REVIEW

Germ–Soma Differentiation in Volvox

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Volvox carteri is a spherical green alga with a predominantly asexual mode of reproduction and a complete germ–soma division of labor. Its somatic cells are specialized for motility, incapable of dividing, and programmed to die when only a few days old, whereas its gonidia (asexual reproductive cells) are nonmotile, specialized for growth and reproduction, and potentially immortal. When a gonidium is less than 2 days old it divides to produce a juvenile spheroid containing all of the somatic cells and gonidia that will be present in an adult of the next generation. The first visible step in germ–soma differentiation is a set of asymmetric cleavage divisions in the embryo that set apart small somatic initials from their large gonidal-initial sister cells. Three types of genes have been found to play key roles in germ–soma specification. First a set of gls genes act in the embryos to shift cell-division planes, resulting in the asymmetric divisions that set apart the large–small sister-cell pairs. Then a set of lag genes act in the large cells to prevent somatic differentiation, while the regA gene acts in the small cells to prevent reproductive development. An inducible transposon was used to tag and recover some of these and other developmentally important genes. The glsA gene encodes a chaperone-like protein that, like another chaperone that is one of its putative binding partners, is associated with the cell division apparatus, although how this leads to asymmetric division remains to be elucidated. The regA gene encodes a somatic-cell-specific nuclear protein that appears to function by repressing genes required for chloroplast biogenesis, thereby preventing somatic cells from growing enough to reproduce. Somatic-cell-specific expression of regA is controlled by three intronic enhancers. © 2001 Academic Press

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"...Volvox displays the two essential features of all multicellular organisms: its cells become specialized, and they cooperate." (Alberts et al., 1994)

INTRODUCTION

Many distinguished 18th and 19th century naturalists, including Antoni van Leeuwenhoek (1700), Charles Bonnet (1762), Lazzaro Spallanzani (1769), Christian Ehrenberg (1838), and August Weismann (1892), realized that the spherical green alga known as Volvox exhibits certain essential features of multicellular organisms in such a diagrammatically simple form that it should provide instructive insights into the reproductive and developmental biology of the more-familiar (but much more complex) land plants and animals. Despite such early and high-profile interest in Volvox as a model, however, detailed studies of its reproduction and development remained elusive until the mid 1960s, when William Darden (then a graduate student in Richard Starr’s laboratory) finally figured out how to maintain a laboratory culture of Volvox for more than a few days or weeks and how to control its entry into the sexual reproductive pathway (Darden, 1966). Starr capitalized on these technical breakthroughs without delay and quickly circled the globe collecting and bringing into culture all of the recognized (and some novel) species of Volvox. After having analyzed key aspects of reproduction and development in many of these isolates (Starr, 1968), he concluded that the prime candidates for establishing a developmental–genetic model system were a mating pair of V. carteri strains that he had collected in Japan (Starr, 1970).

Among the features that caused Starr to single out those two particular strains of V. carteri for detailed study were the following:
• They have the most rapid rate of reproduction in the genus and are able to complete an asexual life cycle (which increases the population size ~16-fold) in less than 2 days. Thus they rapidly produce large clones of genetically similar individuals.

• Their asexual development can be synchronized and controlled with a light–dark cycle, such that one complete cycle is completed every 48 h. Thus, large populations of individuals at any developmental stage of interest can be generated at the same time every second day.

• Segregation of the germ and somatic lineages occurs in a much more visible manner and at a much earlier developmental stage than it does in most other species of Volvox.

• Spontaneous mutations with interesting developmental consequences occur with significant frequency.

• Many spontaneous mutants exhibit substantially modified patterns of germ–soma differentiation and/or reproductive behavior.

• Sexual reproduction can be triggered at will, making Mendelian analysis feasible.

Starr’s assessment of the potential utility of those two strains proved to be on target. Although several other isolates were studied in some detail between 1966 and 1981, none of those other isolates held sustained interest for investigators. Most important, although studies of more than a hundred mutations were reported in Starr’s favored strains during the first decade that they were studied (Starr, 1970; Sessoms and Huskey, 1973; Huskey et al., 1979), only one mutation has ever been described in any other isolate of Volvox [Vande Berg and Starr, 1971; see Kirk (1998) for an extended discussion of this point]. As a consequence, virtually all studies of Volvox development and all studies of Volvox genetics that were reported during the last quarter century were performed with derivatives of the strains of V. carteri from Japan that Starr identified as being worthy of special attention (Kirk, 1998).

THE ASEXUAL PHASE OF THE VOLVOX CARTERI LIFE CYCLE

Each asexual young adult of V. carteri consists of a monolayer of 2000–4000 small, biflagellate, somatic cells embedded in the surface of a transparent sphere of extracellular matrix (ECM) and ~16 large gonidia embedded in the ECM just beneath the somatic cells (Fig. 1).

Volvox somatic cells are highly specialized for motility, phototaxis, and chemotaxis and have a cytoarchitecture extremely similar to that of the most closely related unicell, Chlamydomonas reinhardtii. That is, each cell has an anterior pair of flagella, a posterior chloroplast that contains a conspicuous photosensitive eyespot in one of its anterior extensions, and a nucleus, Golgi elements, and various other organelles arrayed in predictable locations between the chloroplast and flagella. There is one important way in which Volvox somatic cells differ from Chlamydomonas cells, however: whereas the two flagella of a Chlamydomonas cell are oriented so that they beat in opposite directions (permitting the cell to perform “the breast stroke”), the flagella of a Volvox somatic cell are oriented so that they beat in parallel, toward the rear of the spheroid, and slightly to the right (Hoops, 1993). It is this beat direction that accounts for the fact that Volvox spheroids always spin to the left as they swim forward, and it also accounts for the fact that Linnaeus gave the alga the name Volvox, which means “fierce roller.” Studies of Volvox in the wild have shown that such flagellar activity can carry a spheroid on a prodigious diurnal journey to capture essential resources (Sommer and Gliwicz, 1986). Nevertheless, once somatic cells have fulfilled their motile functions, they undergo death and dissolution precipitously when they are just a few days old (Fig. 2) (Pomerville and Kochert, 1981, 1982).

Mature V. carteri gonidia are ~1000 times the volume of somatic cells and differ in other properties as much as they do in size. Gonidia never have functional flagella, so they are completely dependent on somatic cells to keep them up in the sunlight where they can perform photosynthesis (as they must do to survive). In contrast to the highly polarized cytoarchitecture of somatic cells, gonidia exhibit nearly perfect spherical symmetry, with a central nucleus surrounded by radial strands of cytoplasm that are separated by a radiating set of large vacuoles (Kirk, 1998). Patterns of RNA and protein synthesis in the two cell types are as distinctive as their visible features (Kirk and Kirk, 1983;

FIG. 1. Volvox carteri. Each asexual young adult spheroid like the one shown here contains a surface monolayer of many small, biflagellate somatic cells and ~16 large asexual reproductive cells (or “gonidia”) within a transparent sphere of glycoprotein-rich extracellular matrix (ECM).
Of greatest importance, however, is the difference in reproductive potential between the two cell types: whereas no one has ever found a way to force somatic cells to divide once they have begun to differentiate, gonidia can be prevented from dividing only by withholding energy or killing them. Gonidia act as stem cells: each of them divides to generate a juvenile containing a complete new cohort of gonidia and somatic cells (Fig. 2), as described next in more detail.

**Cleavage: Establishing the Germ and Somatic Lineages by Asymmetric Division**

Under the standardized culture conditions used to maintain synchronous development, a *V. carteri* gonidium becomes mature in a little less than 2 days, whereupon it initiates a program of 11–12 stereotyped cleavage divisions that are rapid and synchronous, occur in the absence of growth, and generate all of the cells that will be present in an adult of the next asexual generation (Starr, 1969, 1970; Green and Kirk, 1981, 1982). Under these conditions, cleavage begins 3–4 h before the end of a light period and is competed in the dark. Each division cycle takes ~35 min, and cleavage as a whole takes 6–7 h. Throughout embryogenesis all cells of the embryo are linked by hundreds of cytoplasmic bridges that form as a result of incomplete cytokinesis (Green et al., 1981).

The first five divisions of the embryo are symmetrical, resulting in a 32-cell embryo in which all the cells are the same size and shape (Fig. 3A). At the sixth division, however, 16 cells in the anterior hemisphere (the hemisphere that is adjacent to the overlying somatic cells) divide asymmetrically, producing large–small sister-cell pairs (Fig. 3B). Each large cell is a gonidial initial, destined to give rise to one gonidium, whereas its smaller sister cell (like each of the symmetrically dividing cells of the posterior hemisphere) is a somatic initial that will produce a clone of somatic cells. The gonidial initials continue to divide asymmetrically two more times, producing additional somatic initials each time (Fig. 3C). Then the gonidial initials stop dividing, whereas the somatic initials continue dividing until they have completed a total of 11 or 12 divisions.
Because of the combined effects of asymmetric division and a different number of divisions completed, gonidal initials are 30 times the volume of the somatic initials at the end of cleavage (Fig. 3D). This difference has important consequences because several lines of genetic and experimental evidence have all led to the conclusion that in V. carteri cell size at the end of cleavage determines cell fate (Pall, 1975; Kirk et al., 1993). Perhaps the most compelling of these lines of evidence is that when large cells were produced microsurgically in a region of the embryo that normally produces only somatic cells, every surviving cell that was larger than 8 \( \mu \text{m} \) in diameter developed as a gonidium that cleaved to produce an essentially normal offspring (Kirk et al., 1993). All such studies combined have led to the conclusion that any cell in the embryo that is larger than 8 \( \mu \text{m} \) in diameter at the end of cleavage—no matter where or how it was produced—will activate the gonidial program of gene expression and cytodifferentiation, whereas smaller cells will activate the somatic program. The mechanism by which V. carteri cells assess their size at the end of cleavage and transduce the result into a particular pattern of gene expression remains mysterious. But Volvox is certainly far from unique in this regard: all manner of cells exhibit size-dependent behaviors, although in only a few cases are the underlying transduction mechanisms understood.

**Inversion: Turning the Right Side Out**

By the end of cleavage the embryo contains all of the cells that will be present in an adult, although it is inside out with respect to the adult configuration: its presumptive gonidia are on the outside, and the flagellar ends of its somatic cells are all pointing toward the interior (Fig. 3D). This maladaptive arrangement is soon corrected by a morphogenetic rearrangement known as inversion, in which the embryo turns itself inside out in a gastrulation-like process, to assume the adult configuration (Figs. 3E–3H).
1977; Viamontes et al., 1979; Green et al., 1981; Kirk et al., 1982; Nishii and Ogihara, 1999) and have been reviewed recently elsewhere (Kirk and Nishii, 2001). Mutants blocked at different stages of inversion can be recovered quite readily (Kirk et al., 1982), suggesting that many gene products may play critical stage-specific roles during inversion. The transposon-tagging method described below is now being used to tag and recover inversion-specific genes (I. Nishii and D. L. Kirk, manuscript in preparation).

**Cytodifferentiation: Converting the Germ-Soma Potential to Reality**

By the end of inversion each juvenile spheroid contains the complete complement of cells that will be present in an adult, in their final configuration, but at that stage the cells differ in little more than size. For example, by the end of inversion (which occurs during the dark in synchronized cultures) cells of both sizes have a pair of very short, nonfunctional flagella. Little discernible change occurs in either cell type as long as darkness persists, but at the instant that the lights come on there is a dramatic change in the level and pattern of protein synthetic activity, which takes place on preexisting transcripts (Kirk and Kirk, 1985). (This abrupt transition in protein synthesis is thought to account for the fact that V. carteri development is so readily synchronized by a light–dark cycle.)

Initially the patterns of light-activated protein synthesis are extremely similar in presumptive somatic cells and gonidia (Kirk and Kirk, 1983), and presumably proteins that are involved in demolition of the cytoplasmic bridges are among their early products, because the bridges that have linked all cells throughout embryogenesis quickly begin to break down after the juveniles are illuminated. Certain ECM components are known to be among the very early protein-synthetic products, and timely initiation of ECM assembly is essential to prevent the spheroid from disintegrating when the cytoplasmic bridges break down (see next section).

Evidence of differential gene expression can already be detected in the cells before the cytoplasmic bridges break down (Tam et al., 1991) but this increases dramatically as soon as cytoplasmic continuity between the two cell types is lost, and the patterns of gene expression in the two cell types soon exhibit more differences than similarities at both the RNA level (Tam and Kirk, 1991a) and the protein level (Kirk and Kirk, 1983). The first visible differentiation occurs when the flagellar stubs of the gonidia are resorbed as somatic-cell flagella elongate and become functional (Coggin and Kochert, 1986). Then, as the somatic cells devote themselves to flagellar elongation, formation of eyespots, and ECM deposition, the gonidia devote themselves primarily to rapid growth. Although gonidia start out \( \sim 30 \) times as large as somatic cells, they also grow \( \sim 30 \) times as much as somatic cells, so that by the time they are ready to cleave they are about 1000 times as large as the neighboring somatic cells. We will return to that theme later.

**Construction of the ECM: Architectural Engineering at a Distance**

The ECM, which appears featureless in a live spheroid, consists of a surprisingly complex array of structural elements: several types of fibrous components assemble to form a boundary layer that is continuous over the surface of the spheroid (except where it is penetrated by flagella), whereas others form a honeycomb-like array of cellular compartments that hold the cells in fixed locations and orientations near the surface of the spheroid (Fig. 4), and yet other components form the deeper, noncellular regions (Kirk et al., 1986). The structural components of the ECM that have been analyzed to date are hydroxyproline-rich glycoproteins that are highly modular in their organization, that appear relatively amorphous in the electron microscope. For simplicity, only the proximal part of one flagellum is represented per somatic cell. Note that, although the gonidia never have functional flagella, they do have flagellar channels and peri-flagellar "hillocks" in the spheroid-boundary region that are quite similar to those of the somatic cells. This is because key components of this boundary region are laid down early in the period of cytodifferentiation, while the gonidia and somatic cells still have very similar short flagellar stubs (Hallmann and Kirk, 2000). A system of nomenclature used to identify all of the morphological elements of the ECM is given in Kirk et al. (1986).
is synthesized at the end of embryogenesis plays a critical role in organizing the ECM and orienting the somatic cells therein (Hallmann and Kirk, 2000). As a spheroid enlarges (first as a juvenile and then as a parental spheroid; see Fig. 2) each cellular compartment must be enlarged proportionately, with new components being inserted into its walls at considerable distances from the cell bodies. How such long-distance assembly processes are mediated and regulated remains a matter of mystery.

A number of inducible enzymes have also been discovered within the ECM (Amon et al., 1997; Hallmann, 1999; Hallmann et al., 2001). It is suspected that some of these are involved in resource acquisition and others in ECM remodeling, but in most cases their precise roles remain to be elucidated.

**THE SEXUAL PHASE OF THE VOLVOX CARTERI LIFE CYCLE**

The asexual cycle is the predominant mode of Volvox reproduction in nature. The sexual cycle is not really used as a mechanism of reproduction; its principal function is as a way of producing dormant, resistant cells that will be able to survive adverse conditions from the end of one growing season to the beginning of the next. (In the process, of course, it also provides an opportunity for genetic recombination.) Because V. carteri is haploid throughout the asexual phase, meiosis is not required as a preliminary to gamete formation; however, gametogenesis does require an additional round of asexual reproduction in which cleavage patterns are modified and in which the large cells that are produced by asymmetric division subsequently differentiate into eggs or packets of sperm (Fig. 5). Although both types of gametes can be produced by a single clone in some other species of Volvox (Starr, 1968), in V. carteri the sexes are genetically distinct, although they are morphologically indistinguishable in the asexual phase. As in other green flagellates, the diploid zygote produced by fusion of V. carteri egg and sperm develops into a dormant zygospore with a thick, impermeable cell coat that permits it to survive desiccation, freezing, and other environmental insults. When favorable conditions return, the zygospore undergoes meiosis to produce one viable germling and three polar bodies. The germling then cleaves to start a new asexual phase of proliferation.

**FIG. 5.** The sexual cycle of Volvox carteri. Male and female V. carteri strains are genetically distinct, but indistinguishable in the asexual phase. Their differences become apparent, however, after exposure to the sex-inducing pheromone. Under these conditions, gonidia of both sexes execute one final round of asexual embryogenesis, but with modified sex-specific patterns of asymmetric division that result in production of different numbers and types of germ cells. The female embryo produces ~48 large cells that will develop into eggs, whereas the male produces 64 or 128 large cells called androgonidia that will each execute an additional round of cleavage to make a packet of 64 or 128 sperm. The sperm then swim about in search of fertile females. Eggs that are successfully fertilized develop into diploid zygospores that constitute the overwintering form of the species in nature. When favorable conditions return, the zygospore undergoes meiosis to produce one viable germling and three polar bodies. The germling then cleaves to start a new asexual phase of proliferation.
Moreover, the sexual phase has a built-in amplification system of stupendous proportions. As each sexually mature male spheroid (∼10⁻⁶ L in volume) releases its sperm, it also releases enough pheromone to induce 500,000,000 asexual males and females in 10³ L of water to join in the orgy (Gilles et al., 1984). But how does such an orgy begin? There are two mechanisms: (i) The rate at which spontaneous mutations causing constitutive sexuality appear in male strains is 10-fold higher than the rate at which other mutations occur in either males or females (Callahan and Huskey, 1980; Weisshaar et al., 1984). (ii) Heat shock (as might occur in a vernal pond that is drying up in midsummer) causes all cells of both sexes to immediately make and release enough pheromone to trigger sexual development (Kirk and Kirk, 1986).

The V. carteri sexual pheromone is a 30-kDa glycoprotein that has been thoroughly characterized in terms of both primary sequence and secondary modifications (Mages et al., 1988; Balshüseemann and Jaenicke, 1990), and it has been expressed in active form in both yeast (Haas and Sumper, 1991) and mammalian cells (Jaenicke et al., 1993). However, its mechanism of action remains mysterious, despite the fact that it has been studied more than any other aspect of Volvox biology during the last quarter century (reviewed in Kirk, 1998; Hallmann et al., 1998). The enigmas uncovered by such studies are fascinating but beyond the scope of this review.

GENETIC ANALYSIS OF GERM-SOMA DIFFERENTIATION

Mutations affecting nearly every aspect of V. carteri development have been described (reviewed extensively in Kirk, 1998). Here, however, the focus is on the insights provided by three categories of mutants in which the germ–soma division of labor is specifically abrogated in one or both of the cell types.

The first such mutant discovered was what is now called a Reg (somatic-regenerator) mutant, in which the germ–soma division of labor is abrogated in the somatic cells (Starr, 1970). Embryogenesis proceeds normally in Reg mutants, and the small cells produced by asymmetric division appear to differentiate as normal somatic cells. Later on, however, Reg somatic cells “regenerate”: instead of entering the cell death pathway, they grow, dedifferentiate, and redifferentiate as fully functional gonidia that divide to produce juveniles with the same Reg phenotype. All of the >100 Reg mutations that have been analyzed map to the same locus, called regA (Huskey and Griffin, 1979; Kirk et al., 1999). Reg somatic cells not only are capable of redifferentiating as gonidia but also are capable of redifferentiating as gametes after they have been exposed to the sexual pheromone (Starr, 1970). In short, a regA mutation makes cells that are otherwise incapable of even dividing capable of executing all aspects of both asexual and sexual reproduction. By conventional genetic logic it follows that the wild-type regA product must act in somatic cells as a negative regulator of a process or processes that are essential for both asexual and sexual reproduction.

A Lag (late gonidia) mutation has the complementary effect: in Lag mutants it is the large cells produced by asymmetric division that behave as the small cells of Reg mutants do. That is to say, they differentiate first as large somatic cells and then they redifferentiate as (“late”) gonidia (Kirk, 1990, 1998). The four known lag genes apparently act in a common pathway because any combination of lag mutations results in the same phenotype. It follows that the product(s) of the lag pathway must normally act in presumptive gonidia to suppress all aspects of somatic cell differentiation.

Because regA mutations have no effect on the true gonidia, and lag mutations have no effect on somatic cells, Reg and Lag mutants each have one set of cells that are phenotypically wild-type. On the other hand, Gls (gonidialess) mutants have only one cell type: symmetric cell division proceeds normally in Gls mutants, although a switch to asymmetric division never occurs; all cells divide symmetrically until they are too small to develop as gonidia and thus they all develop as biflagellate somatic cells. This would be a lethal defect on a wild-type background, so Gls mutants are recovered and maintained in the presence of a regA mutation, which permits the somatic cells to take over the job of reproduction (Tam and Kirk, 1991b). Because there is no discernible defect in the cell division process per se in Gls mutants—only a failure to ever divide asymmetrically—it seems most likely that the products of the wild-type gls genes function to shift the cell-division apparatus away from the center of the cell, causing the cell to divide asymmetrically.

A working hypothesis that incorporates these three kinds of genes into a minimal genetic program for V. carteri germ–soma differentiation is diagrammed in Fig. 6. According to this hypothesis, the gln loci act to cause the production of large and small sister cells, then the regA gene acts in the small cells to repress the genes required for gonidial differentiation and reproduction, while the lag genes act in the large cells to repress the genes that are required for somatic differentiation.

Why might an organism use two negative regulators (rather than two positive ones) to program dichotomous differentiation of germline and somatic cells? A useful insight is provided by considering the evolutionary context. Molecular phylogenetic analyses of several different types confirm that “the volvocine algae” (Chlamydomonas reinhardii plus the family Volvocaceae) constitute a surprisingly recent radiation of very closely related green flagellates that span the entire range of complexity from unicellular Chlamydomonas to multicellular Volvox (Kirk, 1999). In all of the smaller members of this group (Chlamydomonas plus the small colonial forms such as Gonium, Pandorina, and Eudorina) in which there is a single cell type, all cells initially develop as biflagellate motile cells resembling Volvox somatic cells, and then all of those cells...
transform into asexual reproductive cells resembling Volvox gonidia that then execute a sequence of rapid cleavage divisions. This first-biflagellate-then-reproductive sequence clearly constitutes the ancestral program of development in the volvocine lineage. However, in *V. carteri* the ancestral program has been converted into a dichotomous program, apparently by addition of genes that generate two cell lineages, turn off the biflagellate half of the ancestral program in one lineage, and turn off the reproductive half of the ancestral program in the other lineage.

**MOLECULAR ANALYSIS OF THE GERM–SOMA PROGRAM**

Cloning and sequencing of *nitA*, which is the gene encoding nitrate reductase, and the only *V. carteri* gene for which it is possible to actively select both loss-of-function and gain-of-function mutations (Gruber et al., 1992), brought *V. carteri* into the molecular age. The *nitA* gene has provided both a trap for a transposon (Miller et al., 1993) that has proven useful for tagging and recovery of genes of interest and a selectable marker used in a nuclear transformation system (Schiedlmeier et al., 1994) that is now exploited for functional analysis of such genes.

**GlsA: A Chaperone for the Asymmetric-Division Dance**

The *V. carteri* transposon referred to above jumps so well, and in such a controlled manner, that it was named Jordan (Miller et al., 1993). It is relatively stable under optimum growth conditions but its transposition rate can be increased as much as 40-fold by a mild stress, such as low-temperature growth. One of the first ways Jordan was exploited was to tag and recover *glsA*, a gene required for asymmetric division (Miller and Kirk, 1999). The GlsA protein encoded by this gene contains four putative protein-binding domains, including a highly conserved J domain that is a characteristic feature of the Hsp40 class of chaperones. Like a human J protein with 60% sequence similarity (mitotic phosphoprotein 11) (Matsumoto-Taniura et al., 1996), GlsA is associated with the mitotic spindle in dividing cells (Miller and Kirk, 1999).

The only known function of a J domain is as a binding site for a chaperone of the Hsp70 class. The J protein then determines where its Hsp70 partner will localize in the cell, what substrates it will bind to, and what type of functions it will execute (Kelley, 1998). An Hsp70 is required for normal centrosome function and mitotic spindle assembly in mammalian cells (Brown et al., 1996), and it has now been established that an Hsp70 co-localizes with GlsA to the *V. carteri* cell-division apparatus (S. M. Miller, personal communication). Furthermore, the Volvox Hsp70A gene has recently been cloned, and efforts are underway to determine the mechanism by which a GlsA–Hsp70 complex may act (probably in conjunction with other, yet-to-be-identified binding partners) to shift the cell division plane from the center of the cell to one side (S. M. Miller, personal communication).

**RegA: Terminal Differentiation via Active Repression of Growth**

The transposon Jordan was also used to tag and recover the *regA* gene (Fig. 7), which was then found to encode a somatic-cell-specific nuclear protein, RegA, with features of an active transcriptional repressor (Kirk et al., 1999). This result, which is consistent with the model presented in Fig. 6, raised the question of what the targets of RegA regulation are.
might be. Earlier, a differential cDNA screen (Tam and Kirk, 1991a) led to the identification of 18 genes with expression patterns that made them reasonable candidates as targets of RegA repression: their transcripts are accumulated to high levels in gonidia and regA::somatic cells, but not in regA::somatic cells (Tam and Kirk, 1991b). Sequencing revealed that all 16 of these genes that encode recognizable products fall into the same category: they are nuclear genes encoding chloroplast proteins (Choi et al., 1996; Meissner et al., 1999). Their products include proteins required for most of the major metabolic activities of the chloroplast (such as light harvesting, water photolysis, electron transport, ATP generation, the Calvin cycle, protein synthesis, etc.). In short, either by direct or indirect action, RegA prevents expression of genes whose products are essential for chloroplast biogenesis and maintenance.

The significance of this finding arises from the fact that Volvox is an obligate photoautotroph in which cell growth is photosynthesis limited and, hence, chloroplast limited. During cleavage, each presumptive somatic cell inherits <0.05% of the chloroplast that was present in the precleavage gonidium. Obviously, it must be enough chloroplast to supply the energy required for cellular maintenance and flagellar activity. But unless somatic cells were able to enlarge their chloroplasts, they probably could not grow, and if they could not grow, they obviously could not reproduce. So the secret underlying the fact that a single gene, regA, is able to block both asexual and sexual reproduction appears to be that what its product actually inhibits is cellular growth, which is a necessary prerequisite for any type of reproduction. Because it is known that nuclear and chloroplast genes required for chloroplast biogenesis are controlled coordinately by a complex set of interactions and feedbacks (Goldschmidt-Clermont, 1998), it remains to be determined which genes are the direct and which are the indirect targets of RegA regulation.

Expression of regA is tightly controlled in a cell-type-specific manner: transcripts are present in somatic cells through most of the life cycle but never in gonidia. This expression pattern has recently been ascribed to cis-regulatory elements in three of its seven introns (Stark et al., 2001). Introns 1, 2, 4, and 6 (Fig. 7) can be removed with no effect, but introns 3 and 5 both must be present for regA to be expressed in somatic cells. The elements in these introns fit the classical definition of enhancers: they function in a position- and orientation-specific manner. So does the promoter-specific element in intron 7, although it acts as a negative regulator (a “silencer”) that is required to prevent expression of regA in gonidia. When regA is expressed in gonidia, either because intron 7 has been removed from it or because its coding region has been put under the control of a constitutive promoter, gonidal growth is strongly inhibited and the strain quickly dies out (Stark et al., 2001), consistent with the hypothesis that the function of regA is to inhibit processes essential for cellular growth.

**FUTURE CHALLENGES**

Volvox has begun to reveal some of the secrets underlying its simple germ–soma division of labor, but many areas for future investigation remain. Among these one should include (i) cloning of the lag genes and analysis of the way their products act to cause presumptive gonidia to bypass the first half (the motile phase) of the ancestral developmental pathway, and (ii) resolution of the mysteries that still surround the process of sexual induction. Some of the most interesting remaining questions, however, involve evolutionary issues: What are the evolutionary origins of the genes that control germ–soma differentiation in V. carteri? Have the species of Volvox that are now known to have arisen independently of V. carteri, but from similar ancestors (Kirk, 1999), evolved a similar or entirely different regulatory strategy to program their germ–soma division of labor?

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