

# Cell-type specific photoreceptors and light signaling pathways in the multicellular green alga *volvox carteri* and their potential role in cellular differentiation

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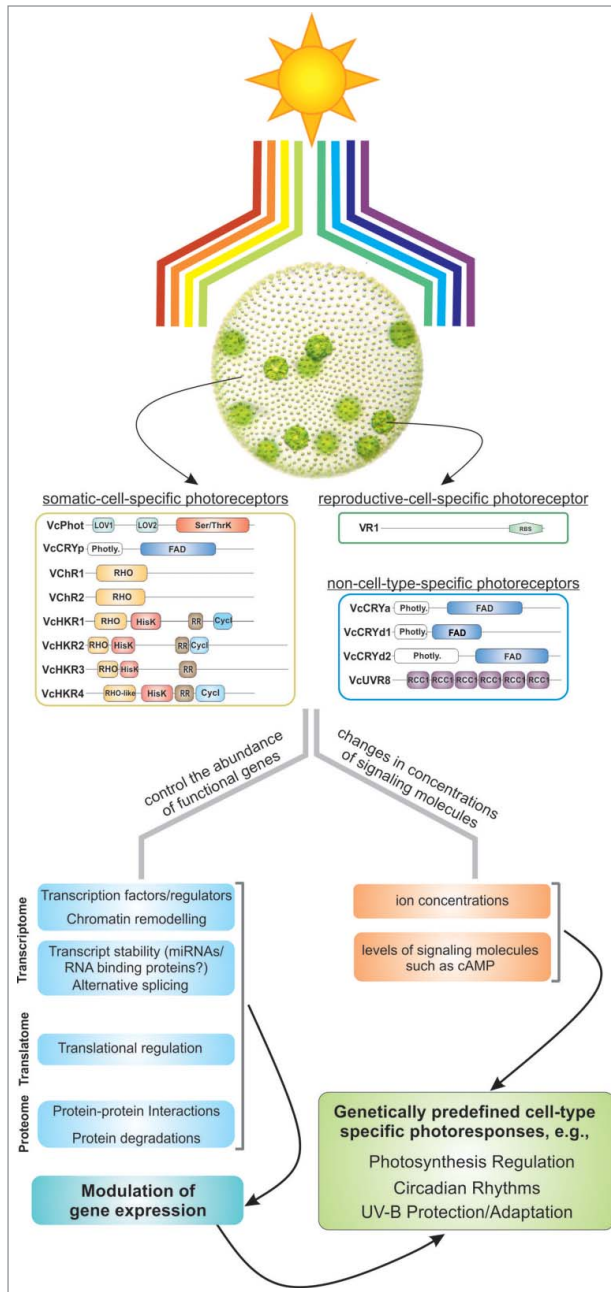
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This publication is dedicated to David Kirk, because of his pioneer role in establishment of *Volvox* as an interesting model organism for investigation of cellular differentiation and evolution of multicellularity.

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**T**he formation of multicellular organisms requires genetically predefined signaling pathways in various cell types. Besides differences in size, energy balance and life time, cell types should be able to modulate appropriate developmental and adaptive responses in ever-changing surrounding environment. One of the most important environmental cues is light which regulates a variety of physiological and cellular processes. During evolution, diverse light-sensitive proteins, so-called photoreceptors, and corresponding signaling pathways have evolved, in almost all kingdoms of life, to monitor light continuously and adjust their growth and development accordingly. However, considering the fact that different cell types should be able to trigger distinct light signaling pathways according to their needs, cell-type specific light signaling pathways are required to guarantee cell type-matched modulation of cellular and developmental processes in response to different light signals. The multicellular green alga *Volvox carteri*, which has only 2 cell types with clear division of labor, possesses cell-type specific photoreceptors and light signaling pathways which allow differential regulation of genes involved in various cellular and metabolic pathways in response to environmental light. The existence of cell-type specific light signaling pathways in multicellular organism like *Volvox* reflects an early development of cell-type specific signaling mechanisms during evolution to ensure maintenance of differentiation.

A fundamental question in biology is how multicellular organisms regulate cellular and physiological processes in response to environmental signals such as light cues in a tissue/cell-type specific manner. Considering the fact that different cell types should be able to trigger distinct light signaling pathways according to their needs, genetically predefined cell-type specific light signaling pathways should have developed during evolution. Although light regulated developmental and adaptive responses are widely observed phenomenon, we know little about the molecular mechanisms underlying cell-type specific light signaling as well as the function of photoreceptors and associated components during and in cellular differentiation. The simply organized model system *Volvox carteri* (hereafter *Volvox*) composed of only 2000–4000 biflagellate motile, terminally differentiated somatic cells, which build a monolayer at the surface of a spheroid, and around 16 much larger immotile reproductive cells (stem cell-like gonidia), which lie just below the somatic cell sheet; the cells are embedded in a transparent sphere of glycoprotein-rich extracellular matrix (ECM)<sup>1,2</sup> (Fig. 1). This multicellular alga belongs to the group of volvocine algae, a group of chlorophytes including unicellular *Chlamydomonas reinhardtii* (hereafter *Chlamydomonas*), colonial *Gonium pectorale*, *Pandorina morum* and *Eudorina elegans* (all 3 without a division of labor) and multicellular *Volvox* (which exhibits germ-soma differentiation), that have been used to understand genetic mechanisms behind evolutionary transition from unicellular



**Figure 1.** Model illustrating of the role of cell-type specific photoreceptors in multicellular *Volvox*. Photograph of multicellular *Volvox* and domain composition of its photoreceptors. Two different cell types, i.e., large dark green reproductive cells and small pale biflagellate somatic cells are located below and at the surface, respectively. *Volvox* makes use of no less than 13 photoreceptors, i.e., 7 rhodopsin-like photoreceptors (VR1, VChR1, VChR2, VcHKR1, VcHKR2, VcHKR3 and VcHKR4), one UV-B photoreceptor (VcUVR8), 4 cryptochromes (VcCRYa, VcCRYp, VcCRYd1 and VcCRYd2) and one phototropin (VcPhot). Photoreceptors are categorized in 3 groups, i.e., somatic-, reproductive- and non-cell-specific photoreceptors, on the basis of their cell-type specific transcript levels. Photoreceptor-mediated control of gene expression occurs at multiple regulatory steps including transcription, translation and post-translational level, allows to optimize cellular and developmental processes in response to environmental light signals. Some algal photoreceptors such as *Volvox* channerhodopsins (e.g., VChR1) and histidine kinase rhodopsins (e.g., VcHKR1) can change the concentration of signaling molecules such as calcium ions and cAMP, respectively. Domain abbreviations are phyto. (photolyase domain), FAD (flavin adenine dinucleotide binding domain), LOV (Light-oxygen-voltage), Ser/ThrK (serine/threonine kinase), RBS (retinal binding site), RHO (rhodopsin), HisK (histidine kinase), RR (response regulator), Cycl (adenylate/guanylate cyclase domain), RCC1 (regulator of chromosome condensation). Figure is modified according to.<sup>15,28</sup>

organisms into a multicellular one.<sup>3</sup> However, considering the fact that unicellular *Chlamydomonas* and multicellular *Volvox* have the same number of protein-coding genes,<sup>4,5</sup> regulation of gene expression (e.g., microRNAs<sup>6</sup> and alternative splicing<sup>7</sup>) seems to be a critical step for cell type specification and differentiation. In fact, complex eukaryotic organisms have evolved through selective expression of specific fraction of the same genome in different cell types in response to developmental and environmental cues.<sup>8</sup> In *Volvox*, for example, both cell types represent differential expression pattern of genes from various functional classes.<sup>9,10</sup> However, little is known regarding cell-type specific changes of gene expression in response to light signals, which is one of the most important environmental signals for controlling growth and development including final cellular differentiation in *Volvox*.<sup>11-13</sup>

The importance of light as an environmental factor affecting survival is also reflected in the number of photoreceptors genes in the genome of this free swimming alga. *Volvox* makes of none less than 13 photoreceptors which are required for accurate light-monitoring during its fast life cycle and to adapt their physiological activities to environmental changes.<sup>14,15</sup> However, the study of photoreceptors genes in *Volvox* was almost always accompanied by questions regarding their cell-type specific functions. Eight photoreceptors, i.e., a phototropin (*VcPhot*), a plant-like cryptochrome (*VcCRYp*), channelrhodopsin-1 (*VChR1*) and -2 (*VChR2*) and 4 histidine kinase rhodopsins (*VcHKR1*, *VcHKR2*, *VcHKR3* and *VcHKR4*), are highly expressed in the somatic cells,<sup>11,13-16</sup> while only one photoreceptor, i.e., volvoxopsin-1 (*VR1*), has been found to predominantly express in the reproductive cells (Fig. 1).<sup>16</sup> The cell-type specificity of photoreceptors is of particular interest because the abundance variability of genes involved in input pathways (e.g., light signaling) contributes to the specificity of the signal response in different cell types.<sup>17</sup> And in fact, it seems that cell-type specific photoreceptors act as foundation for development of cell-type specific light signaling pathways in the multicellular *Volvox*. The organism enables this way to use the same light signal to

provoke distinct signal processing toward regulation of fundamental cellular and developmental processes in a cell-type specific fashion (Fig. 1). In response to 2 important wavelengths, which have an major impact on algal development, i.e., blue and red light,<sup>18</sup> transcripts of genes encoding proteins involved in chlorophyll and carotenoid biosynthesis, light-harvesting complexes and cell cycle control change differentially depends on cell type.<sup>15</sup> In this regard, blue light tends to be effective to accumulate transcripts in the somatic cells; while red light leads to accumulate transcripts predominantly in the reproductive cells. Surprisingly, blue light also induced marked accumulation of 2 components of circadian rhythms only in the somatic cells, indicating that each cell type has its own genetically predefined circadian rhythm.<sup>15</sup> In addition, development of cell-type specific light signaling pathways is not only restricted to visible light, but also includes invisible lights ultraviolet (Kianianmomeni, unpublished observations) and far-red.<sup>15</sup>

Cell-type specific abundance of input (light) receptors has a great impact on modulation of cell-type-specificity of signaling.<sup>17</sup> But in addition, it is also conceivable that a part of components involved in transmission (e.g., mediators and cofactors) and output (e.g., transcription factors) layer express differentially in various cell types of multicellular organisms. The abundance of downstream transcriptional cofactors in different cell types is an important determinant of signaling pathway activation.<sup>19-21</sup> But the main point is: what is the “biological significance” of cell-type specific light signaling pathways? Special types of cells normally have different structure and are specialized to perform specific function. Accordingly, they have different energy balance (e.g., depends on their photosynthetic activity), possess their own circadian rhythms and cell division mechanisms. Moreover, depends on their localization in organism, they are subjected to various light intensities (e.g., UV-B irradiation). Thus, distinct cell-type specific light signaling pathways could ensure cell type-matched modulation of cellular and developmental processes in response to ever-changing light conditions (Fig. 1). It is worth

noting, however, that some photoreceptors/light signaling pathways could be deeply involved in initial differentiation processes as discussed before.<sup>11,13</sup> In summary, it might be reasonably assumed that distinct cellular light signaling in multicellular organisms reflects an early development of cell-type specific signaling mechanisms during evolution to ensure maintenance of differentiation (Fig. 1).

## Future Perspectives

The photobiology of algae is extremely rich and fruitful. Some of the algal photoreceptors display new properties, which are far from the classical picture of animal and plant photoreceptors, and have direct impact on our understanding of the evolution and function of light-sensitive proteins.<sup>11,22</sup> Moreover, algal molecular photobiology has the potential to open up new research avenues which can raise and deal with new questions. However, to overcome the backwardness of algal photobiology compared with photobiology research in plants, more functional analysis based on clear mutants should be performed. Although the functional analysis of light signaling pathways (e.g., with focus on cellular differentiation) could be investigated using “reverse genetics” by the application of RNAi, this approach could only partially resolve significant questions about the function of photoreceptors and light signaling pathways. On other hands, because the success of homologous recombination is very limited in (volvocine) algae and the establishment of a gene targeting system using CRISPR/Cas9 method seems to be a stony way,<sup>23</sup> an alternative available approach to dealing with this issue is insertional mutagenesis based either on transposons<sup>24</sup> or plasmid insertion.<sup>25</sup> However, transposon mutagenesis<sup>26,27</sup> seems to be the most promising approach to do “forward genetic” in multicellular *Volvox* to identify previously unknown key components involved in light signaling and cellular differentiation.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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