Cell-type specific alternative transcripts facilitate multicellularity in the alga *Volvox carteri*

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***Short Abstract* — The volvocine algae, from single celled *Chlamydomonas* to *Volvox,* which has differentiated somatic and gonidial cells, are model systems for the evolution of complex multicellularity. We establish that the volvocines use cell-type specific alternative transcript isoforms (CTSAI) – whereby different cell types express the same gene, but produce different transcripts from the locus – as a mechanism for multicellularity. Specifically, analysis of the complete *Volvox carteri* transcriptome, along with confirmatory experiments, reveal 15 genes displaying significant CTSAI, with metabolic and structural functions that likely impact the development and maintenance of differentiated cell-types from a single-celled ancestor.**

Keywords* — Volvocine algae, somatic, gonidial, differentiated cell types, cell-type specific alternative transcript isoforms (CTSAI), *Volvox carteri

CELL type specialization, a feature of multicellular organisms enabling development of complex forms and functions, is exemplified by plants and animals with hundreds of cell types forming complex tissues and organs [1,2]. All multicellular eukaryotes evolved from single-celled ancestors; hence, a major question in biology is to understand how cell type specialization evolved [1,3]. In the case of animals and plants, their complexity and ancient divergence from unicellular ancestors makes it challenging to infer how cell type specialization arose.

The volvocine algae are a taxonomic group of green algae within the order Chlamydomonadales that is a model for the step-wise acquisition of multicellular organization [4]. This group includes several multicellular genera with increasing developmental complexity, including large multicellular organisms with some differentiation (e.g. *Volvox carteri* with 2000-6000 cells and two clearly differentiated cell types), and a closely related single-cellular outgroup species *Chlamydomonas reinhardtii*. The divergence between *Chlamydomonas* and *Volvox* is estimated to be around 250 MY, which is recent compared to other lineages that evolved complex multicellularity [4].

Previous work on differentiation within *Volvox* has revealed several mechanisms that could generate unique cell types, including differential gene expression, differential use of microRNAs, and use of differentiation factors [5-7]. However, many complex eukaryotes also employ cell-type specific alternative transcript isoforms in which different cell types express the same gene, but produce different transcripts from the locus through alternative splicing of mRNA and/or alternative transcription start/termination sites [e.g., 8]. Different transcript isoforms may specify production of structurally and functionally different proteins from the same locus, most dramatically exemplified by complex alternative splicing of some neuronal transcripts [9]. We demonstrate that CTSAI is employed by *Volvox carteri*, establishing the volvocines as small model organisms for understanding this phenomenon and its role in the evolution of multicellular life.

In detail, we analyzed *V. carteri* transcript expression data from [5]. Applying restriction criteria, statistical tests, and manual curation to remove mis-predicted transcripts, we isolated 15 highly expressed genes having two transcripts with opposite differential expression in the two *Volvox* cell types. We experimentally verified this prediction of CTSAI in *V. carteri,* by selectively amplifying alternatively-spliced regions in three candidate genes. The identified CTSAI genes show functions ranging from controlling metabolism to extra-cellular matrix production, suggesting pathways for the evolution of multi-cellularity.

The small number of CTSAI genes and cell types, along with homologs in a single-celled relative, *Chlamydomonas,* make *Volvox* a good model for studying how CTSAI arises as a cell-type differentiation mechanism. We also identified cases of convergent transcription, another mechanism for cell-type differentiation amenable to future study.

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