-Mode Reader was used to image the cells at 340nm and 380nm, which correspond to the activation threshold of Fura-2. After a 6 minute baseline, 5 µl of CaCl2 was added to both conditions. (n=6; **** p<0.001)

FIGURE 3: yBxi1p-GFP is permeable to calcium in a pH-dependent manner in *E. coli.*

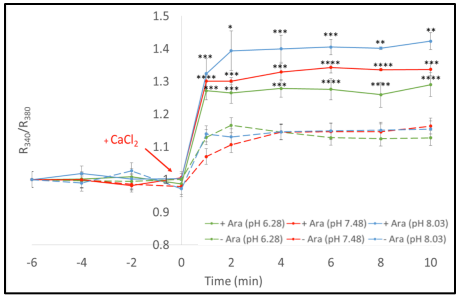

FIGURE 3: Bxi1p-GFP *E. coli* expressing cells were cultured for six hours, with and without arabinose. They were then collected, washed, and incubated with Fura-2 for two hours. Cells were then placed in buffers of various pH. 250 µl of cells were aliquoted into a 96-well plate. A Cytation3 Cell Imaging Multi-Mode Reader was used to image the cells at 340nm and 380nm, which correspond to the activation threshold of Fura-2. After a 6 minute baseline 5 µl of CaCl2 was added to both conditions. (n=3; **** p<0.001, ***p<0.005, **p<0.001, *p<0.05)

FIGURE 4: Gadolinium inhibits yBxi1p-GFP dependent calcium permeability in *E. coli.*

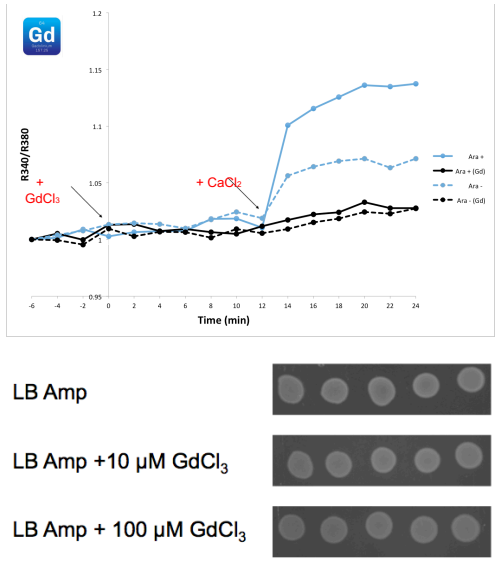

FIGURE 4: A) Bxi1p-GFP *E. coli* expressing cells were cultured for six hours, with and without arabinose. They were then collected, washed, and incubated with Fura-2 for two hours. Cells were then placed in buffers of various pH. 250 µl of cells were aliquoted into a 96-well plate. A Cytation3 Cell Imaging Multi-Mode Reader was used to image the cells at 340nm and 380nm, which correspond to the activation threshold of Fura-2. After a 6 minute baseline, 5 µl of GdCl3 was added to both conditions and after an additional 12 minutes, 5 µl of CaCl2 was added. B) To ensure that Gadolinium does not kill the *E. coli,* cells were grown on plates of different concentrations to test their viability.

CONCLUSIONS

- Our data suggests that yBxi1p-GFP is a calcium leak when overexpressed in *E. Coli.*
- Gadolinium inhibits the flow of calcium in *E. coli.*

LITERATURE CITED

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