The adaptive role of RLS1 in *Chlamydomonas*: a direct test using genomic and RNAi *RLS1* mutants

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RLS1 is part of a volvocine-specific gene family that includes *regA* – the gene responsible for somatic cell differentiation in *Volvox carteri*. In *Chlamydomonas reinhardtii, RLS1* is induced in response to various environmental changes (including dark and nutrient deprivation) when the temporary down-regulation of reproduction is beneficial in terms of survival. Based on its expression pattern, we have previously suggested that *RLS1* acts as an environmentally-induced life-history trade-off gene that promotes survival at a cost to immediate reproduction. However, a direct test of this hypothesis has not been performed. Here, we used genomic and RNAi *RLS1* mutants to directly investigate the effect of *RLS1* loss on several fitness parameters, including reproduction, survival and resistance to stress. Mutant and wild-type strains were grown in various growth conditions (autotrophic and mixotrophic media; continuous light, continuous dark, and 12 h light:12 h dark cycle) and under various stresses (light stress; nutrient deprivation; antibiotics) and their growth rates and survival potentials were compared. This study provides direct evidence for the adaptive role of *RLS1* in *C. reinhardtii* and supports the suggestion that the evolution of somatic cell differentiation in *V. carteri* involved the co-option of an *RLS1*-like gene whose expression in the unicellular ancestor was conditioned on an environmental cue through shifting its expression from a temporal into a spatial (developmental) context.