Identifying the function of GLYMA10, a potential new APC activator in (*Glycine max* L. Merr)

David Grimm and Nicole Dafoe
Department of Biology, Slippery Rock University, Slippery Rock, PA 16057, USA

Introduction

A typical cell cycle is characterized by a growth phase followed by DNA replication and then finally cell division. This process generates genetically identical cells. Alternatively, in the process known as endoreduplication, cells grow, and DNA replication occurs, but the cell does not divide. In plants, CDC20 and CCS52 proteins control when a cell undergoes either mitosis or endoreduplication respectively through activating the anaphase promoting complex (APC). The APC then targets specific proteins called cyclins for degradation to control the progression of a cell cycle. There have been a variety of CDC20 and CCS52 genes discovered, yet more information of these cell cycle regulators needs to be researched. This research project focuses on a potential third type of APC activator in soybean (*Glycine max*), Glyma.10G117000.1 (GLYMA10) and its function. GLYMA10 has been identified in the genome of soybean yet no function is currently known. GLYMA10 shares sequence similarities with CDC20 and CCS52 proteins. Preliminary data showed that Like CDC20 and CCS52 proteins, GLYMA10 has defining traits such as a conserved C-box, WD40 repeats, RVL motif and an IR tail which are all imperative to APC activating proteins, yet there are also distinct differences between these proteins. To confirm that GLYMA10 is expressed, polymerase chain reaction (PCR) was used to amplify GLYMA10 from flower cDNA. The PCR product was cloned into a sequencing vector, which was then transformed into *E. coli*. The plasmid was sequenced confirming that GLYMA10 is expressed in flowers. Quantitative PCR (qPCR) was then used to analyze expression levels of GLYMA10 not only in flowers, but also in apical meristems, trifoliate leaves, unifoliate leaves, stems, roots, seeds, and new and old seed pods. Overall, GLYMA10 was not expressed in trifoliate leaves, unifoliate leaves, roots, or stem tissues and was only very weakly expressed in seeds, flowers and apical meristems. It was, however, very strongly expressed in new and old seed pods, with old seed pods showing the highest expression levels of GLYMA10. In the future, we will continue to study seed pods in an effort to understand the function of this protein.

Materials and Methods

Expression & Sequencing of soybean GLYMA10 in *E. coli*

- Collected flower tissues from *Glycine max* ✓
- Amplified GLYMA10 using PCR ✓
- Separated DNA using Gel Electrophoresis ✓
- Gel purified GLYMA10 ✓
- Cloned GLYMA10 PCR product into sequencing vector ✓
- Transformed *E. coli* with sequencing vector ✓
- Verified transformation ✓
- Sequenced genotype ✓

Localization of GLYMA10 within soybean tissues

- Collected various *Glycine max* tissues such as apical meristems, trifoliate leaves, unifoliate leaves, stems, roots, seeds, and new and old seed pods ✓
- Used qPCR to analyze GLYMA10 expression in *Glycine max* tissues ✓

Results

Table 1. Conserved sequences necessary for APC activating proteins. APC activating proteins require a minimum of three conserved protein sequences. These sequences include a C-Box, RVL Motif within the WD40 repeats, and an IR Tail. GLYMA10 as well as CDC20 genes both have these conserved sequences, indicating that GLYMA10 is a potential APC activator.

Conserved APC Region	Function	Protein Sequence
C-Box	Key to binding to the APC	DRFIRNR
RVL Motif	Regulate cyclin concentrations that regulate cell division	RVL
IR Tail	Key to binding to the APC	IR

Table 2. Expression of GYMA10 based on soybean tissue samples. qPCR was performed on apical meristems, roots, unifoliate leaves, stems, trifoliate leaves, seeds, old bean pods, and new bean pods to determine expression of GLYMA10 within these different tissues. Averages of three replicates for each tissue sample are shown. A lower Cq value signifies higher expression. ND means not detected.

Tissue	Cq	Expression Level
Apical Meristem	34.47997	Low expression
Root	ND	Not expressed
Unifoliate Leaf	ND	Not expressed
Stem	ND	Not expressed
Trifoliate Leaf	ND	Not expressed
Seed	34.81602	Low expression
Old Bean Pods	23.06825	High expression
New Bean Pods	28.82498	High expression

Discussion

- Based on sequence similarities, GLYMA10 appears to be a new type of APC activator
- GLYMA10 is not expressed in trifoliate leaves, unifoliate leaves, roots, or stem tissues
- GLYMA10 was very weakly expressed in seeds, flowers and apical meristems.
- GLYMA10 was very strongly expressed in old and new bean pods with older bean pods having the highest expression

Future Research

- Repeat expression experiment and quantify data using relative qPCR to better understand GLYMA10's expression in seed pods
- Overexpress GLYMA10 gene in soybean and look for phenotype changes
- Continue analyzing expression patterns of GLYMA10 to discover potential function

References

Castro A, Bernis C, Vigneron S, Labbe JC, Lorca T (2005) The anaphase-promoting complex: a key factor in the regulation of cell cycle. Oncogene 24 (3):314-325. doi:10.1038/sj.onc.1207973

Dafoe NJ, Huffaker A, Vaughan MM, Duehl AJ, Teal PE, Schmelz EA (2011) Rapidly Induced Chemical Defenses in Maize Stems and Their Effects on Short-term Growth of Ostrinia nubilalis. Journal of Chemical Ecology 37 (9):984-991. doi:10.1007/s10886-011-0002-9

Fulop K, Tarayre S, Kelemen Z, Horvath G, Kevei Z, Nikovics K, Bako L, Brown S, Kondorosi A, Kondorosi E (2005) Arabidopsis anaphase-promoting complexes - Multiple activators and wide range of substrates might keep APC perpetually busy. Cell Cycle 4 (8):1084-1092

Heyman J, De Veylder L (2012) The Anaphase-Promoting Complex/Cyclosome in Control of Plant Development. Molecular Plant 5 (6):1182-1194. doi:10.1093/mp/sss094

Kevei Z, Baloban M, Da Ines O, Tiricz H, Kroll A, Regulski K, Mergaert P, Kondorosi E (2011) Conserved CDC20 Cell Cycle Functions Are Carried out by Two of the Five Isoforms in Arabidopsis thaliana. Plos One 6 (6). doi:10.1371/journal.pone.0020618

Lima MD, Eloy NB, Pegoraro C, Sagit R, Rojas C, Bretz T, Vargas L, Elofsson A, de Oliveira AC, Hemerly AS, Ferreira PCG (2010) Genomic evolution and complexity of the Anaphase-promoting Complex (APC) in land plants. Bmc Plant Biology 10. doi:10.1186/1471-2229-10-254

Tarayre S, Vinardell JM, Cebolla A, Kondorosi A, Kondorosi E (2004) Two classes of the Cdh1-type activators of the anaphase-promoting complex in plants: Novel functional domains and distinct regulation. Plant Cell 16 (2):422-434. doi:10.1105/tpc.018952

Acknowledgements

We would like to thank the Department of Biology at Slippery Rock University for their wonderful support and the Faculty/ Student Research Grant for funding this research.