Investigating RLS1 Localization and the Evolutionary Origins Of Cellular Differentiation

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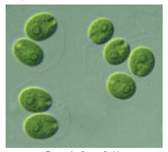
ABSTRACT

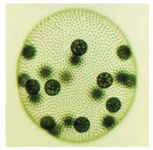
Cellular differentiation is a key attribute of all multicellular organisms, but little is known about the molecular mechanisms that drive its evolution. The goal of this work is to better understand a gene named *RLS1 (regA-like sequence)*, that is believed to have been important for the evolution of 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

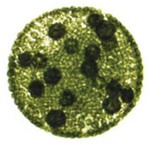
Volvocine algae: *Volvox* and *Chlamydomonas*



C. reinhardtii

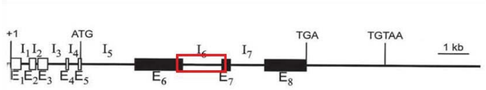


Wild type *V. carteri*

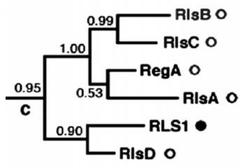


RegA mutant *V. carteri*

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.

ACKNOWLEDGEMENTS

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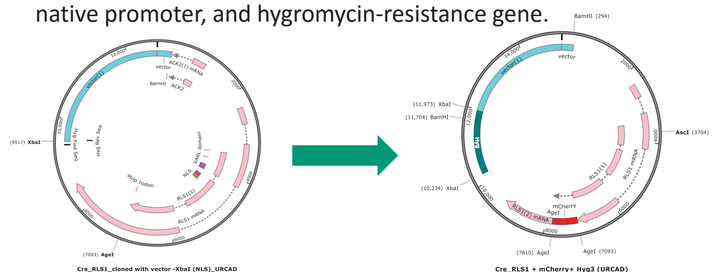
OBJECTIVES

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

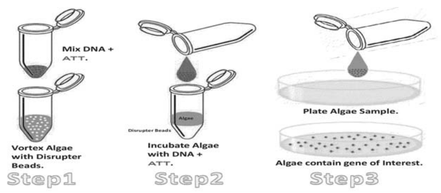
METHODS

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



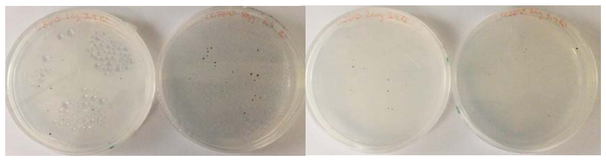
1. Create constructs with the *HSP70A/RBCS (HR)* promoter to overexpress RLS1 and *NIT1* promoter to induce *RLS1* expression in media that contains nitrate.

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

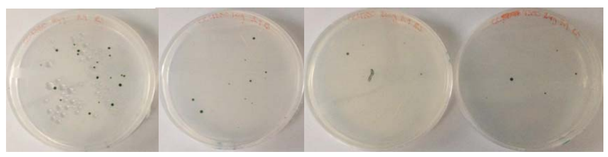


RESULTS

Transformations into CC3395 (selection on hygromycin)



Transformations into CC4350



RESULTS

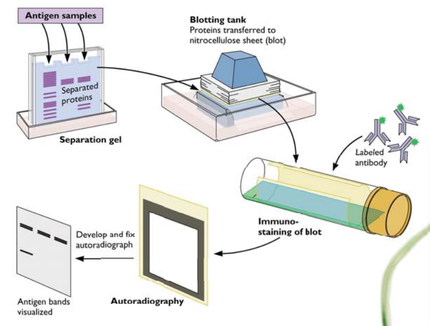
Strain	Transformation Type	Number of Colonies
CC3395	Control- pHyg	56
	1 ug of RLS1+mCherry+Hyg	32
	2 ug of RLS1+mCherry+Hyg	25
CC4350	Control- pHyg	52
	1 ug of RLS1+mCherry+Hyg	38
	2 ug of RLS1+mCherry+Hyg	16

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.



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