

Exploring germ-soma differentiation in *Volvox*

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1. Introduction

As we sat at the bowrail sipping Bloody Marys in the early morning fog 30 years ago, we had the feeling of being suspended in space and time. With the engines of the Delta Queen – the last authentic sternwheeler on the Mississippi river – working at full throttle, the downstream rush of the fall flood waters was so powerful that our upstream movement was barely perceptible. We had planned this trip months earlier, just as a weekend immersion in river nostalgia. But the timing turned out to be perfect: it provided us three days away from ringing telephones and fretting students, and a wonderful opportunity to discuss our long-range scientific goals in a leisurely manner, and to decide which path to take at the fork in the road that we had now reached.

For several years we had been frustrated by our limited ability to address fundamental issues of cellular determination and development in the avian and mammalian models with which we had been working, distressed by the competitiveness and rancor within the particular branch of developmental biology in which we found ourselves, and anxious to identify more rewarding alternatives. With that mindset, we had spent several periods at Stanford's Hopkins Marine Station with Don Abbott, an enthusiastic and exceptionally knowledgeable invertebrate zoologist, looking for interesting and accessible new "developmental models" in the intertidal zone. Although we had identified candidate organisms that we could keep indefinitely in artificial-salt water aquaria at Hopkins, every attempt to keep them alive more than briefly in St. Louis aquaria had failed. So we had turned our attention (somewhat ruefully) to organisms that were less averse to living in the US heartland.

Germ-soma specification and differentiation had long interested us, and at that time *Drosophila*, with its existing stock of *grandchildless* mutants, appeared to provide the most promising avenue for elucidating the genetic and molecular basis of germ cell determination. Because

all offspring of a homozygous *grandchildless* mother lack germ cells, we assumed that the wild-type allele of *grandchildless* probably encoded an essential component of the polar plasm, which had been shown to be capable of triggering germ cell formation (Ilmensee and Mahowald 1974). Therefore, we had been on the verge of applying for the opportunity (and the funds) to spend a sabbatical year in Switzerland with Walter Gehring, to begin studying fruit fly germ-cell biology.

Just as we were about to start writing those proposals, however, Richard Starr visited our department to talk about an organism we had last heard of in high school biology class: *Volvox*. Although leading biologists had been fascinated by *Volvox* since 1700, the first person to succeed in keeping any species of *Volvox* alive in the laboratory for more than a few weeks was one of Starr's first doctoral students, Bill Darden (1965). That breakthrough had so fired Starr's imagination that he had promptly circled the globe to bring into continuous culture, and study, all 18 known and several previously unknown species of *Volvox*. His seminar focused on asexual and sexual development in a male-female pair of *V. carteri* forma *nagariensis* Iyengar¹ strains that he had isolated from a pond in Japan in 1967, and that he subsequently found had "an unusual combination of characteristics" that made them particularly suitable for developmental genetic studies (Starr 1970). The beautiful time-lapse movies of *V. carteri* development that he had projected, and the fascinating developmental mutants that he had described, were still fresh in our minds as we boarded the Delta Queen. Thus, our major preoccupation as we headed upstream was enumerating the pros and cons of *Volvox* vs *Drosophila*

¹Professor M O P Iyengar, a preeminent expert on the green algae in the order Volvocales, and particularly those that are found on the Indian sub-continent (Iyengar and Desikachary 1981), first described this forma (subspecies) of *Volvox carteri* (Iyengar 1933) and provided criteria for distinguishing the various formas of *V. carteri* from one another.

as a system for studying germ-soma determination and differentiation.

Somewhere along the way, as we caught sight of a lone fisherman standing in one of the few breaks in the walls of trees that flank much of the upper Mississippi, Dave was reminded of the advice that his undergraduate research mentor, Jerome Gross, had given him as he left for graduate school: “If you want to be happy in science, find a backwater where there are plenty of big fish to be caught, but where very few people are fishing.” Although there were far fewer folks fishing in the fruit fly waters 30 years ago than there are now, we were certain that even then the banks of the *Drosophila* gene pool were much more crowded than those surrounding *Volvox*.² So . . .

Our discussions continued throughout the weekend, but as soon as we returned to St. Louis we called Richard Starr and arranged to visit him at Indiana University, where he had established the world-class Indiana University Culture Collection of Algae (later to become the UTEX Culture Collection of Algae after he moved to the University of Texas). During our visit, we found Richard to be exceptionally warm and generous: the perfect “Southern gentleman” that his family in rural Georgia had raised him to be, and that he remained until the day he died (in 1998). During that visit he provided us not only with the *Volvox* cultures we had requested, but also with a decade of accumulated insights on how to maintain and work with the organism.

In the intervening 30 years we have looked back on our weekend on the Delta Queen with fondness, and we have never regretted our decision to take “the road less traveled by.”

2. Germ-cell specification in *V. carteri*: a matter of cytoplasmic quality or quantity?

The thing that appealed to us most about *V. carteri* – in addition to the genetic accessibility that Starr (1970) had already demonstrated – was the fact that it presented the germ-soma dichotomy in such a clear and simple form. Each asexual adult (or “spheroid”) of *V. carteri* contains only two cell types: small, biflagellate somatic cells, and large asexual reproductive cells, called gonidia (figure 1). The somatic cells are mortal; once they have provided the organism with motility for a few days they die. The gonidia, in contrast, are potentially immortal; each mature gonidium acts as a stem cell, dividing to produce a juvenile organism containing a new cohort of gonidia and somatic cells. No one has ever found a way to make wild-type

somatic cells divide, but the only way to prevent gonidia from dividing is by withholding energy or poisoning them. Who could ask for a clearer presentation of one of the central issues of developmental biology: how are cells with extremely different phenotypes produced from the progeny of a single cell?

A mature gonidium gives rise to all the cells of both types that will be present in an adult spheroid by initiating a rapid set of synchronous cleavage divisions, certain of which are visibly asymmetric and generate the large–small sister-cell pairs that are the source of the germ and somatic lineages, respectively (Starr 1969, 1970). Under optimum culture conditions, *V. carteri* embryos first cleave symmetrically five times to form a 32-cell embryo in which all cells are similar in size and shape, and then the 16 cells in one hemisphere divide asymmetrically to produce one large gonidial initial and one small somatic initial each (figure 2). After the gonidial initials divide asymmetrically two more times (throwing off additional somatic initials at each division), they withdraw from the division cycle while the somatic initials divide symmetrically about three more times. As a consequence of the asymmetric divisions, plus the different number of divisions completed, by the end of cleavage the gonidial initials are ~ 30 times the volume of the somatic initials.

This raises an obvious question: is germ-cell specification in *V. carteri* a result of the visible difference in the

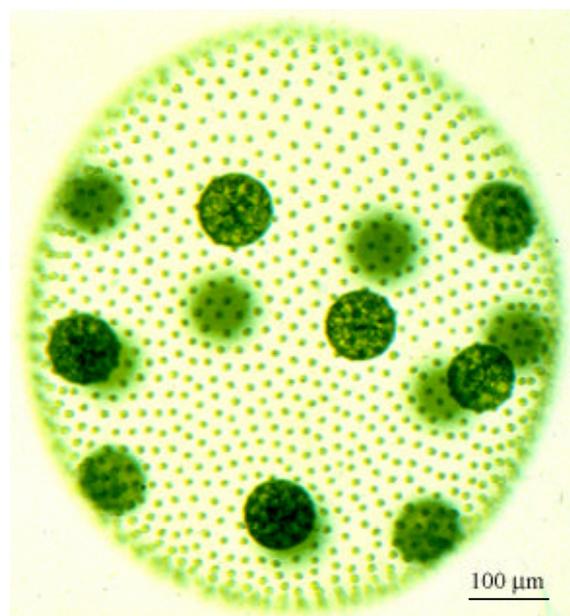


Figure 1. A young adult spheroid of *V. carteri* consists of thousands of small, biflagellate somatic cells that are embedded at the surface of a transparent sphere of extracellular matrix, and about 16 large asexual reproductive cells, called gonidia, that are located just internal to the somatic cells.

²As far as we know there has never been a time when the number of labs around the world that were actively studying *Volvox* was greater than six.

quantity, or some invisible difference in the quality of the cytoplasm that the presumptive gonidia possess? Shortly after we began to work with *V. carteri*, two symposium papers appeared that provided diametrically opposed answers to the preceding question.

Martin Pall (1975) concluded that a quantitative difference in cytoplasm was the basis for germ-soma specification in *V. carteri*. He observed that embryos of three independent premature cessation of division (*pcd*) mutants formed normal numbers of presumptive somatic cells and gonidia via asymmetric division, but then all of the cells stopped dividing after completing only six or seven division cycles, instead of the usual 11 or 12 cycles. As a result, when division ceased, the presumptive somatic cells were distinctly subnormal in number, but above normal in size. These embryos typically developed into adults that had excessive numbers of gonidia as well as a profound deficiency of somatic cells. For example, one embryo that divided only six times turned into an adult with 50 gonidia and only 14 somatic cells. Based on these observations, and his measurements of cell diameters at the end of cleavage, Pall drew two conclusions: (i) that cell size “or something closely related to cell size” (such

as the surface-to-volume ratio, or the cytoplasmic-to-nuclear ratio) is critical for germ-cell specification, and (ii) that a cell diameter of 8 μm constitutes a threshold, above which cells will develop as germ cells.

In contrast, Gary Kochert (1975) concluded that a qualitative difference in cytoplasm was the basis for germ-soma specification. In his review he summarized published studies (Kochert and Yates 1970) indicating that if spheroids were unilaterally UV-irradiated, the gonidia within those spheroids often produced progeny with missing gonidia. He also reported that gonidia in which the cytoplasm had been rearranged by centrifugation produced spheroids with an altered distribution of gonidia. Thus, he proposed that germ cell specification in *V. carteri* was mediated by a particulate, UV-sensitive morphogen, analogous to the “germinal plasm” of frogs (Smith 1966), or the “polar plasm” of insects (Ilmensee and Mahowald 1974), that was prelocalized in those regions of a gonidium from which the next generation of germ cells would be derived.

Emotionally, we favoured Kochert’s interpretation, because one of our motivations for getting into *Volvox* research was a hope that by isolating a *Volvox* germ-cell determinant, and establishing its mechanism of action, we might shed light on the mechanism of germ-cell determination in animal embryos. Intellectually, however, we realized that the evidence supporting the existence of such a germ-cell determinant was less than compelling. Kochert and Yates (1970) had UV-irradiated sizable groups of randomly oriented spheroids, and then had examined gonidial distribution patterns in randomly selected members of the next generation. Thus, they had no way of determining whether there was any correspondence between the region of an offspring from which gonidia were missing and the region of its parental gonidium that had received the highest level of UV irradiation. To eliminate that shortcoming, we followed the development of the progeny of individual gonidia that had been irradiated in known orientation. To our disappointment, we found that there was no necessary relationship between the regions of a gonidium that had received the highest UV dose, and the regions of the offspring from which germ cells were missing. No matter where the gonidium had received the greatest UV dose, members of one particular tier of four gonidia (near the equator) were most likely to be missing in the resulting offspring (our unpublished results). An explanation for this initially baffling outcome became apparent several years later, when the same spatial pattern of gonidial deficiencies was reported for spheroids that had developed in crowded or nutrient-depleted cultures (Gilles and Jaenicke 1982). It seems that there is a predictable sequence in which blastomeres that would divide asymmetrically under optimum conditions are most likely to fail to divide asymmetrically under any set of suboptimal or stressful conditions.

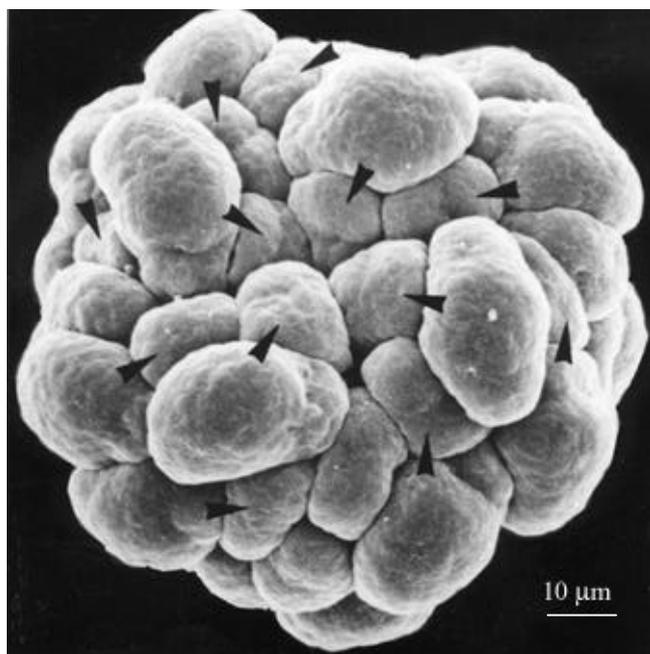


Figure 2. A 64-cell *V. carteri* embryo. The arrowheads connect large–small sister-cell pairs that have just been produced by the first asymmetric cleavage division. Incipient cleavage furrows can be seen near the counter-clockwise ends of several of the large cells, indicating that a second round of asymmetric divisions is about to occur. Each large cell will produce one gonidium while each small cell will produce a clone of somatic cells.

We also failed to find any conditions under which centrifugation changed the spatial distribution of gonidia without also causing other major abnormalities. So, lacking any very promising way to address the problem of germ-soma specification, we pushed that problem toward the back burner, and began concentrating on development of tools to analyse the details of germ-soma differentiation (a problem that held our attention for several years).

When we did eventually return to the germ-cell specification problem, we focused on trying to falsify Pall's "cell-size" hypothesis, rather than seeking additional support for Kochert's "germ-plasm" hypothesis. Ironically, however, the harder we worked to falsify Pall's hypothesis, the more support we generated for it – and not just for his general concept that in *V. carteri* cell size determines cell fate, but also for his more specific conclusion that a cell diameter of ~ 8 µm constitutes a threshold, above which *V. carteri* cells will develop as germ, and below which they will develop as soma. Of the six sets of experimental approaches and results that we and our associates reported (Kirk *et al* 1993), the three that seemed to us to be most persuasive were the following: (i) Wild type embryos were heat-shocked to arrest cleavage at various stages. When cleavage stopped at the 128-cell stage, which is after two rounds of asymmetric division (and thus after any "germ-cell determinants" should have been segregated to the gonidial initials) but while most somatic initials are still ~ 8 µm in diameter, adults were produced that contained as many as 124 gonidia and very few somatic cells. However, embryos in which cells completed just one more round of division produced adults with normal numbers of gonidia (although they still had severe somatic cells deficiencies). (ii) A gonidialess/somatic regenerator double mutant, in which all cells routinely develop first as somatic cells, and then later redifferentiate as gonidia (Tam and Kirk 1991b) was heat-shocked to interrupt cleavage at various stages. In embryos in which division stopped while all cells were still above 8 µm in diameter, all of them developed directly into gonidia. In contrast, if they divided one more time, so that all of the cells were below 8 µm in diameter, all of them developed as somatic cells that redifferentiated as gonidia a day later. (iii) Andy Ransick, a visiting scientist who had previously performed similar experiments in a related species, *V. obversus*, produced large cells microsurgically in a region of the embryo where normally only somatic cells develop. All cells > 8 µm in diameter that survived Ransick's surgical procedure developed as gonidia, despite the fact that all of the cytoplasm that they contained was cytoplasm that would normally be found in somatic cells (Kirk *et al* 1993). Thus, despite our initial bias, we were driven to the conclusion that Pall (1975) was correct, and that in *V. carteri* any cell that has a sufficient quantity of cytoplasm at the end of cleavage becomes a germ cell

rather than a somatic cell. There is as yet no evidence that any special type of cytoplasm is required for this to occur.

3. The genetic control of germ-soma differentiation in *V. carteri*

Within a year after Richard Starr had described normal development in the *V. carteri* forma *nagariensis* strains that he had isolated in 1967 from a pond in Japan (Starr 1969), he described several spontaneous mutants that had interesting abnormalities of asexual and/or sexual development, established that Mendelian analysis of such mutants was feasible, and indicated that these were clearly the *Volvox* isolates that would be most amenable to a detailed developmental-genetic analysis (Starr 1970). So, naturally, when Robert Huskey decided to switch from T-even bacteriophage genetics to *Volvox* genetics, he used the strains that Starr recommended, and soon had recovered an additional assortment of interesting developmental mutants (Sessoms and Huskey 1973). As a result of those early successes, all of the genetic studies, as well as nearly all of the non-genetic studies of *Volvox* that have been reported in the past 35 years have employed descendants of those same two isolates from Japan.

The mutant that most excited our interest when we first learned of Starr's pioneering work was one that he called "the fertile somatic cell mutant." In this mutant, which later became known as the "somatic regenerator" or "Reg" mutant, the somatic cells appeared to develop normally at first, but then they dedifferentiated and redifferentiated as fully functional gonidia that divided to produce progeny with the same phenotype (figure 3). Furthermore, Starr (1970) showed that under conditions that induce sexual reproduction, Reg somatic cells can produce gametes. Thus, somatic cells, which normally would have no reproductive potential whatsoever, gained the capacity for both asexual and sexual reproduction as a result of a single mutation. It was immediately obvious that the wild-type allele of this gene must act as what would now be called a "master control gene" that prevents all aspects of reproductive development in somatic cells. A developmental biologist's "dream gene"! Later Huskey and Griffin (1979) found that all 39 Reg strains that they analysed had mutations that mapped to the same locus, which they named *regA*. This appeared to indicate that *regA* exerted its profound effect on somatic cell development more or less single-handedly.

Naturally, from the moment we first heard about Reg mutants we were extremely curious how a single gene could exert such sweeping control of both the asexual and the sexual reproduction pathways. But in that pre-recombinant-DNA era, it never occurred to us to dream that we would live long enough to get the *regA* locus in a bottle

(so to speak) and analyse its nucleotide sequence and its molecular mechanism of action.

For several years after we began working with *Volvox* we had a very rewarding, collegial relationship with Bob Huskey, in which we regularly exchanged information, strains, methods, etc. but were perfectly happy to leave the formal genetics of *Volvox* to him and his associates (Huskey *et al* 1979a), just as he was happy to leave the cell biology and biochemistry of *Volvox* to us (Kirk *et al* 1982; Kirk and Kirk 1983). However, when Bob succumbed to the siren call of academic administration and eventually left research in the mid 1980's, we realized that if the genetic analysis of *Volvox* development was not going to be allowed to die out, we would probably have to resuscitate it ourselves. So we expanded our genetic activities greatly, eventually devoting a major portion of our resources to projects ranging from mutant screens to development of a moderately detailed linkage map of the genome (Kirk 1998).

Naturally, as we took up the genetics baton, one of our first goals was to define the genetic program for germ-soma differentiation. So we set about identifying as many mutants as possible in which the germ-soma division of labour was abrogated in one or both cell types. Of the dozens of interesting mutant categories that we subsequently identified, only three met that criterion. These were: (i) The now-familiar Reg mutants, in which the small cells produced by asymmetric division develop

initially as motile, biflagellate cells and then redifferentiate as functional gonidia. (ii) Lag (late gonidia) mutants, in which the large cells produced by asymmetric division develop initially as biflagellate cells and then redifferentiate as gonidia. By the same sort of logic used above for the *regA* locus, we concluded that the function of the wild-type *lag* loci must be to prevent development of all somatic-cell features (such as flagella and eyespots) in presumptive gonidia. (iii) Gls (gonidialess) mutants in which embryos at the end of cleavage have no cells large enough to activate the gonidial program of development – because there are no asymmetric cleavage divisions – and so all cells develop as somatic cells. (Obviously the absence of gonidia would be a lethal defect on a wild type background, so Gls mutants are isolated and maintained on a Reg background in which the somatic cells redifferentiate as reproductive cells; Tam and Kirk 1991b.) The function of the *gls* loci appears to be to shift the division plane from the middle of the cell to one side at certain times and places, thereby producing the large and small cells that will activate the *lag* and *regA* loci, respectively.

These three sets of gene functions were then combined to form a working hypothesis regarding the genetic program for germ-soma differentiation (figure 4) (Kirk 1988). We knew very well at that time that there were many other genes involved in this program, including, for example, at least four “pattern-forming” genes that determine in which cells and during which cleavage cycles the *gls* genes will

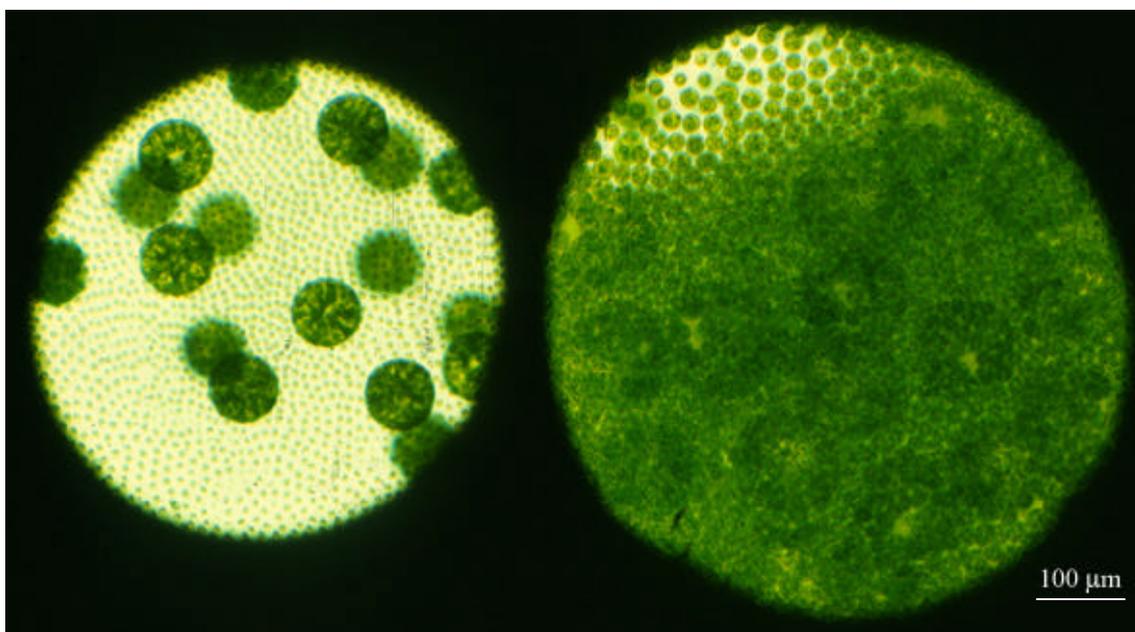


Figure 3. A somatic-regenerator, or Reg, mutant. In young adults of this strain (left) the somatic cells appear quite normal, but later on (right) instead of dying, these cells become darker green, enlarge, and transform into gonidia that will eventually divide to produce offspring of similar phenotype.

act – and thereby determine the number and locations of gonidia that will be formed (Kirk 1990, 1998). Nevertheless, the hypothesis diagrammed in figure 4 has held up well as a skeletal model for genetic control of the germ-soma dichotomy.

It might seem surprising to one unfamiliar with the volvocine green algae that a program for germ-soma differentiation would involve two kinds of negative regulators: one that blocks reproductive functions in presumptive somatic cells and another that blocks somatic functions in presumptive gonidia. Why not two positive regulators that activate somatic development in prospective somatic cells and activate reproductive development in prospective gonidia? The answer appears to lie in the evolutionary history of *Volvox*. All of the various species of *Volvox* are recently evolved members of the family Volvocaceae of green flagellates (Nozaki 2003), in which members of all of the basal genera contain a single cell type. In these more primitive volvocaceans with a single cell type, every cell first goes through a motile, biflagellate phase in which it grows 2^n -fold, and then redifferentiates and enters a reproductive phase in which it divides rapidly n times to

produce a daughter colony containing 2^n cells. Thus, the ancestral developmental program for volvocine algae is a biphasic (“first biflagellate and then reproductive”) program. But in *V. carteri* this biphasic program has been converted to a dichotomous one by the addition of two kinds of negative regulators: the product of the *regA* gene blocks the reproductive half of the ancestral program in somatic cells, while the products of the *lag* genes block the biflagellate half of the ancestral program in gonidia.

4. A fruitful trans-Atlantic collaboration in *Volvox* molecular genetics

By the mid 1980’s the recombinant-DNA bandwagon was really rolling, and we were not anxious to be left behind. Among other things, we had begun a project to clone the *regA* gene via a chromosome walk from a linked restriction fragment polymorphism. However, before long a variety of unforeseen technical difficulties had made this project feel more like a tortuous crawl through quicksand than a brisk walk in the park. Moreover, with Bob Huskey having switched his focus from research to administration, we had no colleagues with whom we could talk shop, and were beginning to feel very isolated and discouraged. Just about that time, however, we learned that Rudy Schmitt, a bacterial geneticist/molecular biologist in Regensburg, Germany (figure 5) had recently developed interests in *Volvox* that complemented our own. After initial contacts had been made, Rudy and Dave arranged to meet in 1986 at a snowy conference in the mountains of Colorado. They struck a harmonious chord at once, agreed to cooperate in developing molecular-genetic tools of the type that would be needed to bring *Volvox* genetics into the modern era, and laid plans for funding a series of exchange visits. It was agreed that the two groups would continue their hitherto unsuccessful molecular-genetic projects (positional cloning in our case, and transformation of *Volvox* with antibiotic-resistance genes in Rudy’s case) but would also cooperate on a new approach that would begin with cloning of the *nitA* (nitrate reductase-encoding) gene of *V. carteri*.

The reason for our interest in *nitA* was that it was (and still is) the only *Volvox* locus for which we knew how to select for both loss-of-function and gain-of-function mutations. (Loss-of function *nitA* mutations confer chlorate resistance, whereas gain-of-function reversions restore the ability to grow on nitrate as a sole nitrogen source; Huskey *et al* 1979b.) We reasoned that if we could clone and characterize the *nitA* locus, we could use it both as a homologous selectable marker in a nuclear transformation system and as a trap for transposons that might be useful for tagging and recovering developmentally important genes.

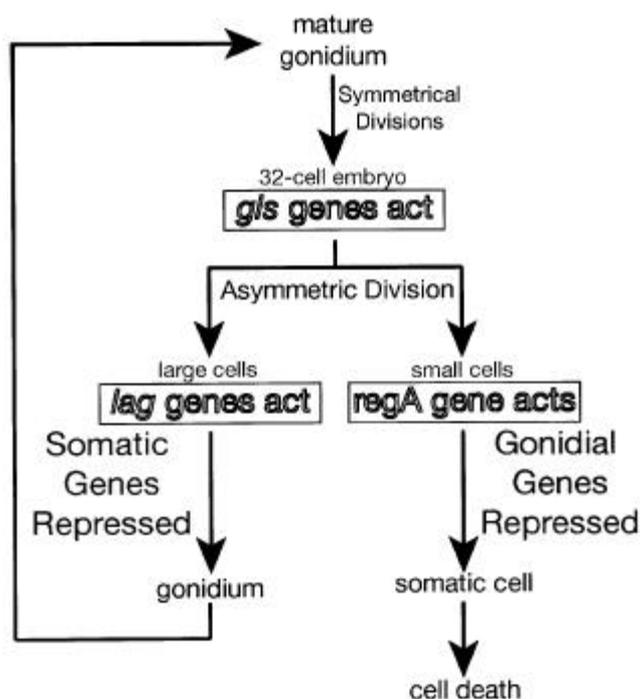


Figure 4. The skeletal genetic program for germ-soma differentiation in *V. carteri*. At the 32-cell stage the *gonidialless* (*gls*) genes act to cause asymmetric division and the formation of large–small sister-cell pairs. Then the *late gonidia* (*lag*) genes act in the large cells to prevent development of somatic features, while the *somatic regenerator A* (*regA*) gene acts in the small cells to suppress all aspects of reproductive development.

The *nitA* project turned out to be much more fraught with technical difficulties, and therefore much more time-consuming, than we had imagined initially, but the way it came to fruition illustrates the close and productive nature of our collaboration. The two groups started out on complementary paths, with each group screening the genomic library it had made previously. The Schmitt group screened their library with synthetic oligonucleotides corresponding to conserved regions of land-plant nitrate reductase-encoding genes, while we probed our library with a cloned fragment of the corresponding *Chlamydomonas* gene, *nit1*, that had been generously provided to us by Pete Lefebvre. For a considerable period of time nothing but false-positive, cross-hybridizing clones were recovered by either lab. During this long, dry period, Rudy Schmitt visited us, and on his first day in our lab he spent time with a new graduate student who was working on the *nitA* project. She told him she had just sequenced another false positive, but as she showed him her handwritten sequence and the autoradiograph from which it had been obtained, he realized that she had read the autorad backwards! When they read the sequence in the correct direction Rudy immediately suspected that she had hit gold. So he quickly faxed the sequence to Heribert Gruber, the graduate student who had been pursuing the *nitA* locus in Regensburg. Before long the answer came back from Heribert that the clone encoded a peptide so similar to a portion of the *Arabidopsis* nitrate reductase that there could be little doubt that it was part of the *nitA* gene! Despite this breakthrough, however, we were never able to find any overlapping clones in our genomic library. Had we been working alone, the project might

have ended there . . . but it did not, because the Schmitt group used our initial clone to retrieve overlapping clones from their library. And so it went, with aspects of the project being passed back and forth from one lab to the other, a bit like a trans-Atlantic game of catch. Finally, and fittingly, the sequencing and characterization of the gene was completed by Rudy's student, Heribert Gruber, in our lab (Gruber *et al* 1992).

As had been predicted years earlier during that blizzard-struck conference in the Colorado mountains, our continued trans-Atlantic collaboration soon led to use of the *nitA* gene both as a homologous selectable marker in a nuclear transformation system (Schiedlmeier *et al* 1994) and as a transposon trap (Miller *et al* 1993).

We were able to take the first step toward development of a transformation system in St. Louis, because our linkage-mapping project had left us with a large collection of multiply-marked strains, among which we found a group of wild-type and morphologically mutant strains that were good transformation candidates because they all carried a non-revertible *nitA* mutation (a partial deletion). We provided proof-of-principle by obtaining the first unambiguous *nitA* transformant with one of these target strains, but were unable to translate this initial success into a user-friendly system. That came only when Rudy and his student Bernhard Schiedlmeier developed a homemade particle gun that could deliver DNA to the targets with far greater efficiency than the delivery method that we had used. They and their colleagues then went on to make the extremely important demonstration that the *nitA*-based system could be used for efficient co-transformation of unselected markers (Schiedlmeier *et al* 1994).



Figure 5. Four *Volvox* investigators mentioned in the text, left to right: David Kirk, Marilyn Kirk, Rüdiger Schmitt and Stephen Miller.

Meanwhile, Steve Miller (figure 5) had come to our lab as a postdoc, determined to develop a transposon-tagging system that he could use to tag one or more of the *gls* genes that are required for asymmetric division. Early in his time with us, he spent several months in Regensburg, where another of Rudy's students, Andreas Lindauer, was using a reverse-transcriptase probe to fish retrotransposons out of the *Volvox* genome (Lindauer *et al* 1993). Unfortunately, however, such retrotransposons did not prove to be useful for tagging *Volvox* genes. So when Steve returned to St. Louis he concentrated on using the *nitA* locus to trap a mobile element. The one he found turned out to jump so well under appropriate conditions that he named it after his basketball hero, *Jordan* (Miller *et al* 1993).

Marilyn subsequently used *Jordan* to tag and recover the *regA* locus – the Holy Grail of *Volvox* biology that we had been pursuing for nearly a quarter of a century – while Steve was using *Jordan* to clone his favorite gene, *glsA*. Of course, compelling evidence that these two projects had been successful came by curing appropriate mutants via co-transformation, using *nitA* as the selectable marker. Back-to-back publication of those two success stories (Kirk *et al* 1999; Miller and Kirk 1999) brought a thrill that could scarcely have been imagined on the day that we first brought Starr's *Volvox* cultures into our lab.

By no means did the St. Louis-Regensburg collaboration end there. Critical parts of the unexpectedly difficult task of sequencing and characterizing the *regA* locus were performed by Rudy's associates, and two of those associates (Klaus Stark, a predoctoral student, and Waltraud Müller, a former technician) made some of their most important contributions after coming to our laboratory. As we anticipated, the sequence of *regA* that was obtained in this highly collaborative way (Kirk *et al* 1999) suggested that it encoded a transcriptional repressor, and raised the question of what target gene(s) it repressed to account for the fact that it blocked both asexual and sexual reproduction. At that point, other students in Rudy's lab sequenced more than a dozen putative *regA* targets that we had identified earlier in a differential cDNA cloning experiment (Tam and Kirk 1991a). They found, to our initial surprise, that all of those genes that had recognizable protein products were nuclear genes encoding chloroplast proteins (Meissner *et al* 1999). This led to our current working hypothesis: namely, that RegA, the product of the *regA* gene, prevents all aspects of reproductive development in somatic cells by blocking chloroplast biogenesis, thereby preventing cellular growth. (And if the somatic cells cannot grow, they obviously cannot reproduce.) Furthermore, Klaus Stark's analysis of *cis*-regulatory elements that regulate cell-type specific expression of the *regA* gene was begun in St. Louis and completed in Regensburg (Stark *et al* 2001).

It is highly likely that without this fruitful 18-year collaboration between St. Louis and Regensburg, neither group would have succeeded in developing the tools required for a modern molecular approach to development, and *Volvox* developmental genetics would have ground to a halt many years ago.

5. Lessons from *V. carteri*: model systems often serve best as models of themselves

When we came to *V. carteri* from our backgrounds in animal biology and development, we thought of the organism as a potential model system that would do what we thought all good model systems were supposed to do: provide information that could be usefully extrapolated to other systems that are less amenable to detailed analysis. More specifically, we began our studies of gonidial determination in *Volvox* with naïve hopes that our findings would provide useful insights into the mechanism of germ-cell determination in animals, a problem that had fascinated developmental biologists for at least a century. Indeed, initial reports by Kochert and Yates (1970) had seemed to support such hopes by suggesting that gonidial specification in *V. carteri* was mediated by UV-sensitive morphogenic particles resembling the germinal granules of frogs, or the polar granules of fruit flies.

However, many years later, when we had been driven to the realization that Pall (1975) had been correct, and that gonidial specification in *V. carteri* was based on the quantity, and not the quality of the cytoplasm that an embryonic cell contains at the end of cleavage (Kirk *et al* 1993), we realized that *V. carteri* could not even serve as an instructive model for germ-cell specification in most other species of *Volvox*, let alone for animals! This was because only two of the 18 species of *Volvox*, namely, *V. carteri* and *V. obversus*, execute any asymmetric division during embryogenesis. In all the other species of *Volvox*, prospective gonidia are not distinguishably larger than prospective somatic cells at the end of cleavage, so they must have a different mechanism of gonidial specification than *V. carteri* does. Furthermore, before Andy Ransick came to our lab to perform microsurgery on *V. carteri* embryos and provide one of the most compelling pieces of evidence supporting our "cell size determines cell fate" hypothesis, he had performed similar surgery on *V. obversus* and obtained quite a different result (Ransick 1991). He had shown that *V. obversus* cells develop as gonidia only if they are both greater than 8 μm in diameter, and also contain cytoplasm from the region of the embryo, where the gonidia are normally formed! Thus, in the final analysis it is clear that the only species of *Volvox* in which the *V. carteri* mechanism of gonidial specification is observed is *V. carteri* itself.

Such a finding (though admittedly it was somewhat disconcerting initially) is certainly not without precedent. Few insect embryos pattern their body segments just as *Drosophila* does, few nematodes establish cell fates during vulval development just as *C. elegans* does, and few operons – even in *Escherichia coli* – are regulated in just the same way that the *E. coli lac* operon is. Does that strip such model systems of all instructive value? Surely not. Indeed, it could be argued that it is by guiding the discovery of the differences observed in other systems that such models take on their greatest heuristic value. In the present era we tend to emphasize – perhaps over-emphasize – the unity of life. But surely the diversity of life is at least as striking and awe inspiring as its unity, and the handful of model systems that have been studied intensively so far have barely begun to reveal the richness of the diversity that evolution has generated by playing multiple variations on a relatively small number of unifying themes.

In any case, although we thought of *Volvox* as a “model” that could shed light on other more complex systems as we cruised up the Mississippi on the Delta Queen 30 years ago, we had not worked with *Volvox* for very long before we began to think of it as a member of our family, and had shifted our goals to understanding its developmental mechanisms for their own sake. Someone who has never had the privilege of developing a long-term relationship with a particular organism (especially one that is so aesthetically pleasing!) probably can not comprehend the thrill that comes from having it occasionally respond to questions by sharing a few of its well-guarded secrets. We are still quite astonished (but extremely grateful) that society would pay us to engage in such hedonic pursuits.

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