Transmembrane protein Opy2 interacts with Mig2

Unnati Dev, Sheela Karunanithi and Dr. Paul Cullen
Department of Biological Sciences, University at Buffalo

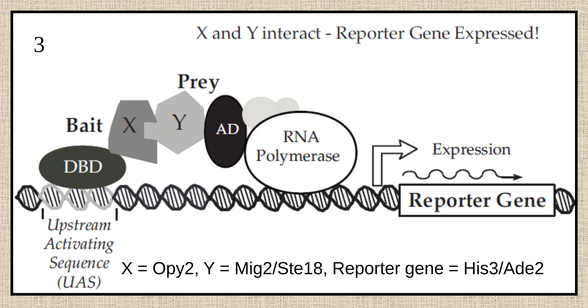
Abstract

All living forms must satisfy their nutritional requirements to survive. Organisms have developed a wide range of mechanisms for obtaining and assimilating essential nutrients such as glucose. Budding yeast, *Saccharomyces cerevisiae*. undergoes filamentous growth under limited glucose conditions. The cytoplasmic tail of a trans-membrane protein receptor Opy2 was shown to interact with Ste50, and activate the MAPK pathway to induce filamentous growth (Sheela Karunanithi, unpublished data). Mig1, a transcription factor that represses glucose/galactose genes, was found to interact with Opy2 to link the glucose regulatory pathway and the filamentous growth pathway. Mig2 is another transcription factor that represses glucose/galactose expression and is homologous to Mig1. We tried to determine if Opy2 and Mig2 interact, using a yeast-two hybrid assay. The *MIG2* gene was cloned into pGAD-C1 and transformed into yeast cells, PJ69-4a, along with the pre-cloned *OPY2* gene (in pGBDU). When spotted on media lacking histindine, the cells containing both pGAD-C1-*MIG2* and pGBDU-*OPY2* showed growth, indicating an interaction between Mig2 and Opy2 proteins leading to the association of the activating domain (AD) to the binding domain (BD) and transcription of histidine gene. This helps illustrate a connection between two evolutionarily conserved signaling pathways: one that senses glucose, the other that promotes differentiation.

Filamentous Growth

Basics of Yeast Two-Hybrid Assay

The yeast two hybrid assay, invented by Dr. Stan Fields, is a very widely used technique of determining in-vivo protein-protein interactions. PJ69-4a, the strain used in our two-hybrid analysis, was synthesized by Dr. Philip James and is auxotrophic for uracil and leucine biosynthetic genes. It can grow only if the media contains uracil and leucine or if plasmids containing these genes are transformed into the strain. The gene encoding one of the proteins of interest (*OPY2*) is cloned into Gal4 DNA binding domain on a plasmid (pGBDU) such that BD-Opy2 fusion protein is obtained[1]. This plasmid contains a reporter gene for uracil. The gene encoding the second protein of interest (*MIG2* and *STE18*) is cloned into Gal4 DNA activating domain on another plasmid (pGAD) such that AD-Mig2 and AD-Ste18 fusion proteins are obtained[1]. The plasmid pGAD has a leucine reporter gene. These plasmids are transformed into PJ69-4a strain. If the two fusion proteins interact, the AD binds to the BD and RNA polymerase is recruited to transcribe the *His3* and *Ade2* reporter genes of PJ69-4a and allows growth on media lacking these amino acids.[2].



Methods

Cloning

- *MIG2* and *STE18* genes were isolated from the yeast genomic DNA and amplified by Polymerase Chain Reaction.
- Vector (plasmid) pGAD and purified *MIG2* and *STE18* were digested using *SalI* and *BamHI* restriction enzymes, followed by electrophoresis on sea plaque agarose gel.
- Bands corresponding to digested vector, *MIG2* and *STE18* were cut, melted and ligated.
- Plasmids *pGAD-MIG2* and pGAD-*STE18* were transformed into E. coli and analyzed by sequencing.

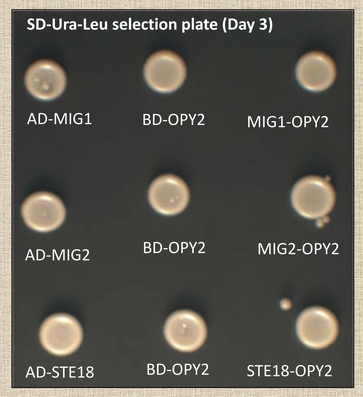
Yeast Two-Hybrid Assay

The following plasmids were transformed into PJ-69 4a strain of yeast and plated on SD-Ura-Leu selective agar plates:

- pGAD-*MIG2* + pGBDU-*OPY2* → Experimental
- pGAD-*MIG2* + pGBDU → Experimental
- pGAD-*STE18* + pGBDU-*OPY2* → Experimental
- pGAD-*STE18* + pGBDU
- pGAD-*MIG1* + pGBDU-*OPY2* → Positive Control
- pGAD-*MIG1* + pGBDU

Cells were spotted on SD-Ura-Leu, SD-Ura-Leu-His, SD-Ura-Leu-His+ATA and SD-Ura-Leu-Ade selection agarose plates

Results



Results

Plate → / Plasmids	SD-Ura-Leu	SD-Ura-Leu-His	SD-Ura-Leu-His + ATA	SD-Ura-Leu-Ade
pGAD-MIG1 + pGBDU	+	+	-	-
pGAD-MIG2 + pGBDU	+	+	-	-
pGAD-STE18 + pGBDU	+	+	-	-
pGBDU-OPY2 + pGAD	+	Slight	-	-
pGAD-MIG1 + pGBDU-OPY2	+	+	+	+
pGAD-MIG2 + pGBDU-OPY2	+	+	+	Slight
pGAD-STE18 + pGBDU-OPY2	+	+	-	-

Conclusions

- SD-Ura-Leu plate tests the plasmid transformation efficiency. Growth of all colonies indicates that transformation was successful. Uracil and leucine genes on the plasmids were expressed. The media provided the amino acids histidine and adenine for growth.
- SD-Ura-Leu-His plate selects the colonies that can synthesize the reporter histidine gene. If the two fusion proteins interact, they cause the AD and the BD to associate. This enhances the histidine gene transcription and expression. At times, a single fusion protein is sufficient to enhance reporter gene expression, which is probably why all strains are growing on this plate. 3-Amino-1,2,4-triazole (ATA), controls this background expression observed on SD-Ura-Leu-His plates.
- ATA allows the growth of only those colonies with an enhanced histidine gene expression and provides a more reliable two-hybrid result. Hence growth on the SD-Ura-Leu-His+ATA plate indicates an interaction between the cloned proteins. Our results demonstrate that Mig2 and Opy2 interact. Both the Mig1-Opy2 (positive control) and Mig2-Opy2 containing colonies show growth while Ste18-Opy2 colony does not.
- Testing the expression of Adenine reporter gene on SD-Ura-Leu-Ade is a more stringent test of protein interaction. These results confirm an interaction between Mig2 and Opy2 when compared to Mig1-Opy2 (positive control).
- An in-vivo interaction between Mig2 and Opy2 links the glucose sensing pathway to the cell differentiation pathway leading to filamentous growth of yeast cells.
- Ste4, a protein in the cell mating pathway interacts with Ste18 as well as with Opy2. Our results show that Ste18 does not interact with Opy2 even though it is in the same mating pathway as Ste4.

References

1 R. Daniel Gietz, Barbara Triggs-Raine, Anne Robbins, Kevin C. Graham and Robin A. Woods. Identification of proteins that interact with a protein of interest: Applications of the yeast two hybrid system. Molecular and Cellular Biochemistry 172: 67–79, 1997.

2 P. James, J. Halladay, and E. A. Craig. Genomic Libraries and a host strain designed for a highly efficient two hybrid selection in yeast. Genetics 144(4): 1425–1436, 1996.

3 Figure from Invitrogen ProQuest 2 hybrid system product manual (Catalog nos. PQ10001-01 and PQ10002-01)

Saccharomyces Genome Database. http://www.yeastgenome.org/ March 12, 2012.