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# ***Volvox* (Chlorophyta, Volvocales) as a Model Organism in Developmental Biology**

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**Abstract**—Model systems based on two or more related species with different types of development are finding increasing use in current comparative embryology. Green algae of the genus *Volvox* offer an interesting opportunity to study sex pheromones, morphogenesis as well as the formation of a somatic cell line undergoing terminal differentiation, senescence, and death as well as a line of reproductive cells, which at first grow and then undergo a series of consecutive divisions that give rise to new organisms. However, almost all studies of the recent years were conducted on a single species, *Volvox carteri* f. *nagariensis*. The goal of this publication was to advertise the cosmopolitan alga *V. aureus* as a model species in developmental biology. Published data on *V. aureus* are briefly reviewed in comparison with the development of *V. carteri* and outlooks of further studies are specified. In particular, the expediency of collecting new *V. aureus* strains from nature to study their development in clonal culture is outlined.

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Current evolutionary and comparative embryology usually compares the development of representative species from different types or classes of Metazoa. Recently, Raff and Love (2004) pointed to the relevance of model systems with related species (optimally, within the same genus) with different modes of early development. Such ontogenetic rearrangements are relatively common in evolution of different groups of marine invertebrates, for instance, sea urchins *Heliocidaris erythrogramma* and *H. tuberculata* (Raff, 1987) or ascidians *Molgula oculata* and *M. occulta* (Jeffery, 1997).

This comparative approach seems promising for lower eukaryotes, primarily *Volvox*, whose cleavage resembles early cleavage in multicellular animals. The asexual cycle of *Volvox* development includes growth of gonidia (asexual reproductive cells), subsequent series of synchronous gonidial divisions, inversion (turning inside-out) of the formed young colonies, their growth within the parent, release from it, etc.

Experiments on clonal cultures of this freshwater alga, whose colonies consist of several hundred or thousand somatic cells and a small number of reproductive cells, were primed more than 40 years ago with the discovery of a glycoprotein pheromone controlling sexual differentiation in *Volvox aureus* (Darden, 1966). This species was the subject of experimental studies for several subsequent years. In particular, the properties

and action mechanism of the sex pheromone (Darden, 1968, 1980; Ely and Darden, 1972), ultrastructural properties of gametogenic differentiation (Deason et al., 1969; Deason and Darden, 1971), cellular mechanisms of colony motility (Hand and Haupt, 1971) and inversion (Kelland, 1977), and dynamics of nucleic acid synthesis during asexual development (Tucker and Darden, 1972) were studied. In addition, the cell cycle properties were studied during gonidial cleavage (Desnitskiy, 1981, 1982, 1985) and a model of *V. aureus* colony growth was developed (Smolyaninov and Maresin, 1972).

In the recent 25–30 years, nearly all studies on *Volvox* development preferred *V. carteri* forma *nagarensis* (Huskey et al., 1979; Kirk, 1998; Kirk and Nishii, 2001; Schmitt, 2003; Kirk and Kirk, 2004). This was the only *Volvox* form where numerous spontaneous and induced mutations were obtained; other representatives of the genus proved to be unsuitable for formal genetic analysis.

In the meantime, the genus *Volvox* includes 18 species, many of which demonstrate specific pattern of development and cell differentiation (Starr, 1968, 1970; Desnitskiy, 1995, 2006, 2008). We would like to reattract the attention of researchers to *V. aureus* (synonyms *V. dioicus*, *V. lismorensis*, *V. minor*, *V. sphaerosira*, *Janetosphaera aurea*) as a model species in developmental biology.

First of all, let us consider the main morphological and physiological differences between two species

mentioned above (Desnitskiy, 2006). In *V. carteri*, gonidia after long-term growth reach a considerable size ( $> 50\text{--}60 \mu\text{m}$  in diameter) before series of divisions, which is at least 6–8 times that of somatic cells. On the other hand, in *V. aureus*, the period of gonidial growth is short, and they reach a relatively small size (20–25  $\mu\text{m}$ ) exceeding somatic cells no more than 3–4-fold in diameter. Cells continue to grow in the intervals between consecutive divisions. In addition, all cells in an adult *V. aureus* colony are connected with thin cytoplasmic bridges; while these degrade in early ontogeny of *V. carteri* (soon after the series of divisions and young colony inversion). The preservation of intercellular bridges in *Volvox* colonies correlates with the small size of mature gonidia (Hoops et al., 2006). Apparently, both these characters are evolutionarily derived. Presumably, nutrients are transported from somatic cells to gonidia and cleaving embryos in *V. aureus* and several other *Volvox* species with small gonidia; however, it has not been ultimately confirmed yet.

In this context, it is noteworthy to mention the publications (Mita, 1983; Rott, 1987) proposing the critical role of the nuclear/cytoplasmic ratio in the regulation of cell division and other events in early ontogeny of multicellular organisms. Indeed, there are grounds to believe that reaching the critical mass of gonidial cytoplasm (i.e., the minimum critical nuclear/cytoplasmic ratio) is a prerequisite for cleavage start. On the other hand, since gonidia and somatic cells are connected into a common system in *V. aureus*, the critical mass of the cytoplasm (common for two cell types in this *Volvox* species) is reached “prematurely,” after a substantially reduced period of gonidial growth. Clearly, this reasoning assumes that the terminally differentiated and incapable of division nuclei in *V. aureus* somatic cells are not taken into account in the minimum critical mass ratio between the gonidial nuclei and total syncytial–colonial cytoplasm. Thus, this hypothesis agrees with the possibility of nutrient transport from somatic cells to gonidia and embryos in *V. aureus*. The preservation of intercellular bridges could favor the decrease in the size of mature gonidia and be among the factors of evolutionary rearrangement of developmental mode and organization of the *Volvox* colony.

Specific segregation of cell lines is another significant distinction between asexual developmental cycles in these two species. In *V. carteri*, the gonidial rudiments result from unequal (asymmetrical) division of 16 cells in the anterior part of the embryo during the transition from the 32-cell stage to the 64-cell stage (Starr, 1970). In contrast, gonidia become morphologically distinct much later in *V. aureus*—only after the completion of the series of divisions and young colony inversion (Darden, 1966). The molecular genetic mechanisms of differentiation into somatic cells and gonidia are well understood in *V. carteri* f. *nagariensis* (Kirk, 1998; Schmitt, 2003; Kirk and Kirk, 2004); however, no such data are available for *V. aureus*.

Noteworthy, the methods of formal genetics proved to be inapplicable in the above-mentioned successful studies on the evolution of related sea urchins of the genus *Heliocidaris* (Raff, 1987) as well as related ascidians of the genus *Molgula* (Jeffery, 1997). Thus, thorough studies of the organization and development of asexual colonies in *V. aureus* in comparison with *V. carteri* and other related species of the genus are also of apparent interest. Such studies can shed light on the origin and early evolution of primitive multicellular condition. To date, only one detailed comparative study of the rate, diel rhythms, and light/dark control of cell divisions during the asexual developmental cycle in *V. aureus*, *V. carteri* f. *nagariensis*, and some other *Volvox* species has been conducted (Desnitskiy, 1995, 2006, 2008).

Let us consider sexual reproduction in *Volvox*. Similar to *V. carteri* f. *nagariensis*, in the American M5 strain of *V. aureus* it is controlled by a species-specific glycoprotein pheromone. The latter is synthesized by male individuals and released to culture medium (Darden, 1966; Ely and Darden, 1972). At the same time, heat shock, mechanical damage, and other stress factors can stimulate the transition to sexual reproduction through the synthesis of the sex pheromone in somatic cells of asexual individuals in *V. carteri* f. *nagariensis* (Amon et al., 1998; Kirk, 1998; Nedelcu, 2005). It would be interesting to conduct such experiments on *V. aureus* (as well as on other *Volvox* species).

Several American strains of *V. aureus* are known for a long time, where males are missing or extremely rare, and in old cultures a fraction of gonidia is transformed without fertilization into orange parthenospores resembling dormant zygotes (Darden, 1968; Starr, 1968; Darden and Sayers, 1969; Starr and Zeikus, 1993). Such old cultures proved to contain the pheromone inducing the parthenospore formation in young cultures of the same *V. aureus* strains or the formation of male colonies in *V. aureus* M5 culture. In reciprocal experiments, the sex pheromone from *V. aureus* M5 culture induced the parthenospore development in the young cultures of parthenoporic strains. Note that parthenospores have not been reported in any other representatives of the genus *Volvox*.

In summer 1996, we started a clonal culture of *V. aureus* collected from a small temporary pool in the southern part of the Leningrad Region (Desnitskiy, 2000, 2002). Male colonies have never been observed within 10 years of thorough observation, although 50–80% of colonies in old cultures contained resistant dormant cells (presumably, parthenospores). Thus, our data agree with analogous data on American parthenoporic strains of *V. aureus*.

The problems that can be analyzed using parthenoporic strains of *V. aureus* primarily include senescence and apoptosis. Pommerville and Kochert (1982) proposed that *V. carteri* had an endogenous genetic program of senescence, since somatic cells lived several

days longer in female colonies than in asexual ones. We observed the same pattern in somatic cells of *V. aureus* colonies containing parthenospores.

In summary, the opportunities of *V. aureus* as a model species in developmental biology of lower eukaryotes have not been completely realized. This alga is the most widespread and the only cosmopolitan representative of the genus *Volvox* (Desnitskiy, 2003). This can be due to the occurrence of numerous populations with parthenospores. It is a common knowledge that animal and plant species which lost the ability for sexual reproduction often have more broad distribution (Maynard Smith, 1981).

*V. aureus* can be easily found in small ponds or temporary pools, since its populations reach the maximum density there. Culture methods (under both sterile and non-sterile conditions) are simple and were described elsewhere (Darden, 1966; Starr and Zeikus, 1993). It is not improbable that one will succeed to get from nature such populations of *V. aureus*, in which the mutations affecting morphogenesis can be induced. In addition, the capacity to synthesize pheromones controlling *Volvox* differentiation is often lost after many years of cultivation. This fact once more necessitates the isolation of material for new cultures from natural populations. Thus, the intensification of studies on *V. aureus* can contribute to further progress in our understanding the evolution of multicellularity and development in the whole genus *Volvox*.

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