ABSTRACT

Cellular differentiation is a key attribute of all multicellular organisms, but little is known about the molecular mechanisms that drive its evolution. The goal of this work is to better understand the gene named RLS1 (regA-like sequence), that is believed to have been important for the evolution of cell types in the volvocine green algae. This family includes unicellular Chlamydomonas reinhardtii, which has no cell differentiation, and multicellular Volvox carteri. RLS1 is the closest C. reinhardtii homolog of RegA, a Volvox protein essential for cell differentiation; however, little is known about RLS1. Our immediate goal is to learn more about the accumulation and localization of the RLS1 protein during the C. reinhardtii life cycle by developing a construct that expresses mCherry-tagged RLS1 protein. We PCR-amplified a hygromycin-resistance-encoding gene fragment, which was subcloned into an RLS1-containing plasmid. An mCherry fragment was synthesized and will be subcloned to create the completed construct. That construct will be transformed into C. reinhardtii and western analysis will be used to identify transformants that express m-Cherry-RLS1. These transformants will be analyzed to determine how RLS1 accumulates during the C. reinhardtii life cycle, thus providing insights into its developmental function and the evolution of multicellularity.

BACKGROUND

Volvocine algae: Volvox and Chlamydomonas

regA gene

VARL domain

• RegA contains a conserved region, the VARL (Volvocine Algal RegA Like) domain that is found in homologs in both V. carteri and C. reinhardtii. RLS1 is the closest C. reinhardtii homolog of RegA.

• RLS1 mRNA is expressed more when C. reinhardtii is light- or nutrient-deprived, which could mean that RLS1 plays an important role in regulating photosynthesis.

• RLS1 mRNA is present throughout the life cycle under normal growth conditions.

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OBJECTIVES

To better understand RLS1 and evolutionary origins of multicellularity in Volvocine algae by analyzing and manipulating protein expression.

METHODS

1. Create a construct that expresses mCherry-tagged RLS1 via native promoter, and hygromycin-resistance gene.

2. Transform all three constructs separately into the CC3395 and CC4350 strains of C. reinhardtii via glass bead transformation.

RESULTS

Transformations into CC3395 (selection on hygromycin)

Transformations into CC4350

CONCLUSIONS

• So far the mCherry+ Hyg+ RLS1 construct has been made and transformed into C. reinhardtii.

• From the transformations into CC4350 and CC3395, overall there were more transformants using less DNA (1ug).

FUTURE GOALS

• Complete the HR and NIT1 promoter constructs by inserting the hygromycin-resistant gene at the XbaI restriction site.

• Analyze transformants via confocal microscopy for red fluorescence (RLS1 protein).

• Perform Western blot analysis to analyze accumulation of the mCherry-RLS1 protein through out the life cycle.

REFERENCES


