Functional analysis of regA paralogs rlsA-rlsD

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Abstract:

regA encodes a somatic-cell-specific nuclear protein (RegA) that represses growth and reproduction and is essential for maintenance of the somatic cell fate in *Volvox carteri*. RegA paralogs share with RegA a single conserved region, the ~110-aa Volvocine Algal RegA-like (VARL) domain that is related to the DNA-binding SAND domain present in several plant and animal proteins. Information about the functions of these paralogs is lacking, but could shed light on cell differentiation and how it evolved in *V. carteri*. Of the four closest paralogs of *regA*, three (*rlsA*-*rlsC*) have transcript accumulation patterns that roughly parallel those of *regA*, while *rlsD* transcripts are not developmentally regulated during the vegetative life cycle. Here, we report results from several approaches to investigate the functions of these RegA paralogs. In addition to other experiments, we used RNA hairpin-expressing transgenes to attempt gene knockdowns, and we tested the ability of chimeric versions of RegA with paralog VARL domains to rescue a *regA* mutant. RNA hairpins targeting *rlsA*, *rlsB*, or *rlsC* have not yielded detectable phenotypes, suggesting that *rlsA*, *rlsB*, and *rlsC* individually might not be essential for somatic cell differentiation. On the other hand, an RNA hairpin targeting *rlsD* leads to severe defects in growth and reproduction, suggesting that in stark contrast to *regA*, *rlsD* promotes growth and reproduction. Meanwhile, none of the chimeric RegA proteins with paralog RegA VARL domains rescued a *regA* mutant, suggesting that the RegA VARL domain has a different function than the paralog VARL domains. Considered together, our results indicate that RegA is functionally different from its close paralogs, and that the VARL domain is key to this difference.