

Molecular mechanisms of life history trade-offs and the evolution of multicellular complexity in volvocalean green algae

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21.1 Introduction

Although life history trade-offs are recognized as central to life history evolution, the mechanisms underlying trade-offs are not well understood (e.g., Roff 2007b, Monaghan *et al.* 2009). Both artificial selection and experimental evolution have demonstrated the presence of genetically-based trade-offs, but relatively few studies have been able to pinpoint the underlying molecular mechanisms (see Roff 2007b and Chapters 1 and 2 for discussion). Often, the functional basis of a trade-off is understood in terms of competition for limited resources among competing traits—such as reproduction, somatic growth, and maintenance—within an organism (also known as adaptive resource allocation) (Zera and Harshman 2001, Chapter 24). Searches for genes that affect, in opposite ways, two life history traits (i.e., “trade-off” genes) suggest that life history trade-offs could be the result of adaptive resource allocation at the organismal level (Bochdanovits and de Jong 2004, St-Cyr *et al.* 2008), and that this differential allocation might be caused by a trade-off between protein biosynthesis (growth) and energy metabolism (survival)—likely mediated by signal transduction pathways at the level of cellular metabolism (Bochdanovits and de Jong 2004). However, several studies propose that trade-offs could, in fact, be the result of signaling genes or pathways that simultaneously regulate two life history traits in opposite directions, independent of

resource allocation (Leroi 2001, Chapter 11). Furthermore, trade-offs can also be produced as a consequence of the performance of one activity generating negative consequences for other traits; for instance, aerobic metabolism generates reactive oxygen species that, if not fully neutralized, can be damaging to biological molecules and negatively affect other activities (Monaghan *et al.* 2009; also see Chapter 15).

Because the loci involved in life history trade-offs are expected to show antagonistic pleiotropy (Stearns 1992), life history trade-offs are generally thought to limit the set of possible trait combinations, and thus restrict the range of possible evolutionary trajectories and end-points (Roff 2007b). Life history trade-offs gain unique significance during evolutionary transitions in individuality (Box 21-1), such as the transitions from unicellular to multicellular individuals and solitary individuals to eusocial societies. Specifically, it has been suggested that trade-offs constraining the evolutionary trajectories of solitary individuals can be uncoupled in the context of a group through the evolution of specialized cells in multicellular individuals (Michod 2006, Michod *et al.* 2006) and castes in eusocial insects (Roux *et al.* 2009). In this way, traits (and fitness components) negatively correlated in previously solitary individuals can be optimized independently and simultaneously in a group, and new levels of fitness at the group level can emerge. The ability to break life history trade-offs through

Box 21-1 Glossary**Chlamydomonas**

A polyphyletic genus that includes many unicellular species of bi-flagellated green algae. The most well-known species is *Chlamydomonas reinhardtii* (Fig. 21-1A), which is also a close relative of multicellular volvocine algae.

Eudorina

Spherical colonial forms comprising 16–32 undifferentiated cells separated by a considerable amount of extracellular matrix (Fig. 21-1C).

Evolutionary transitions in individuality

An evolutionary process whereby a group of previously independent individuals become stably integrated into a new functional, physiological, and reproductively autonomous and indivisible evolutionary unit; that is, a new individual. Such transitions took place during the evolution of the eukaryotic cell, the evolution of multicellularity, and the evolution of eusociality.

Flagellation constraint

Stems from the fact that in flagellated cells a single structure—known as the microtubule organizing center (MTOC)—has to perform two distinct functions: during the growth phase, MTOCs act as flagellar basal bodies (and organize the flagellar microtubules), whereas during cell division MTOCs act as centrioles (and direct the formation of the mitotic spindle). This constraint was proposed to have been at the origin of differentiated multicellularity in early metazoans (Margulis 1981). Notably, the flagellation constraint in volvocalean algae (Koufopanou 1994) has a slightly different structural basis than the one invoked in the origin of metazoans. Specifically, in contrast to other protists, in most green flagellates MTOCs can move laterally while attached to the flagella, and can act simultaneously as basal bodies and centrioles during cell division. However, in volvocalean algae, due to a 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 remaining attached to the flagella.

Gonium

Colonial forms comprising 8–16 undifferentiated cells organized as flat or slightly curved sheets in a single layer (Fig. 21-1B).

Palintomy

The process during which a giant parental cell undergoes a rapid sequence of repeated divisions, without intervening growth, to produce numerous small cells.

Pleodorina

Spherical colonial forms consisting of 64–128 cells, including up to 50% differentiated somatic cells at the anterior pole (Fig. 21-1D).

Volvoclean algae

A group of green algae (order Volvocales) in the class Chlorophyceae, comprising both unicellular species (such as those in the genus *Chlamydomonas*) and multicellular species with various levels of morphological and developmental complexity (spanning many genera, including *Gonium*, *Pandorina*, *Eudorina*, *Pleodorina*, *Volvox*; Fig. 21-1). Most genera are poly- or paraphyletic, as many characters used to define specific genera have evolved independently several times (Herron and Michod 2008).

Volvox

A polyphyletic genus that comprises large spherical colonies with a high ratio of somatic to reproductive cells. *Volvox* species differ in many developmental traits, including the timing of germ-line segregation (early versus late in development) and the presence/absence of cytoplasmic bridges in the adult. The most studied species is *Volvox carteri* (Fig. 21-1E), which is a species with complete and early separation between somatic and reproductive cells, and loss of cytoplasmic bridges by the end of the embryonic development (Kirk 1998).

the evolution of specialization and division of labor is likely to have contributed to the evolutionary success of multicellularity and sociality.

During transitions in individuality, fitness becomes reorganized. For instance, in unicellular individuals, the same cell contributes to both fitness components, typically these contributions being separated in time. In multicellular groups, however, cells can specialize in either component, and this leads to the differentiation of reproductive cells (germ) and survival-enhancing cells (soma). Thus during the evolution of multicellularity, new levels of fitness can emerge at the higher level of organization (i.e., the multicellular individual) following the reorganization of survival- and reproduction-related functions between somatic and reproductive cells (Michod and Nedelcu 2003, Michod 2006, Michod *et al.* 2006). From a mechanistic point of view, we have argued that the evolution of germ-soma separation in multicellular individuals involved the co-option of molecular mechanisms underlying life history trade-offs in unicellular lineages, by changing their expression from a temporal into a spatial context (Nedelcu and Michod 2006, Nedelcu 2009).

Here, we discuss further this proposal using the volvoclean green algal group as a model system. First, we introduce the volvoclean algae and discuss the aspects of their life history that are relevant to the evolution of multicellularity in this lineage. Then, we briefly review what is known about the mechanistic basis of acclimation—a specific adaptive response to environmental changes that magnifies the survival–reproduction trade-off in these algae. We suggest that the molecular basis for this trade-off is independent of resource allocation. Instead, this survival–reproduction trade-off is mediated through photosynthesis, whose down-regulation has a pleiotropic effect on the two fitness components; that is, it promotes survival (through avoiding the production of damaging reactive oxygen species (ROS) at a cost to immediate growth and reproduction. Lastly, we propose a hypothesis for the evolution of somatic cells in which, by simulating the general acclimation signal (i.e., a change in the redox status of the cell) in a spatial rather than temporal context, a life history trade-off gene can be co-opted into a “specialization” gene. In particular,

we suggest that the gene responsible for the differentiation of sterile somatic cells in the multicellular alga, *Volvox carteri*, evolved from a gene involved in the down-regulation of photosynthesis during acclimation to stressful environmental conditions, as a means to ensuring survival at a cost to immediate reproduction.

21.2 The volvoclean green algal group

21.2.1 Overview

The volvoclean group comprises photosynthetic bi-flagellated green algae with discrete generations and a single reproductive episode that marks the end of the generation. The so-called “volvocine lineage” contains closely related unicellular (*Chlamydomonas*-like) and multicellular species that show an increase in cell number, volume of extra-cellular matrix per cell, division of labor, and ratio between somatic and reproductive cells (Larson *et al.* 1992; Fig. 21-1). Cell specialization evolved multiple times in this group, and the different levels of complexity among volvoclean species 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 (Larson *et al.* 1992, Herron and Michod 2008).

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. All volvoclean algae share the so-called “flagellation constraint” (Koufopanou 1994; Box 21-1); that is, due to a rigid cell wall, the flagellar basal bodies cannot move laterally and take the position expected for centrioles during cell division while remaining attached to the flagella. 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., up to five cell divisions).

The presence of a cell wall is coupled with the second conserved feature among volvoclean algae, which is their unique way of reproduction—namely, palintomy (Box 21-1). Volvoclean cells do not

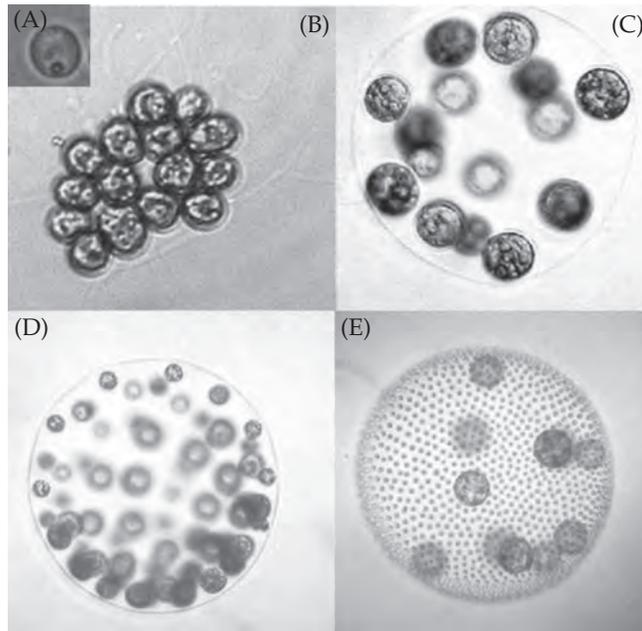


Figure 21-1 Unicellular (A, *Chlamydomonas reinhardtii*) and multicellular volvoclean algae without (B, *Gonium pectorale* and C, *Eudorina elegans*) and with (D, *Pleodorina californica* and E, *Volvox carteri*) cell differentiation (smaller cells are somatic bi-flagellated cells; larger cells are nonmotile reproductive cells).

double in size and then undergo binary fission. Rather, each cell grows about 2^n -fold in volume, then a rapid and synchronous series of n divisions (multiple fission) without intervening growth is initiated (under the mother cell wall) to produce 2^n small cells. Because clusters, rather than individual cells, are produced in this way, this type of reproduction is believed to have been an important precondition facilitating the evolution of multicellularity in this group (Kirk 1998). Indeed, while in the unicellular members of the group—such as *Chlamydomonas* (Fig. 21-1A; Box 21-1)—the daughter cells (2^2 – 2^4) separate from each other after division; in the multicellular species, the cluster of 2^n cells does not disintegrate, and coenobial forms (a type of multicellular organization in which the number of cells in the adult is determined by the number of cleavage divisions during embryogenesis; i.e., there is no increase in number of cells in the adult; Kirk 1998) are produced. For instance, in *Gonium*, the resulting cells (2^2 – 2^5) stay together and form a convex discoidal colony (Fig. 21-1B; Box 21-1), whereas in *Eudorina*,

Pleodorina and *Volvox* (Fig. 21-1C, D and E; Box 21-1), the cells (2^4 – 2^6 , 2^6 – 2^7 , and 2^9 – 2^{16} , respectively) form spherical colonies up to 3 mm in size.

The two selective pressures that are thought to have contributed to the increase in complexity in volvocine algae 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 activity (to access to the euphotic/photosynthetic zone and nutrients, and to achieve better mixing of the surrounding environment) (Bell *et al.* 1985, Sommer and Gliwicz 1986, Solari *et al.* 2006a,b). Interestingly, given the background imposed by the volvoclean type of organization presented above, namely the flagellar constraint and the palintomic mode of reproduction, it is difficult to achieve the two selective advantages—larger size and motility—simultaneously. Indeed, the larger the colonies (and the number of cells), the larger the mother cell and the number of cell divisions (up to 15–16 in some *Volvox* species). Consequently, the motility (and thus

survival) of a colony whose reproduction requires more than five cell divisions is negatively impacted during the growth and reproductive phase. In other words, the larger the size of the colonies, the more acute the trade-off between reproduction and viability.

The negative impact of the flagellation constraint can be overcome by cellular specialization/division of labor: some cells become involved mostly in motility, while the rest of the cells specialize 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 none in *Gonium* and *Eudorina*, to up to one-half in *Pleodorina* and more than 99% in *Volvox* (Larson *et al.* 1992) (Fig. 21-1). In *Volvox carteri*, the division of labor is complete (Fig. 21-1 E): the reproductive cells (gonidia)—which give rise to new daughter colonies—are set apart early during development; the somatic cells are terminally differentiated and undergo cellular senescence and death once the progeny is released from the parental colony (Kirk 1998). Overall, the present diversity in morphological and developmental complexity in the volvoclean algae reflects distinct strategies and solutions to the same set of constraints, selective pressures, and life history trade-offs (discussed further below).

21.2.2 Life history trade-offs and the evolution of multicellularity in volvoclean algae

Volvoclean algae are constrained by life history trade-offs that are similar to those in other lineages (e.g., the trade-off between reproduction and survival mediated through body size and developmental time). These trade-offs are also rather dynamic; they can change during development, in response to the environment, and can evolve. Notably, in this group, some of the common life history trade-offs, especially in relation to survival and reproduction, are amplified by developmental traits (i.e., palintomy), constraints (i.e., the flagellar constraint), and selective pressures specific to volvoclean algae (i.e., the need to maintain flagellar activity throughout their entire life-cycle).

As in other lineages in which fecundity increases with body size (e.g., most invertebrates, plants, and some small mammals), in unicellular volvoclean algae, fecundity is directly correlated to (and can be predicted from) body size at the end of the growth phase; that is, the number of offspring is dependent on the maternal body size, specifically on how many times the mother cell increases in volume. However, as attaining a large body size requires a longer time to maturity, survival rates may decrease as the time to reproduction increases. This trade-off between reproduction and survival mediated through body size and developmental time may therefore limit the increase in fecundity (Fig. 21-2A). In environments in which predation imposes a strong selective pressure, a large body size can also be beneficial in terms of survival (Morgan *et al.* 1980). In such cases, the survival benefits of a large body size can offset the survival cost incurred due to longer developmental times. Nevertheless, the decrease in the cell surface-to-volume ratio (which affects metabolic exchanges) puts a limit on the maximum size a unicellular organism can reach. Further survival benefits associated with escaping predators can only be achieved if the increase in body size is attained by forming multicellular groups—such as through the failure of the offspring (daughter cells) to separate at the end of cleavage (Fig. 21-2B). Indeed, it has been shown experimentally that predation by a phagotroph selects for eight-celled colonies in the green alga, *Chlorella vulgaris* (Boraas *et al.* 1998).

Such a scenario is postulated to have happened during the evolution of multicellularity in this group (Kirk 1998), and is reflected in the presence of colonial forms developed from a single mother cell—such as in the genera *Gonium* and *Eudorina*, comprised of up to 32 cells (Fig. 21-1; Box 21-1). However, an additional increase in size (and a potential increase in survival through avoiding a different class of predators) will be constrained by new trade-offs set up by the cost of maintaining, moving, and reproducing a large colony. For instance, as the flagellation constraint puts a limit on the number of cell divisions (i.e., five) that can take place without negatively affecting the survival of the colony during the reproductive phase, an increase in the number of cell divisions above five

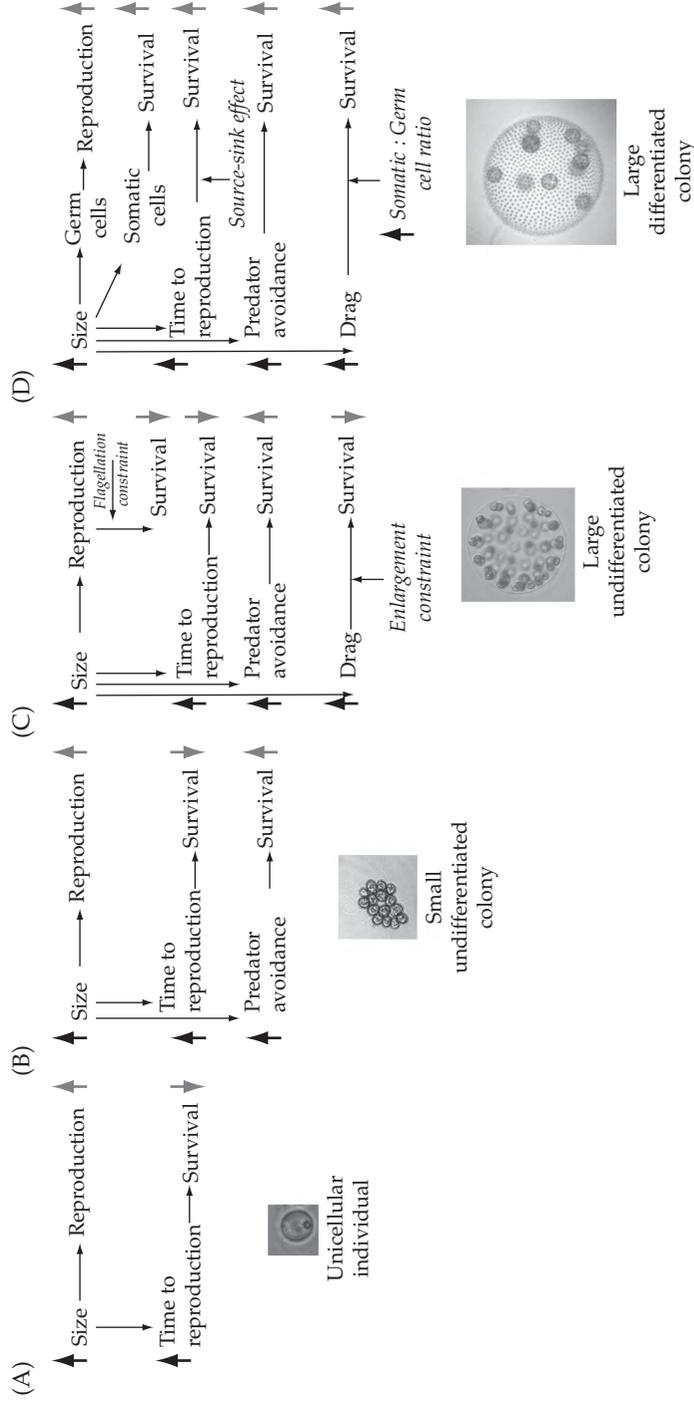


Figure 21-2 Life history trade-offs during the evolution of multicellularity in the volvocine lineage (see text for discussion). Solid black upward arrows denote an increase in the value of the life history trait or cost/benefit indicated on the right; gray upward and downward arrows, respectively, indicate positive and negative effects on the two components of fitness. A, Trade-offs in unicellular individuals. Basic survival–reproduction trade-off mediated through body size and time to reproduction in unicellular volvocine algae such as *Chlamydomonas*. B, Trade-offs in small undifferentiated multicellular colonies. The survival benefits of size in terms of predator avoidance can offset the survival cost of reproduction in terms of increased time to reproduction in small colonial volvocine (8–16 cells) algae such as *Gonium*. C, Trade-offs in larger undifferentiated colonies. Increased colony size increases the survival costs of reproduction via the flagellation and enlargement constraints in volvocine colonial species with more than 32 cells, such as *Pleodorina*. D, Release from trade-offs in species with differentiated somatic and germ cells. In large multicellular *Volvox* species, the survival costs of reproduction imposed by the flagellation and enlargement constraints and by the increased time to reproduction are alleviated through the specialization of somatic and germ cells, an increased somatic-to-germ cell ratio, and the source-sink effect.

(and an increase in size of the offspring above 32 cells) will result in the mother colony losing motility during the reproductive phase. Furthermore, due to their specific way of reproduction (autocolony, i.e., each cell in a colony will produce a daughter colony), each cell in the colony needs to grow 2ⁿ-fold in volume in order to produce a daughter colony of 2ⁿ cells. This massive increase in volume of all cells in the colony will impose a high cost on motility (and survival) as the mass (and the drag) of the colony will increase. This results in the motility of the colony being negatively affected even before the division phase (the enlargement constraint; Solari *et al.* 2006a,b). Thus, the survival cost of moving a large body size and the survival cost during the reproduction phase (imposed by the flagellation constraint) will likely offset any survival benefits of large size (such as escaping predators) (Fig. 21-2C). The trade-off between reproduction and viability becomes more and more acute as body size increases and at some point it becomes untenable to have further increases in body size, unless the trade-off can be broken and the two components of fitness (reproduction and survival) maximized independently.

The evolution of specialized sterile somatic cells, in colonies comprising more than 32 cells, released multicellular volvocalean algae from the constraints imposed by the survival–reproduction trade-off specific to this group (Fig. 21-2D). By not growing and by not reproducing, the somatic cells alleviate the survival costs of moving and reproducing a large colony, thus allowing the benefits of a large body size to be realized further. The benefits of soma specialization are multiple and include:

- colony motility while reproducing (overcoming the flagellation constraint)
- motility while large (overcoming the enlargement constraint)
- increased resource uptake due to the “source-sink” effect (in which somatic cells serve as the source and the germ cells act as the sink) (Bell *et al.* 1985, Koufopanou and Bell 1993)
- enhanced uptake of resources and removal of waste by flagellar beating (Solari *et al.* 2006a,b).

Germ specialization can also provide additional benefits, such as decreased generation time,

increased productivity by specialization at photosynthesis, and hydrodynamic advantages stemming from the location of germ in the interior of the colony (Solari *et al.* 2006b). The specialization of germ and soma also imposes some direct costs, as germ specialization reduces the number of cells available for vegetative functions (survival cost), and soma specialization reduces the number of reproducing cells (fecundity cost). However, these costs are overcome by the benefits of germ–soma separation, and are mediated by the ratio between the number of germ and somatic cells in a colony. The observed increase in the soma-to-germ ratio among species with increasing numbers of cells is thought to be a reflection of the trade-off between survival and fecundity imposed by the cost of reproduction as body size (in terms of number of cells) increases.

21.3 Mechanisms of life history trade-offs and the evolution of multicellularity in volvocalean algae

21.3.1 Overview

Trade-offs can occur between physiological traits (whether expressed at the same or at different times in the life cycle), and result from variations in genetic factors (pleiotropy), environmental factors, or combinations of these two types of factors (Zera and Harshman 2001, Chapter 24). As in other systems, little is known about the causal mechanisms underlying life history trade-offs (and how trade-offs are manifested or modulated at the genetic level) in volvocalean algae. A special case is the trade-off between survival and reproduction during the reproductive phase, which is mediated through the flagellation constraint. In this case, the functional basis of the trade-off between motility and cell division is understood in terms of competition for the same structure, as the microtubule-organizing centers act both as flagellar basal bodies and centrioles (Box 21-1). In other cases, trade-offs between survival and reproduction can be thought of in terms of adaptive allocation of limited internal resources. This might be especially true in cases where somatic and reproductive cells are connected through cytoplasmic bridges (such as in some

species of *Volvox*, e.g., *Volvox aureus*) and resources are believed to be transferred from somatic cells into gonidia (Kirk 1998). Differential allocation of limited internal resources to survival and reproduction can also be invoked during embryonic development in volvoclean species in which cell size at the end of embryonic cleavage is indicative of cell fate (e.g., *V. carteri*); that is, small cells are destined to become somatic cells while larger cells develop into gonidia (Kirk 1995). This strategy can be interpreted as a type of maternal control whereby internal resources are differentially allocated (during embryonic cell division) among cells that will specialize for either survival- or reproduction-related functions.

Reduced nutrient availability is known to substantially magnify, while increased nutrient availability can diminish (or obviate), an apparent trade-off; such plastic responses are thought to be determined by priority rules that govern the relative allocation of resources to organismal processes as a function of nutrient input (Zera and Harshman 2001). In some lineages, laboratory and field experiments showed that, under nutrient-poor or stressful conditions, allocation to maintenance or storage take precedence over allocation to reproduction (see Zera and Harshman 2001). For instance, in yeast, high nutrient conditions favor high levels of cellular cAMP and cell division, while nutrient-deprived environments trigger a drop in the concentration of cAMP and cells cease to divide (Eraso and Gancedo 1985). Yeast strains that cannot properly regulate cAMP level continue to divide during nutrient deprivation, and ultimately die due to starvation (Wilson and Tatchell 1988).

21.3.2 Acclimation and life history trade-offs in *Chlamydomonas*

In volvoclean 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 (Grossman 2000). 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. However, the mecha-

nistic basis underlying this trade-off is very different from that described above in yeast.

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 metabolic slowdown, and cessation of cell division (Grossman 2000, Wykoff *et al.* 1998). 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 (Pfanschmidt *et al.* 2009).

The down-regulation of photosynthesis is critical for sustaining cell viability under conditions of nutrient deprivation (Davies *et al.* 1996, Wykoff *et al.* 1998). 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 (Wykoff *et al.* 1998, Grossman 2000). Furthermore, because NADPH is not rapidly recycled (due to the slow-down 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 ROS. ROS are a series of partially reduced and highly reactive forms of oxygen, including the superoxide anion (O₂⁻), the hydroxyl radical (OH·), and hydrogen peroxide (H₂O₂). Although ROS are by-products of normal metabolism and can act as secondary messengers in various signal transduction pathways (e.g., see Van Breusegem *et al.* 2001, Mittler 2002, Eberhard *et al.* 2008; also see Chapter 15), increased intracellular levels of ROS (i.e., oxidative stress) can alter cellular functions and damage many biological structures, most importantly DNA (e.g., Marnett and Plataras 2001).

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 (Grossman 2000, Chang *et al.* 2005).

Several mutants that affect general acclimation responses have been isolated in *Chlamydomonas reinhardtii* (Davies *et al.* 1996, Chang *et al.* 2005, Moseley *et al.* 2006). These mutants are unable to down-regulate photosynthetic electron transport and die sooner than the wild-type. Notably, their death appears to be the consequence of the accumulation of photo-damage. Consistent with this suggestion is the fact that an electron-transport inhibitor that can induce the down-regulation of photosynthesis can rescue these mutants (Davies *et al.* 1996, Moseley *et al.* 2006). Moreover, if maintained in the dark, these mutants can survive nutrient deprivation as well as the wild-type strains do (Davies *et al.* 1996).

Overall, the available data suggest that, in *Chlamydomonas*, survival under nutrient limitation is primarily determined by the ability to down-regulate the photosynthetic electron transport to avoid light-induced oxidative damage. How is this accomplished? Changes in the status of the photosynthetic apparatus during acclimation to nutrient limitation result in changes in the redox potential of the chloroplast. Photosynthetic redox signals (including photosynthetically generated ROS) are then transduced in the chloroplast or cytosol and affect the expression of nuclear genes coding for various proteins involved in acclimation, including chloroplast light-harvesting proteins (Moseley *et al.* 2006, Pfannschmidt *et al.* 2009). Since, in *Chlamydomonas*, cell division is dependent on cell size (Umen and Goodenough 2001), and cell growth is dependent on photosynthesis, the down-regulation of photosynthesis in order to ensure survival will also suppress

cell growth and thus reproduction. In other words, the down-regulation of photosynthesis has a pleiotropic effect on two life history traits: it promotes survival at a cost to immediate reproduction. Therefore, the observed trade-off between survival and reproduction under nutrient limitation does not appear to be a direct consequence of resource allocation (although, as a side-effect, the inhibition of growth and reproduction might release resources that could, in theory, be re-allocated to survival-related functions).

Recently, we have identified in *C. reinhardtii* a gene—currently known as *rls1* (Duncan *et al.* 2007), which is induced under specific nutrient limitation (including phosphorus and sulfur-deprivation), during the stationary phase, as well as under light-deprivation (Nedelcu and Michod 2006, Nedelcu 2009). Furthermore, we showed that the induction of *rls1* coincides with the down-regulation of a nuclear-encoded light-harvesting protein (Nedelcu and Michod 2006) and with the decline in the reproduction potential of the population under limiting conditions (Nedelcu 2009). 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*. Supporting this suggestion is the finding that an inhibitor of the photosynthetic electron flow that triggers general acclimation-like responses (Wykoff *et al.* 1998) also induces the expression of *rls1* (Nedelcu 2009).

Thus, altogether, *rls1* appears to be specifically induced under environmental conditions where the temporary down-regulation of photosynthesis is beneficial in terms of survival though costly in terms of immediate reproduction. In other words, *rls1* might act as a life history trade-off gene (i.e., a gene that affects, in opposite ways, two life history traits, Bochdanovits and de Jong 2004).

21.3.3 The genetic basis for cell differentiation in *Volvox carteri*

V. carteri consists of approximately 2000 permanently biflagellated somatic cells and up to 16

nonflagellated reproductive cells. Terminal differentiation of somatic cells in *V. carteri* involves the expression of *regA*, a master regulatory gene that encodes a transcriptional repressor (Kirk *et al.* 1999), thought to suppress nuclear genes coding for chloroplast proteins (Meissner *et al.* 1999). Consequently, the cell growth (dependent on photosynthesis) and division (dependent on cell growth) of somatic cells are suppressed. Interestingly, the closest homolog of *regA* in *C. reinhardtii* is *rls1* (Nedelcu and Michod 2006, Duncan *et al.* 2007). *RegA*, like *rls1*, contains a SAND domain, which is found in a number of nuclear proteins, many of which function in chromatin-dependent or DNA-specific transcriptional control. 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 (Carles *et al.* 2005).

Mutations in *regA* result in the somatic cells retaining reproductive abilities, which in turn results in them losing their flagellar capabilities (e.g., Starr 1970, Kirk *et al.* 1987). As flagellar activities are very important for these algae, the survival and reproduction of *V. carteri* individuals in which such mutant somatic cells occur is negatively affected (Solari *et al.* 2006b). Although *regA* belongs to a gene family that comprises 14 members in *V. carteri* (Duncan *et al.* 2007), *regA* is currently known as the only locus that can mutate to yield Reg mutants (Kirk *et al.* 1999).

The expression of *regA* is strictly determined by the size of cells at the end of embryogenesis; cells below a threshold size develop into somatic cells (Kirk *et al.* 1993). Which cells express *regA* and differentiate into somatic cells is determined during development through a series of symmetric and asymmetric cell divisions. 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* (Kirk 1995). *RegA* is induced in very young somatic cells immediately after the end of embryogenesis, but is never expressed in gonidia (Kirk *et al.* 1999). 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 ele-

ments identified in three of the introns (i.e., two enhancers and one silencer) and act in concert to either silence or induce *regA* expression (Stark *et al.* 2001).

21.4 Co-opting mechanisms underlying environmentally induced life history trade-offs for cell differentiation

The evolution of specialized cells in multicellular volvocalean algae can be understood in terms of the need to break survival–reproduction trade-offs, such that survival and reproduction can be maximized independently and simultaneously. We have previously suggested that the evolution of soma in multicellular lineages involved the co-option 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). This, we suggested, happened through shifting their expression from a temporal (environmentally induced) into a spatial (developmental) context (Nedelcu and Michod 2004, 2006; Fig. 21-3). Furthermore, we have reported that the closest homolog of *V. carteri regA* in its unicellular relative, *C. reinhardtii*, is *rls1*—a life history gene that is involved in the general acclimation response to various environmental stresses (Nedelcu and Michod 2006, Nedelcu 2009).

How can general acclimation responses in unicellular organisms be co-opted 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 (e.g., Wykoff *et al.* 1998). 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 (e.g., Wykoff *et al.* 1998, Pfannschmidt *et al.* 2009; Fig. 21-4). Other environmental factors (e.g., cold, water stress) are also known to result in changes

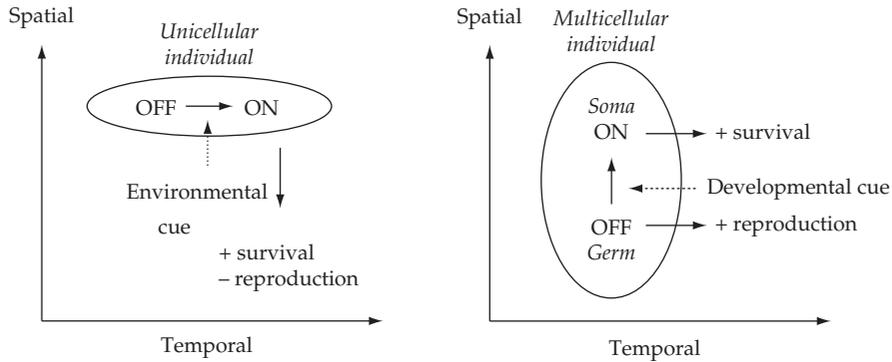


Figure 21-3 Schematic representation of the change in expression pattern from a temporal context (environmentally-induced) into a spatial context (developmentally-induced) of a life-history trade-off gene in a unicellular individual as it becomes a "specialization" gene in a multicellular individual (adapted from Nedelcu and Michod 2006).

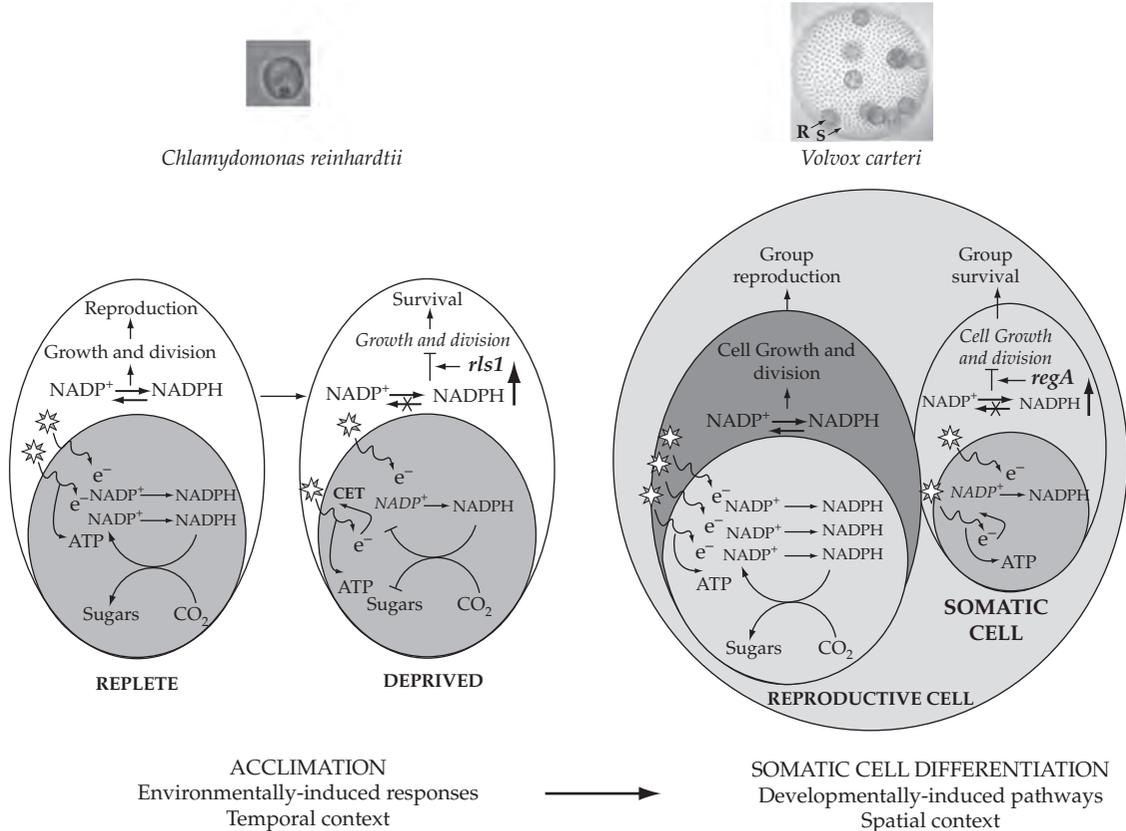


Figure 21-4 A model for the co-option of acclimation responses into somatic cell differentiation in *Volvox carteri* (R and S denote reproductive and somatic cells, respectively; see text for discussion). Although many components are involved, for simplicity, changes in redox status are symbolized by 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 (Eberhard *et al.* 2008) and possibly in *Volvox* somatic cells, is also indicated (adapted from Nedelcu 2009).

in the cellular redox status and trigger similar acclimation responses (e.g., Eberhard *et al.* 2008). 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.

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 (Kirk *et al.* 1993). 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) changes relative to the ratio in larger cells. Specifically, there will be an excess of membrane-bound proteins (Nedelcu 2009; Fig. 21-4). 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 intra-cellular redox status and the induction of acclimation-like responses, culminating in the suppression of division (Fig. 21-4). Supporting this scenario is the fact that cytodifferentiation in *V. carteri* is light-dependent (Stark and Schmitt 2002).

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. 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 in which volvoclean algae live (Kirk 1998), 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*; Reboud and Bell 1997), such environments are likely to be more conducive to the evolution of somatic cell dif-

ferentiation. In this context, it is noteworthy that cast differentiation in social wasps is also thought to have evolved in variable environments, and specific adaptations to seasonal environments that control sequential shifts between life-cycle phases (such as diapause; Chapter 18) have been proposed as prerequisites to the evolution of sociality in this group (Hunt and Amdam 2005).

21.5 Conclusion

Volvoclean algae are an excellent model system with which to study life history trade-offs from both evolutionary and mechanistic perspectives. They exhibit a diverse range of morphological, developmental, and life history traits, inhabit different habitats (soil, temporal water bodies, permanent lakes), and are subjected to various ecological pressures. In addition, the genomes of *C. reinhardtii* (Merchant *et al.* 2007) and *V. carteri* (Prochnik *et al.* 2010) have been recently sequenced, which will allow the integration of life history theory with genomics, facilitate the development of mechanistic models of life history trade-offs, and address the extent to which evolutionary trajectories are deterministic versus stochastic (Chapter 2). Perhaps the unique feature of this system is the possibility to address the evolution of life history traits and trade-offs during evolutionary transitions in individuality. Although we focused here on the transition from unicellular to multicellular life, we argue that similar principles are likely to apply to other evolutionary transitions as well.

21.6 Summary

1. Although life history trade-offs are generally thought to limit the possible set of trait combinations and can constrain the evolutionary trajectory of a lineage, during evolutionary transitions in individuality such trade-offs can be uncoupled by differentiation or specialization, and thus can promote evolution at the new level of organization.
2. Understanding the molecular mechanisms of life history trade-offs in solitary individuals is important for understanding evolutionary transitions in individuality and the evolution of new traits at the higher level (such as sterile somatic cells during the evolution of multicellularity and sterile castes

during the evolution of eusociality), in both proximate and ultimate terms.

3. Life history trade-offs can be caused by factors other than resource allocation. In volvocalean algae, the trade-off between survival and reproduction in nutrient-limited environments is mediated through photosynthesis, whose down-regulation has a pleiotropic effect on the two fitness components, that is, it promotes survival (by avoiding the overproduction of damaging reactive oxygen species) at a cost to immediate reproduction.

4. We propose that during the transition to multicellularity and the evolution of germ–soma separation, life history trade-off genes associated with ensuring long-term survival at a cost to immediate reproduction have been co-opted into “specializa-

tion” genes by changing their expression patterns from a temporal into a spatial context. In particular, we suggest that the gene responsible for the differentiation of sterile somatic cells in the multicellular alga, *Volvox carteri*, evolved from a gene involved in the down-regulation of photosynthesis during acclimation to stressful environmental conditions.

21.7 Acknowledgments

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