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^{4-5}-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
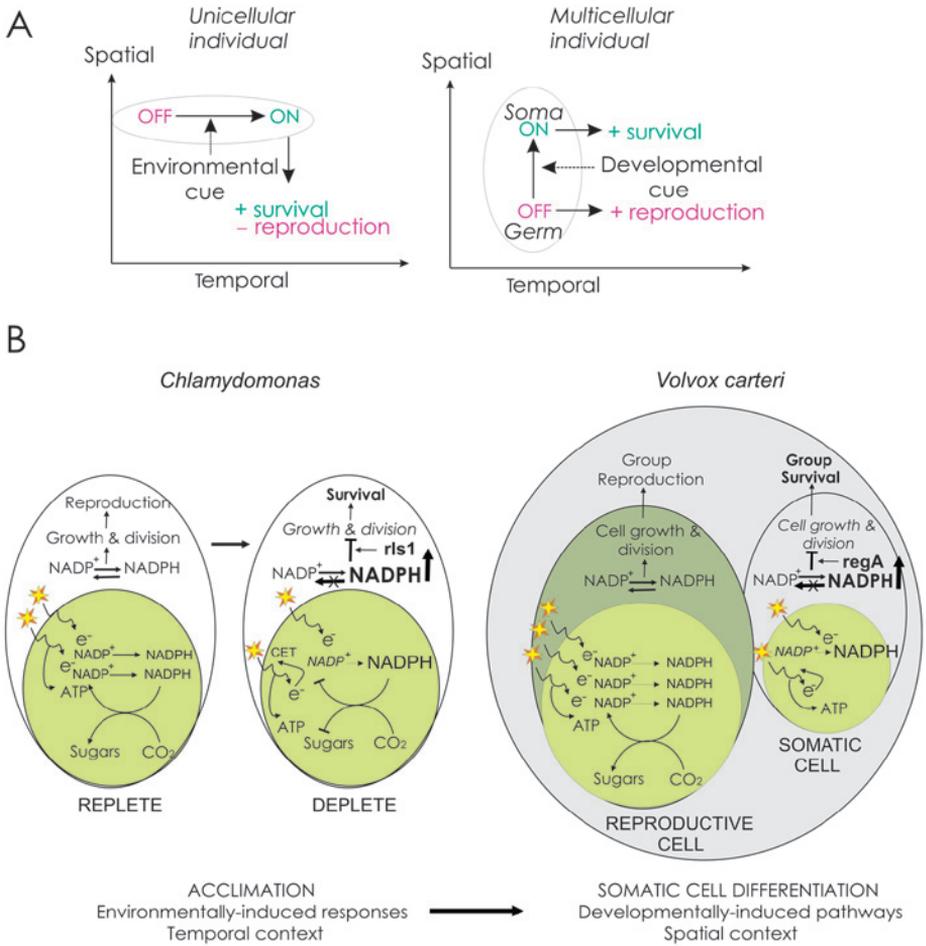
Figure 3. The evolution of germ-soma separation during the transition to multicellularity. A) General schematic representation of the change in expression pattern of a life-history trade-off gene from a temporal context (environmentally-induced)—in a unicellular individual, into a spatial context (developmentally-induced) 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·) 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.

ACKNOWLEDGMENTS

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