CHAPTER 2

THE EVOLUTION OF SELF DURING THE TRANSITION TO MULTICELLULARITY

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Abstract: The notion of "self" is intrinsically linked to the concepts of identity and individuality. During evolutionary transitions in individuality—such as, for instance, during the origin of the first cell, the origin of the eukaryotic cell and the origin of multicellular individuals—new kinds of individuals emerged from the interaction of previously independent entities. The question discussed here is: How can new types of individuals with qualities that cannot be reduced to the properties of their parts be created at a higher level? This question is addressed in the context of the transition to multicellularity and using the volvocine green algae—a group of closely related unicellular and multicellular species with various degrees of physiological and reproductive unity—as a model system. In this chapter, we review our framework to addressing the evolution of individuality during the transition to multicellularity, focusing on the reorganization of general life-traits and cellular processes and the cooption of environmentally-induced responses.

INTRODUCTION

In philosophy, "self" is broadly defined as the essential qualities that make a person distinct from all others; the particular characteristics of the self determine its identity. The notion of "self" is, thus, intrinsically linked to the concepts of identity and individuality. Individuals are entities that are distinct in space and time. In biology, individuals have been defined based on several additional criteria including genetic uniqueness, genetic homogeneity, or physiological autonomy.[1] Going back to the root of the word individual (i.e., "not divisible"), individuals can also be thought of as the smallest units that cannot be divided into parts that maintain the essential properties of

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the whole. Lastly, from an evolutionary perspective, individuals are units of selection that possess the properties of heritable variation in fitness.[2]

During evolutionary transitions in individuality—such as, for instance, during the origin of the first cell, the origin of the eukaryotic cell and the origin of multicellular individuals—new kinds of individuals emerged from the interaction of previously independent entities. Such associations can involve similar entities (such as during the transition to multicellularity) or rather distinct entities (such as during the evolution of the eukaryotic cell) and be based on a wide range of ecological interactions (from commensalism and mutualism to exploitation and parasitism; for discussion see ref. 3). The initial interactions can be facilitated by either aggregation (e.g., the formation of multicellular fruiting bodies in slime molds and myxobacteria) or the failure of offspring individuals to separate (which is the case during the development of most multicellular organisms). The long-term stability of these associations and the subsequent integration of previously independent units into higher-level individuals are dependent on the frequency of cooperative interactions and the mediation of the inherent conflicts among lower levels.[3] At a mechanistic level, during transitions in individuality, a new genotype-phenotype map has to be created to reflect the emergence of a new kind of individual (and a new "self"/identity) at the higher level. The way in which the lower-level genotype-phenotype maps are reorganized at the higher level can influence the potential for evolution of the newly emerged multilevel system.[4]

The question discussed here is: How can a new kind of individual with qualities that cannot be reduced to the properties of its parts be created at a higher level and how does this process affect the lower levels (i.e., the previously independent individuals) in terms of their own individualities and identities? We address this question in the context of the transition to multicellularity and using the volvocine green algae—a group of closely related unicellular and multicellular species with various degrees of physiological and reproductive unity—as a model system. For the purpose of this discussion, we define an individual as the smallest unit that is physiologically and reproductively autonomous. This definition restricts the term multicellular individual to organisms with two types of cells: reproductive (germ) cells and nonreproductive (somatic) sterile cells. In contrast to multicellular forms in which all cells have reproductive abilities—and thus each cell (part) can reproduce the group (the whole), in multicellular organisms with a germ-soma separation, not all cells are able to recreate the whole; the evolution of nonreproductive cells renders the group indivisible and thus a true individual.

We have approached the questions posed above from many perspectives: multilevel selection (in terms of cooperation, conflict and conflict mediation),[3] fitness trade-offs and fitness reorganization,[2] life history trade-offs,[5] reorganization of general life-traits and cellular processes[4] and the cooption of environmentally-induced responses.[6] Below, we review our framework to addressing the evolution of individuality during the transition to multicellularity, focusing on the two latter perspectives. Specifically, we have argued that the emergence of individuality at a higher level (and the emergence of a new genotype-phenotype map) requires (i) the dissociation of certain processes, traits and functions at the lower level and their reorganization at the higher level, (ii) the cooption of lower-level processes and pathways for new functions at the higher level and (iii) changes in gene expression patterns, from a temporal into a spatial context.[4,6,7] We have also suggested that some of the differences among extant multicellular lineages (including differences in their evolutionary potential) can be explained by the way in which the reorganization of these processes and traits (and the emergence of the new

genotype-phenotype map) has been achieved during the transition to multicellularity and the evolution of individuality at the higher level.[4] Volvocine algae exemplify well these suggestions. In this group, the transition to multicellularity embraced unique paths, partly due to the constraints inherited from their unicellular ancestors.

THE VOLVOCINE ALGAE AS A CASE STUDY

"Few groups of organisms hold such a fascination for evolutionary biologists as the Volvocales. It is almost as if these algae were designed to exemplify the process of evolution".[8]

Diversity

Volvocine algae are photosynthetic biflagellated green algae in the order Volvocales, comprising closely related unicellular (*Chlamydomonas*-like) and multicellular forms that show a progressive increase in cell number, volume of extracellular matrix per cell, division of labor and ratios between somatic and reproductive cells[9] (Fig. 1). Interestingly, somatic cell specialization and higher-level individuality evolved multiple times in this group and the different levels of complexity are thought to represent alternative stable states (among which evolutionary transitions have occurred several times during the evolutionary history of the group), rather than a monophyletic progression in organizational and developmental complexity.[9,10] The observed morphological and developmental diversity among volvocine algae appears to result from the interaction of conflicting structural and functional constraints and strong selective pressures.

CONSTRAINTS

All volvocine algae share the so-called "flagellation constraint",[11] which has a different structural basis than the one invoked in the origin of metazoans.[12] Specifically, in volvocine algae, because of their coherent rigid cell wall the position of flagella is fixed and thus, the basal bodies cannot move laterally and take the position expected for centrioles during cell division while still remaining attached to the flagella (as they do in "naked", wall-less green flagellates). Therefore, cell division and motility can take place simultaneously only for as long as flagella can beat without having the basal bodies attached (i.e., only up to five cell divisions).

The presence of a coherent cell wall is coupled with the second conserved feature among volvocine algae—namely, their unique way of cell division. In this green algal group, cells do not double in size and then undergo binary fission. Rather, each cell grows about 2^n-fold in volume, followed by a rapid, synchronous series of n divisions under the mother cell wall; this type of cell division is referred as to multiple fission or palintomy (i.e., the process during which a giant parental cell undergoes a rapid sequence of repeated divisions, without intervening growth, to produce numerous small cells). Because clusters, rather than individual cells, are produced in this way, this type of division was suggested to have been an important precondition facilitating the

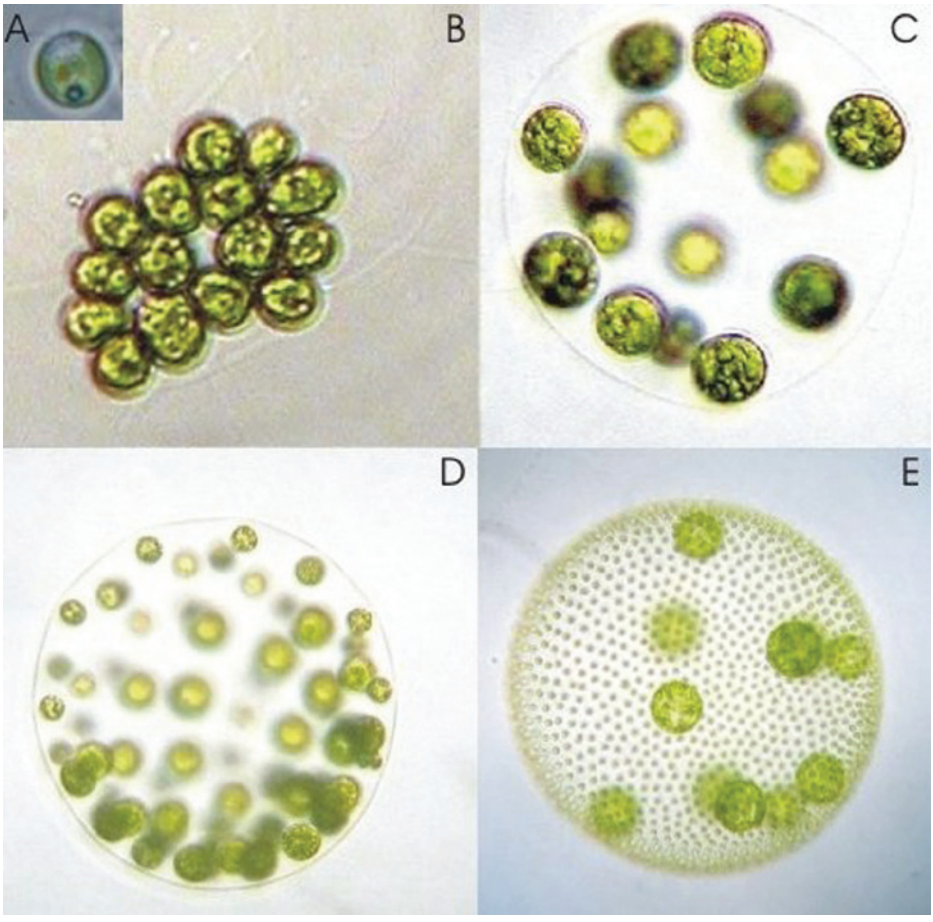

Figure 1. A subset of volvocine green algae that show a progressive increase in cell number, volume of extracellular matrix per cell, division of labor between somatic and reproductive cells and proportion of vegetative cells. A) *Chlamydomonas reinhardtii*; B) *Gonium pectorale*; C) *Eudorina elegans*; D) *Pleodorina californica*; E) *Volvox carteri*. Where two cell types are present, the smaller cells are the vegetative (somatic) cells, whereas the larger cells are the reproductive (gonidia) cells.

formation of multicellular colonies in this group.[13] In the unicellular species, such as *Chlamydomonas*, the daughter cells (2^2-2^4 cells) separate from each other after division. However, in many species, the cluster of 2^n daughter cells does not disintegrate and coenobial forms (i.e., a type of multicellular organization in which the number of cells is determined by the number of divisions that went into its initial formation, without any further cell additions)[13] are produced. For instance, in *Gonium*, the resulting cells (2^2-2^5) stay together and form a convex discoidal colony. In *Eudorina* and *Pleodorina* the cells (2^4-2^6, 2^6-2^7, respectively) are separated by a considerable amount of extracellular matrix and form spherical colonies. Finally, in *Volvox*, a high number of cells (2^{15}-2^{16}) form colonies up to 3 mm in size (Fig. 1).

SELECTIVE PRESSURES

The two selective pressures that are thought to have contributed to the increase in complexity in all volvocalean lineages are the advantages of a large size (potentially to escape predators, achieve faster motility, homeostasis, or better exploit eutrophic conditions) and the need for flagellar motility (e.g., to optimally position themselves in the water-column and to achieve better mixing of the surrounding environment).[8,14,15] Interestingly, given the background offered by the volvocalean type of organization presented above, namely the flagellar constraint and the multiple fission type of cell division, it is difficult to achieve the two selective advantages simultaneously. As the colonies increase in size and number of cells, also does the number of cell divisions (up to 15-16 in some *Volvox* species); consequently, the motility of the colony during the reproductive phase is negatively impacted for longer periods of time than are acceptable in terms of the need to access the euphotic zone. In larger species, this negative impact of the flagellation constraint is overcome by division of labor: some cells are involved mostly in motility, while the rest of the cells become specialized for reproduction. The proportion of cells that remain motile throughout most or all of the life cycle is directly correlated with the number of cells in a colony: from up to one-half in *Pleodorina* to >99% in *Volvox*.[9] In *Volvox*, the division of labor is complete: the motile (somatic) cells are sterile, terminally differentiated and undergo cellular senescence and death once the progeny is released from the parental colony;[16] only the reproductive cells (termed gonidia) form new colonies.[17]

THE GENETIC BASIS FOR CELL DIFFERENTIATION IN *VOLVOX CARTERI*

Volvox carteri is the most studied member of the multicellular volvocine algae[13] (Fig. 1). It consists of 2,000-4,000 permanently biflagellated somatic cells and up to 16 nonflagellated reproductive cells (Fig. 2). Terminal differentiation of somatic cells in *V. carteri* involves the expression of *regA*, a master regulatory gene that encodes a transcriptional repressor[18] thought to suppress nuclear genes coding for chloroplast proteins.[19] Consequently, the cell growth (dependent on photosynthesis) and division (dependent on cell growth) of somatic cells are suppressed. *regA* contain a SAND domain, which is found in a number of nuclear proteins, many of which function in chromatin-dependent or DNA-specific transcriptional control.[7] Proteins containing a SAND domain have been reported in both animal and land plants; one such protein, ULTRAPETALA1, acts as a key negative regulator of cell accumulation in *Arabidopsis* shoot and floral meristems.[20]

Mutations in *regA* result in the somatic cells regaining reproductive abilities—which in turn results in them losing their flagellar capabilities.[21,22] As motility is very important for these algae, the survival and reproduction of *V. carteri* individuals in which such mutant somatic cells occur is negatively affected.[14] Interestingly, although *regA* belongs to a gene family that comprises 14 members in *V. carteri,*[23] *regA* is currently known as the only locus that can mutate to yield Reg mutants.[18]

The expression of *regA* is strictly determined by the size of cells at the end of embryogenesis; cells below a threshold size will develop into somatic cells.[24] Which cells will not express *regA* and differentiate into germ cells is determined early in development

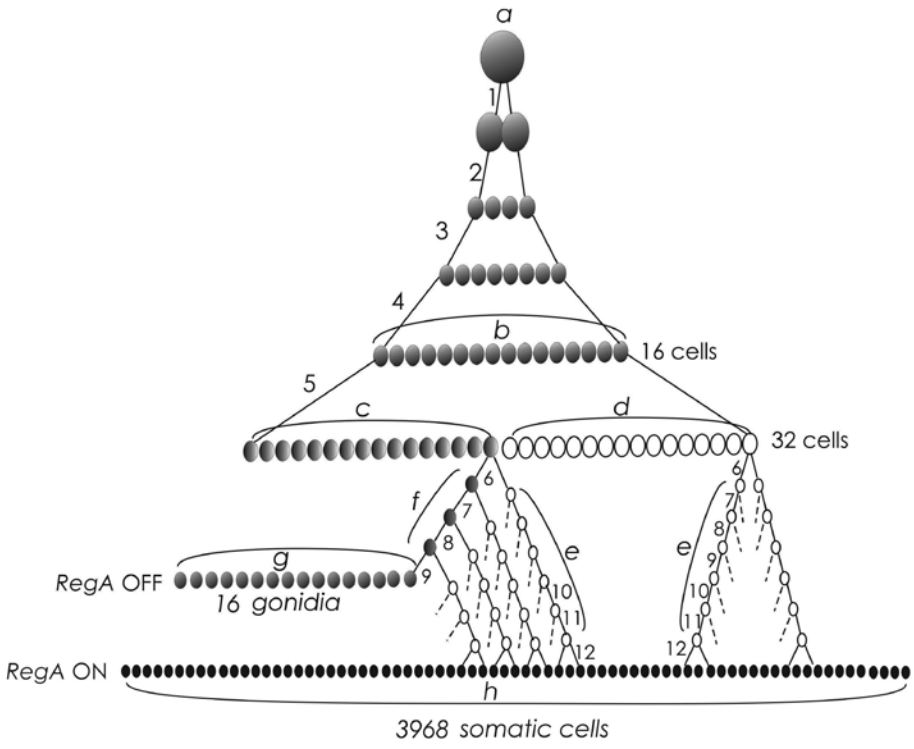

Figure 2. Schematic representation of asexual development and germ-soma separation in *Volvox carteri*. Gray ellipses denote totipotent (the "mother" gonidium—*a* and the 16 totipotent blastomeres—*b*), pluripotent cells (*c* and *f*) and the next-generation gonidia (*g*); white ellipses indicate unipotent (i.e., the somatic blastomeres and the somatic initials: *d* and *e*, respectively); black ellipses denote terminally differentiated somatic cells (*h*). Numbers mark the succession of cell divisions in the embryo. Cells are not represented at scale (*a* is ca. 2^9-fold larger than *g* and there is a ½-reduction in cell size with every symmetric cell division); all divisions take place under the mother cell wall, in a rather rapid fashion without intervening growth (i.e., palintomy and multiple fission) (adapted from ref. 4).

through a series of asymmetric cell divisions (Fig. 2). The asymmetric divisions ensure that some cells (i.e., the germ line precursors) remain above the threshold cell size associated with the expression of *regA*.[25] *RegA* is induced in very young somatic cells immediately after the end of embryogenesis but is never expressed in gonidia.[18] The mechanism underlying the differential expression of *regA* (i.e., ON in the somatic cells and OFF in the gonidia) is not known; it has been postulated that specific transcription factors bind to the *cis*-regulatory elements identified in three of the introns (i.e., two enhancers and one silencer) and act in concert to either silence or induce *regA* expression.[26]

UNICELLULARITY VERSUS MULTICELLULARITY

Many general life-properties and traits (such as immortality, totipotency, growth and reproduction) as well as cellular processes (such as cell division) are expressed differently

in unicellular versus multicellular individuals (see below). In the next section we discuss how these basic life properties and cellular processes have been reorganized during the transition to multicellularity and the emergence of individuality at the higher level, and apply these concepts to the evolution of multicellularity in volvocine algae.

General Life-Properties and Traits

Vegetative and Reproductive Functions

Any biological entity features two main sets of functions, vegetative (i.e., nutrition and growth) and reproductive, which correspond to the two basic components of fitness, survival and reproduction. These basic biological functions are coupled at the level of the individual, as a physiological and reproductive unit. However, the two sets of functions are realized differently between a unicellular and a multicellular individual. In unicellular forms, the same cell is responsible for both vegetative and reproductive activities (i.e., they are coupled at the cell level). Nevertheless, at the level of the individual, these functions do not take place simultaneously as they are dissociated in time: the vegetative phase precedes the reproductive phase. In undifferentiated multicellular forms, all cells perform both vegetative and reproductive functions and—as in their unicellular ancestors, these functions are separated in time, both at the cell level and multicellular entity level. On the other hand, in multicellular individuals with germ-soma separation, the two sets of functions are uncoupled at the cell level; some cells perform only vegetative functions, whereas other cells are specialized for reproductive functions. Consequently, the two sets of functions can take place simultaneously (i.e., they need not be separated in time anymore).

Growth is an important property of life. Interestingly, growth has different implications in unicellular versus multicellular individuals. In the former, growth is coupled with reproduction; growth to a specific cell size will generally trigger the reproduction of the individual and vice versa, reproduction requires achieving a preset cell size. In multicellular individuals, on the other hand, growth and reproduction of the individual can be uncoupled; reproduction is not necessarily dependent on growth and growth does not necessarily trigger reproduction.

Immortality and Totipotency

Immortality and totipotency are two basic life-traits. Here, immortality is used as the capacity of a cell to divide indefinitely and totipotency is defined as the ability of a cell to create a new individual. In contrast to totipotency, the term pluripotent denotes the ability of a cell lineage to produce cells that can differentiate into all cell types (but not into a new functional individual); lastly, multipotency refers to the potential of one cell to differentiate into more than one (but not any) cell type.

In unicellular forms, cells have both the potential to divide indefinitely (i.e., they are potentially immortal) and to create new individuals, either asexually or sexually (i.e., they are totipotent). In unicellular individuals, immortality and totipotency are thus coupled at the cell level. In differentiated multicellular individuals, on the other hand, only one or a few cell lineages manifest both immortality and totipotency; most other cell lineages have only certain degrees and combinations of potential for cell division and differentiation. For instance, in groups without an early segregated germ line (such

as plants and some simple metazoans like *Hydra*), the somatic cell lineages are incapable of continuous division or redifferentiation and thus they have to be replenished from one or a few pluripotent lineages that remain mitotically active throughout ontogeny and can also differentiate into germ cells (e.g., the interstitial I-cells in *Hydra*).[27] In lineages with a germ line that is terminally differentiated early in the development (such as in many animals), various degrees of mitotic capacity (approaching immortality in some stem cell lineages) and/or potential for differentiation are maintained in the many multipotent somatic stem cells (i.e., secondary somatic differentiation).[28]

Cellular Processes and Life-Traits

Cell division is a basic process in all cellular life-forms. The mechanisms controlling cell division are, however, different between unicellular and multicellular individuals. In unicellular individuals, cell division is strictly dependent on cell growth (cells divide when a specific set size is achieved). In many multicellular forms, however, this is not always the case: factors other than cell size (such as intercellular or systemic signals) can trigger or inhibit cell division. In addition, in unicellular forms cells have an unlimited division potential (cell division is strictly coupled with immortality), whereas in multicellular individuals, cells have limited and variable potential in most cell lineages (i.e., they are mortal) and their division potential is under the control of the higher-level individual.

Cellular Processes and Higher-Level Functions

Interestingly, cell division and cell growth have different roles and consequences at the level of the individual in unicellular compared to multicellular forms. In unicellular forms, every cell division results in the reproduction of the individual (cell division is strictly coupled with reproduction). In multicellular forms, on the other, hand, cell division is uncoupled from the reproduction of the individual in most cells (i.e., cell divisions do not necessarily result in the reproduction of the higher level). Also, whereas in unicellular forms, cell growth is the main contributor to the growth of the individual (with the exception of extracellular deposits in some lineages), in multicellular forms, the growth of the individual is mostly achieved through increasing the number rather than the size of cells (with some exceptions in lineages where there is significant increase in volume of extracellular matrix, internal space or even cell size).

TRANSITION TO MULTICELLULARITY: THE EMERGENCE OF A NEW SELF

We have argued that the unicellular-multicellular transition and the emergence of individuality at a higher level requires: (i) reorganizing basic life-traits (such as immortality and totipotency) between and within lower levels, (ii) decoupling processes from one another at the lower level (e.g., cell division from cell growth), (iii) decoupling certain cellular processes from functions and traits (e.g., cell division from reproduction and immortality), (iv) coopting them for new functions at the higher level (e.g., the cooption of cell division for multicellular growth) and (iv) changing the temporal expression of vegetative and reproductive functions into a spatial context.[4] Below, we discuss these concepts and apply them to our study case, the volvocine algae.

Reorganizing Immortality and Totipotency

During the transition to multicellularity and the emergence of individuality at the higher level, immortality and totipotency became restricted to one or a few specific cell lineages, namely those involved in the reproduction of the higher level. However, many cell lineages maintained various degrees and combinations of mitotic and differentiation potential. This required the reorganization (i.e., the differential expression) of these traits both among cell lineages and within a cell lineage. As discussed earlier, this reorganization has been achieved differently among the extant multicellular groups.

In *V. carteri*, immortality and totipotency are restricted to gonidia, the 16 cells following the first 4 embryonic cell divisions (*a* and *b* in Fig. 2)[24,29] and the zygote (after a sexual cycle; not shown). At the 32-cell stage, 16 cells (i.e., the germline blastomeres—*c* in Fig. 2) are pluripotent (i.e., they give rise to both germline precursors—*f* and somatic initials—*e*), while the other 16 cells (i.e., the somatic blastomeres—*d* in Fig. 2) are unipotent and produce solely somatic initials. The germline blastomeres divide asymmetrically for three or four times (each time renewing themselves and producing a somatic initial) and arrest mitosis two or three cell division cycles before the somatic blastomeres do. These 16 cells (*g* in Fig. 2) will differentiate into the germ cells of the next generation. After a total of 11-12 cell divisions, the somatic initials stop dividing and differentiate into somatic cells (*h* in Fig. 2), which have no mitotic or differentiation potential (they are terminally differentiated).

It is interesting that in *Volvox*, although immortality and totipotency have become fully restricted to the germ line (and reproduction and individuality at the higher level emerged), somatic lineages have no mitotic or redifferentiation potential. In other words, the two traits have been reorganized between germ and soma, but not within somatic cell lineages. The two sets of traits are still very linked in *V. carteri*; they are either both fully expressed (in gonidia) or both suppressed (in somatic cells). Noteworthy, the early-sequestration of the germ line was achieved without the evolution of secondary somatic differentiation processes; no multipotent somatic stem cells are present in the adult. This is rather surprising, because it has been suggested that the evolution of an early-defined germ line was possible because, due to the evolution of the multipotent stem cells and secondary somatic differentiation, the ancestral pluripotent germinative lineage was released from the task of producing the somatic tissues and was able to terminally differentiate into germ cells early in development.[28]

Decoupling Cell Division from Cell Growth

In multicellular individuals, to ensure the functionality of the soma, factors other than cell size must be used to determine which cells divide, when and how often. This requirement necessitates decoupling cell division from cell growth; consequently, a better and more finely tuned control on the replicative potential of the lower level can be achieved. However, this has not been accomplished in *V. carteri*; cell division is still strictly dependent on cell growth; reproductive cells have to increase $2^{10\text{-}12}$ fold in volume before dividing 10-12 times to produce the final number of cells in the multicellular individual.

Decoupling Cell Division From Cell Reproduction

Furthermore, to ensure the reproduction of a cell-group (and the heritability of the group traits), cell division has to be uncoupled from cell reproduction (i.e., the reproduction of the previously independent unicellular individual) and be coopted for the reproduction of the higher level (the group). The ability to reproduce the group can be achieved either by all or only some members of the group.

The case in which all cells have higher-level reproductive capabilities is best exemplified by a reproductive mode called autocolony, in which when the group enters the reproductive phase, each cell within the group produces a new group similar to the one to which it belongs; cell division no longer produces unicellular individuals but multicellular groups. This mode of reproduction characterizes the volvocine algae without a germ-soma separation, such as *Gonium* and *Eudorina* (Fig. 1).

In *Eudorina*, all cells (16 or 32) go through a vegetative (growth) and reproductive phase. However, cell division does not anymore result in a number of free unicellular individuals (such as in *Chlamydomonas*), but rather an embryo; cell division has been thus decoupled from cell reproduction and has been coupled with the reproduction of the group in all members of the group. Nevertheless, cell division is still strictly dependent on cell growth: each cell will start dividing only after a $2^{4\text{-}5}$-fold increase in size was attained, and once cell divisions are initiated they will continue synchronously until all the new embryos are formed. Although the stability, heritability and the reproduction of the higher level are ensured in this way, its individuality is not; because every member can be separated from the group and create a new group, such a group is not the smallest physiological and reproductive autonomous unit, thus is not a true individual in the sense used here (i.e., it is divisible).

The case in which only some cells have higher-level reproductive capabilities characterizes lineages with a separation between germ and soma. To achieve this, the coupling between cell division and reproduction is broken in most cells, namely the somatic cells; they reproduce neither themselves (as former free-living unicellular individuals) nor the higher-level unit; cell division is thus decoupled from the reproduction of both the lower and higher levels. In this way, somatic cells loose their individuality as well as the right to participate in the next generation; but in doing so they contribute not only to the emergence of individuality at the higher level but also to the emergence of a new level of organization, the multicellular soma. Soma is thus the expected consequence of uncoupling cell division from reproduction in order to achieve individuality at the higher level. *V. carteri* follows this pathway; however, the way in which germ-soma separation was achieved is rather unique among multicellular forms.

Coopting Cell Division for Growth at the Higher Level

By decoupling cell division from reproduction, this very important process became available for new functions. We suggested that this event was paralleled by the co-option of cell division for a new function at the higher level, namely the growth of the multicellular individual. Later, the use of cell division for more than cell multiplication, (i.e., which "gives rise to more entities of the same kind")[30] may have provided multicellular lineages with an additional advantage, namely cell differentiation; indeed, in many multicellular lineages asymmetric cell divisions are involved in cell differentiation.

Interestingly, in *V. carteri,* although the coupling between cell division and reproduction has been broken in the somatic cells, cell division was not coopted for the post-embryonic growth of the higher-level individual; rather, cell division was simply repressed in somatic cells. Specifically, the somatic cells lack the ability to divide post-embryonically; all the cell divisions responsible for the final number of cells in the adult take place during embryonic development (the further growth of the young spheroid is accomplished only through small increases in cell size and through a massive deposition of extracellular matrix). The implications of this outcome are multiple and profound. A direct implication is that soma in *V. carteri* differs from the soma of most multicellular organisms. Because somatic cells do not divide, further growth and/or regeneration of the individual are not possible during ontogeny; in addition, because the somatic cells undergo senescence and death at the age of 5 days,[16,17] the life span of the higher-level individual is limited to the life span of the lower-level somatic cell. Due to this unique type of soma, *V. carteri* is missing more than the ability to grow, regenerate, or live longer. Without a mitotically active multipotent stem cell lineage or secondary somatic differentiation there is less potential for cell differentiation and further increases in complexity.[4]

Changing Expression Patterns from a Temporal to Spatial Context

As discussed above, during the transition to multicellularity and the emergence of individuality at a higher level, some cells loose both their own individuality as well as the right to participate in the next generation. But why would cells give up their own reproduction (i.e., reproductive altruism)? The evolution of specialized somatic and reproductive cells can be understood in terms of the need to break survival-reproduction trade-offs, such that the survival and reproduction of a multicellular group can be maximized independently and simultaneously, and the benefits of a large size can be realized.[2] For instance, in undifferentiated multicellular flagellated algae, the reproductive phase is paralleled by the loss of motility—which can negatively affect the survival of the individual, especially in multicellualr groups whose reproduction will require a large number of cell divisions. On the other hand, in differentiated multicellular forms—such as *Volvox*, the spatial dissociation of reproductive and vegetative functions between gonidia and somatic cells allows the two sets of functions to take place simultaneously.

At a mechanistic level, we suggested that the evolution of germ-soma separation involved a change in the expression of vegetative and reproductive functions from a temporal (as in unicellular individuals) to a spatial context.[4] We have further argued that the evolution of soma in multicellular lineages involved the cooption of life-history trade-off genes whose expression in their unicellular ancestors was conditioned on environmental cues (as an adaptive strategy to enhance survival at an immediate cost to reproduction), through shifting their expression from an environmentally-induced context into a developmental context[4,7] (Fig. 3A).

Indeed, in volvocine algae—as in other photosynthetic organisms, nutrient-poor or stressful environments trigger a series of metabolic alterations—collectively known as acclimation, which favor survival when the potential for cell growth and division is reduced.[31] One of the consequences of this complex series of responses is a temporary inhibition of cell division (and thus reproduction), to ensure long-term survival. Acclimation involves both specific responses (e.g., scavenging for a specific nutrient) and general responses. The general responses include: a decline in the rate of photosynthetic activities, the accumulation of starch (diverting energy and fixed carbon from cell growth), a general

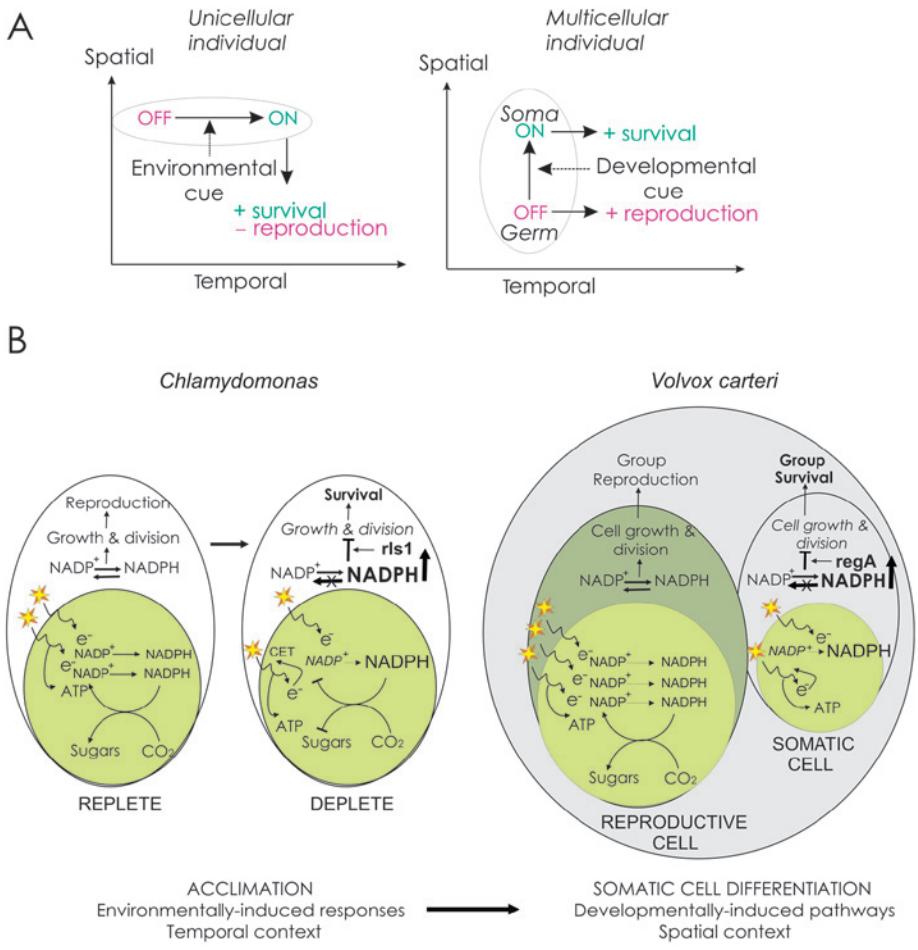

Figure 3. The evolution of germ-soma separation during the transition to multicellularity. A) General schematic representation of the change in expression pattern of a life-history trade-off gene from a temporal context (environmentally-induced)—in a unicellular individual, into a spatial context (developmentally-induced) in a multicellular individual. Adapted from Nedelcu AM et al. The evolutionary origin of an altruistic gene. Mol Biol Evol 2006; 23(8):1460-4; with permission of Oxford University Press. B) A model for the cooption of acclimation responses into somatic cell differentiation in *Volvox carteri*; see text for discussion. Although many components are involved, for simplicity, changes in redox status are symbolized by the over-reduction of the NADP pool due to either decreased NADPH consumption—in nutrient-deprived *Chlamydomonas*, or excess of excitation energy (owing to a higher surface/volume ratio)—in *Volvox* somatic cells. The switch to cyclic electron transport (CET), which can maintain ATP synthesis (and thus vital processes) in acclimated *Chlamydomonas* cells[35] and possibly in *Volvox* somatic cells, is also indicated (adapted from ref. 6).

metabolic slowdown and cessation of cell division.[31,32] Photosynthetic organisms use light energy to generate chemical energy (ATP) and reductants (NADPH) that are subsequently used to fix carbon dioxide (which will regenerate ADP and $NADP^+$). This coupling renders photosynthesis and its efficiency highly dependent on environmental conditions; changes in various abiotic factors—including light, temperature, water and nutrient

availability have an immediate impact on photosynthetic activities and subsequently on other metabolic processes.[33]

The down-regulation of photosynthesis is critical for sustaining cell viability under conditions of nutrient deprivation.[32,34] The lack of nutrients in the environment blocks cell growth and limits the consumption of NADPH and ATP generated via photosynthesis. Consequently, the photosynthetic electron transport becomes reduced and the redox potential of the cell increases.[31,32] Furthermore, because NADPH is not rapidly recycled (due to the slowdown of anabolic processes and the decreased demand for reductant in nutrient-poor environments), excited chlorophyll molecules and high potential electrons will accumulate and could interact with oxygen to create reactive oxygen species (ROS). ROS refer to a series of partially reduced and highly reactive forms of oxygen, including the superoxide anion (O_2^-), the hydroxyl radical ($OH\cdot$) and the hydrogen peroxide (H_2O_2). Although ROS are byproducts of normal metabolism and can act as secondary messengers in various signal transduction pathways (e.g., see refs. 35-37 for a review), increased intracellular levels of ROS (i.e., oxidative stress) can alter cellular functions and damage many biological structures, most importantly, DNA.[38]

Consequently, the regulation of the photosynthetic electron transport is an important hallmark of the general response to nutrient deprivation in *Chlamydomonas*. A series of processes including reduced photosynthetic electron transport and the redirection of energy absorbed from photosystem II to photosystem I can decrease NADPH production, favor ATP production through cyclic electron transport and allow a more effective dissipation of the excess absorbed excitation energy. Altogether these changes decrease the potential toxic effect of excess light energy (and thus serve to increase survival) and help coordinate cellular metabolism and cell division with the growth potential of the cell.[31,39]

We have identified in *C. reinhardtii* [7] the closest homolog of *V. carteri regA*—the gene responsible for the permanent suppression of division and reproduction in somatic cells (discussed earlier). Recently, we have also shown that this gene—currently known as *rls1*,[23] is induced under nutrient limitation (including phosphorus-, sulfur-deprivation and during stationary phase) as well as light deprivation.[6] Furthermore, we showed that the induction of *rls1* coincides with the down-regulation of a nuclear-encoded light-harvesting protein[7] and with the decline in the reproduction potential of the population under limiting conditions.[6] The fact that *rls1* is expressed under multiple environmental stresses and its induction corresponds with a decline in reproduction suggests that *rls1* is part of the general acclimation response and might function as a regulator of acclimation in *C. reinhardtii*. To support this suggestion is the finding that an inhibitor of the photosynthetic electron flow that triggers general acclimation-like responses,[32] also induces the expression of *rls1*.[6]

How can general acclimation responses in unicellular organisms be coopted for cell differentiation in multicellular groups? As we discussed above, in photosynthetic organisms, the flux of electrons through the electron-transport system (ETS) has to be balanced with the rate of ATP and NADPH consumption; imbalances between these processes can result in the generation of toxic ROS[32]. When a nutrient (e.g., sulfur, phosphorus) becomes limiting in the environment, ATP and NADPH consumption declines; this results in an excess of excitation energy and a subsequent change in the redox state of the photosynthetic apparatus, which will trigger a suite of short- and long-term acclimation responses[32,33] (Fig. 3B). Other environmental factors (e.g., cold, water stress) are also known to result in changes in the cellular redox status and trigger similar acclimation responses.[35] Thus, in principle, any factor that can elicit a similar redox change could prompt acclimation-like responses and ultimately induce cessation

of cell division. In a group context, if such a change is restricted to a subset of cells and if the suppression of reproduction in this subset of cells is beneficial to the group, sterile somatic cells can evolve and be fixed.

In *V. carteri*, the expression of *regA* is restricted (by an unknown mechanism) to cells whose size at the end of embryonic divisions falls below 8 μm.[24] As cell surface area and volume change at different rates, we proposed that in these small cells the ratio between membrane-bound proteins (including ETS and ETS-associated components) and soluble factors (including $NADP^+$ and ADP) becomes skewed—relative to the ratio in larger cells, towards the former.[6] Consequently, these small cells could experience an imbalance between the flux of electrons and the availability of final acceptors, which would result in a change in the intracellular redox status and the induction of acclimation-like responses, culminating with the suppression of division (Fig. 3B). To support this scenario is the fact that cytodifferentiation is light-dependent in *V. carteri*.[40]

Hence, by simulating the general acclimation signal (i.e., a change in the redox status of the cell) in a spatial rather than temporal context, an environmentally-induced trade-off gene can be differentially expressed between cell types, allowing for the two components of fitness to be maximized independently and simultaneously, and for individuality at the higher level to emerge. This hypothesis also predicts that somatic cell differentiation is more likely to evolve in lineages with enhanced acclimation mechanisms—or more generally, in lineages that can trade-off reproduction for survival in stressful environments. Because environments that vary in time (such as those volvocine algae live in)[13] will select for enhanced and efficient acclimation responses (note that temporally varying environments have been shown to select for phenotypic plasticity—i.e., generalists, in *C. reinhardtii*),[41] such environments are likely to be more conducive to the evolution of somatic cell differentiation.

A New Genotype-Phenotype Map

It is not known how the genotype-phenotype maps are formed nor how they are able to change in evolution.[42] During the unicellular-multicellular transition, a new genotype-phenotype map has to be created to reflect the emergence of individuality at the higher level. We argued that the way in which certain complex sets of traits and the genotype-phenotype maps associated with them are reorganized during the transition affects the flexibility and robustness of the new genotype-phenotype map at the higher level and can interfere with the potential for further evolution of the lineage.[4]

In this context, it is rather intriguing that in *V. carteri*, immortality can be regained and individuality can be destroyed by single mutations. As mentioned earlier, mutations in *regA* result in somatic cells regaining reproductive abilities. Although they start out as small flagellated cells, they later enlarge, loose flagella and redifferentiate into gonidia;[43] in other words, somatic cells regain both immortality and totipotency. In other multicellular lineages, such as humans, multiple mutations (each of which requires a minimum of 20-30 cell divisions) are required for immortality (i.e., cancer cells) to be regained.[44] The fact that single mutations have such large effects on individuality traits suggests that in *V. carteri*, the genotype-phenotype map at the higher level has been realized through a rather small number of genetic changes. Any attempt to increase the evolvability of these lineages has to first affect the current genotype-phenotype map to allow increased variability of the traits associated with immortality and totipotency (so as to decouple them in the somatic cells) without affecting the individuality of the system (e.g., by evolving

mechanisms to control these traits independently, thereby allowing cell replication and/or differentiation in the soma). In other words, the genotype-phenotype map has to at first become more robust (so that small genetic changes will not lead to the recreation of the maps associated with the previously independent lower levels, as it is currently the case) but flexible (so as to allow improvement through mutation and selection).

To gain such properties a number of small-effect mutations, in a very precise order (such that the viability of the individual under selection is not affected) is required. However, the way in which cell division, cell growth, immortality and potency have been reorganized in *Volvox*, as well as the way the genotype-phenotype map has been created at the higher level, makes the evolution of such traits more difficult. For example, the fact that i) the decoupling of cell division from reproduction in somatic cells was not achieved by inventing new ways to control cell division, but rather by blocking it altogether and ii) the suppression of cell division was not achieved through evolving some new mechanisms but rather through inhibiting the growth of the cell, strongly limits the evolution of traits that are dependent on these processes. These important complex sets of processes have not been decoupled from one another through their dissociation at the lower level and their cooption for new functions at the higher level, but rather through the suppression of some of the processes at the lower level; in this way, processes such as cell growth, cell division and differentiation are not represented in the higher-level map and thus cannot contribute to phenotypic variability.

Improvement is expected to come from mutations that, for instance, allow the somatic cells to regain controlled mitotic activity and some degree of differentiation potential during ontogeny. To achieve this, the multiple fission type of division should be replaced by a binary type, such that cell divisions during adulthood do not result in the duplication of the entire organism (as they do in the *V. carteri* mutants in which somatic cells regain mitotic capabilities); in addition, a binary type of cell division would allow a more finely tuned increase in body size, via small increments. In this way, more phenotypic variability can be achieved and become available for selection. It should be mentioned that the multiple fission type of division is a derived trait, which is thought to have evolved through the modification of the cell cycle via very conserved type of proteins involved in the key pathway that controls both cell division and differentiation in animal cells, namely, the retinoblastoma (RB) family of tumor suppressors.[45] Mutations of this gene in *Chlamydomonas reinhardtii* result in the initiation of the cell cycle at a below-normal size, followed by an increased number of cell divisions.[46] Such an alteration of the cell cycle might have been involved in the evolution of the multiple fission type of cell division, which is considered a precondition for the origin of multicellularity in *Volvox*.[13] If this is the case, it would argue for another example of achieving an important trait at the higher level (i.e., multicellularity) through a small number of genetic changes and thus for the potential instability/inflexibleness of the higher-level genotype-phenotype map emerged in this way.

CONCLUSION

During evolutionary transitions in individuality, a new identity (a new "self") emerges at the higher level from the re-organization of the properties displayed by the interacting entities. For instance, the transition from unicellular to multicellular individuals requires the re-organization at the higher level of certain basic life properties, such as growth, reproduction, immortality and totipotency, as well as of the cellular processes associated

with them (e.g., cell division and cell growth). The way in which this re-organization is achieved can affect the flexibility and robustness of the genotype-phenotype map that emerges at the higher level and can interfere with the potential for further evolution of the lineage. During the evolution of multicellularity, some cells gave up not only their own individuality but also their ability to reproduce. This form of extreme reproductive altruism is instrumental to the emergence of individuality at the higher level, as the presence of cells that lack the ability to reproduce the group (i.e., to recreate the whole) renders the multicellular group indivisible and thus an individual. The evolution of soma involved the co-option of life-history genes whose expression in their unicellular ancestors was conditioned on environmental cues (as an adaptive strategy to enhance survival at an immediate cost to reproduction), through shifting their expression from a temporal (environmentally-induced) into a spatial (developmental) context.[4,7] Interestingly, in eusocial insects, caste evolution is also thought to have involved the remodeling of pathways associated with basic life-history traits such as nutrition and reproduction present in their solitary ancestors,[47,48] which argues that the two distinct evolutionary transitions in individuality can be understood in a common framework.

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REFERENCES

1. Santelices B. How many kinds of individual are there? Trends Ecol Evol 1999; 14:152-155.
2. Michod RE, Nedelcu AM. On the reorganization of fitness during evolutionary transitions in individuality. Int Comp Biol 2003; 43:64-73.
3. Michod RE, Nedelcu AM. Cooperation and conflict during the unicellular-multicellular and prokaryotic-eukaryotic transitions. In: Moya A, Font E, eds. Evolution: From Molecules to Ecosystems. Oxford University Press, 2004:195-208.
4. Nedelcu AM, Michod RE. Evolvability, modularity and individuality during the transition to multicellularity in volvocalean green algae. In: Schlosser G, Wagner G, eds. Modularity in Development and Evolution. Chicago:University of Chicago Press, 2004:466-89.
5. Michod RE, Viossat Y, Solari CA et al. Life-history evolution and the origin of multicellularity. J Theor Bio 2006; 239:257-272.
6. Nedelcu AM. Environmentally induced responses co-opted for reproductive altruism. Biol Lett 2009; 5:805-808.
7. Nedelcu AM, Michod RE. The evolutionary origin of an altruistic gene. Mol Biol Evol 2006; 23:1460-1464.
8. Bell G. The origin and early evolution of germ cells as illustrated by the Volvocales. In: Halvorson HO, Monroy A, eds. The Origin and Evolution of Sex. 1st ed. New York: Alan R. Liss, Inc, 1985:221-56.
9. Larson A, Kirk M, Kirk DL. Molecular phylogeny of the volvocine flagellates. Mol Biol Evol 1992; 9:85-105.
10. Herron MD, Michod RE. Evolution of complexity in the volvocine algae: Transitions in individuality through Darwin's eye. Evolution 2008; 62:436-451.
11. Koufopanou V. The evolution of soma in the Volvocales. Am Nat 1994; 143:907-931.
12. Margulis L. Symbiosis in cell evolution. San Francisco:Freeman, 1981.
13. Kirk DL. Volvox. Molecular genetic origins of multicellularity and cellular differentiation. New York: Cambridge University Press, 1998.
14. Solari CA, Kessler JO, Michod RE. A hydrodynamics approach to the evolution of multicellularity: Flagellar motility and germ-soma differentiation in volvocalean green algae. Am Nat 2006; 167:537-554.
15. Solari CA, Ganguly S, Kessler JO et al. Multicellularity and the functional interdependence of motility and molecular transport. Proc Natl Acad Sci USA 2006; 103:1353-1358.
16. Pommerville J, Kochert G. Changes in somatic cell structure during senescence of Volvox carteri. Eur J Cell Biol 1981; 24:236-243.

17. Pommerville J, Kochert G. Effects of senescence on somatic cell physiology in the green alga Volvox carteri. Exp Cell Res 1982; 140:39-45.
18. Kirk M, Stark K, Miller S et al. RegA, a Volvox gene that plays a central role in germ soma differentiation, encodes a novel regulatory protein. Development 1999; 126:639-647.
19. Meissner M, Stark K, Cresnar B et al. Volvox germline-specific genes that are putative targets of RegA repression encode chloroplast proteins. Curr Genet 1999; 36:363-370.
20. Carles CC, Choffnes-Inada D, Reville K et al. ULTRAPETALA1 encodes a SAND domain putative transcriptional regulator that controls shoot and floral meristem activity in Arabidopsis. Development 2005; 132:897-911.
21. Starr R. Control of differentiation in Volvox. Dev Biol Suppl 1970; 4:59-100.
22. Kirk DL, Baran GJ, Harper JF et al. Stage-specific hypermutability of the reg A locus of Volvox, a gene regulating the germ-soma dichotomy. Cell 1987; 18:11-24.
23. Duncan L, Nishii I, Harryman A et al. The VARL gene family and the evolutionary origins of the master cell-type regulatory gene, regA, in Volvox carteri. J Mol Evol 2007; 65:1-11.
24. Kirk M, Ransick A, McRae SE et al. The relationship between cell size and cell fate in Volvox carteri. J Cell Biol 1993; 123:191-208.
25. Kirk DL. Asymmetric division, cell size and germ-soma specification in Volvox. Semin Dev Biol 1995; 6:369-379.
26. Stark K, Kirk DL, Schmitt R. Two enhancers and one silencer located in the introns of regA control somatic cell differentiation in Volvox carteri. Genes Dev 2001; 15:1449-1460.
27. Bode HR. The interstitial cell lineage of Hydra: a stem cell system that arose early in evolution. J Cell Sci 1996; 109:1155-1164.
28. Buss LW. The evolution of individuality. Princeton: Princeton University Press, 1987.
29. Kirk DL. Germ cell specification in Volvox carteri. In: Marsh J, Goode J, eds. Germline Development Ciba Symposium 184. Chichester:Wiley, 1994:2-30.
30. Szathmáry E, Maynard Smith J. From replicators to reproducers: the major transitions leading to life. J Theor Biol 1997; 187:555-572.
31. Grossman A. Acclimation of Chlamydomonas reinhardtii to its nutrient environment. Protist 2000; 151:201-224.
32. Wykoff DD, Davies JP, Melis A et al. The regulation of photosynthetic electron transport during nutrient deprivation in Chlamydomonas reinhardtii. Plant Physiol 1998; 117:129-139.
33. Pfannschmidt T, Brautigam K, Wagner R et al. Potential regulation of gene expression in photosynthetic cells by redox and energy state: approaches towards better understanding. Ann Bot 2009; 103:599-607.
34. Davies JP, Yildiz FH, Grossman A. Sac1, a putative regulator that is critical for survival of Chlamydomonas reinhardtii during sulfur deprivation. EMBO J 1996; 15:2150-2159.
35. Eberhard S, Finazzi G, Wollman FA. The dynamics of photosynthesis. Ann Rev Gen 2008; 42:463-515.
36. Van Breusegem F, Vranova E, Dat J et al. The role of active oxygen species in plant signal transduction. Plant Sci 2001; 161:405-414.
37. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Sci 2002; 7:405-410.
38. Marnett LJ, Plastaras JP. Endogenous DNA damage and mutation. Trends Genet 2001; 17:214-221.
39. Chang CW, Moseley JL, Wykoff D et al. The LPB1 gene is important for acclimation of Chlamydomonas reinhardtii to phosphorus and sulfur deprivation. Plant Physiol 2005; 138:319-329.
40. Stark K, Schmitt R. Genetic control of germ-soma differentiation in Volvox carteri. Protist 2002; 153:99-107.
41. Reboud X, Bell G. Experimental evolution in Chamydomonas. III. Evolution of specialist and generalist types in environments that vary in space and time. Heredity 1997; 78:507-514.
42. Wagner GP, Altenberg L. Complex adaptations and the evolution of evolvability. Evolution 1996; 50:967-976.
43. Huskey RJ, Griffin BE. Genetic control of somatic cell differentiation in Volvox. Dev Biol 1979; 72:226-235.
44. Wright WE, Shay JW. Cellular senescence as a tumor-protection mechanism: the essential role of counting. Curr Opin Genet Dev 2001; 11:98-103.
45. Sage J, Mulligan GJ, Attardi LD et al. Targeted disruption of the three Rb-related genes leads to loss of G1 control and immortalization. Genes Dev 2000; 14:3037-3050.
46. Umen JG, Goodenough UW. Control of cell division by a retinoblastoma protein homolog in Chlamydomonas. Genes Dev 2001; 15:1652-1661.
47. Toth AL, Robinson GE. Evo-devo and the evolution of social behavior. Trends Genet 2007; 23:334-341.
48. Smith CR, Toth AL, Suarez AV et al. Genetic and genomic analyses of the division of labour in insect societies. Nat Rev Genet 2008; 9:735-748.