Genetic and cytological control of the asymmetric divisions that pattern the \textit{Volvox} embryo

DAVID L. KIRK*, MELISSA R. KAUFMAN†, RICHARD M. KEELING and KANDACE A. STAMER

Department of Biology, Washington University, St Louis, MO 63130, USA

*Corresponding author
† Present address: Department of Microbiology and Immunology, University of Tennessee Health Science Center, Memphis, Tennessee 38163, USA

Summary

The highly regular pattern in which approximately 2000 small somatic cells and 16 large reproductive cells (or 'gonidia') are arranged in a typical asexual adult of \textit{Volvox carteri} can be traced back to a stereotyped program of embryonic cleavage divisions. After five symmetrical divisions have produced 32 cells of equal size, the anterior 16 cells cleave asymmetrically, to produce one small somatic cell initial and one larger gonidial initial each. The gonidial initials then cease dividing before the somatic cell initials do. The significance of the visibly asymmetric divisions is underscored by genetic and experimental evidence that differences in size—rather than differences in cytoplasmic quality—are causally important in activating the programs that cause small cells to become mortal somatic cells and large cells to differentiate as reproductive cells. A number of loci, including at least five \textit{mul} ('multiple gonidia') loci, appear to be responsible for determining where and when asymmetric divisions will occur, since mutations at these loci result in modified temporal and/or spatial patterns of asymmetric division in one or more portions of the life cycle. But the capacity to divide asymmetrically at all appears to require a function encoded by the \textit{gls} (gonidialless) locus, since \textit{gls} mutants fail to execute any asymmetric divisions. Second-site suppressors of \textit{gls} that have been identified may encode other functions required for asymmetric division. Cytological and immunocytochemical studies of dividing embryos are being undertaken in an attempt to elucidate the mechanisms by which cell-division planes are established and shifted under the influence of such pattern-specifying genes. Studies to date clearly indicate a central role for the basal body apparatus, and particularly its microtubular rootlets, in establishing the orientation of both the mitotic spindle and the cleavage furrow; but it remains to be determined how behavior of the division apparatus becomes modified during asymmetric division.

Key words: asymmetric division, basal body apparatus, cell size, cell determination, cytokinesis, cytoskeleton, germ-soma differentiation.

Introduction

The simplicity and regularity of the cellular patterns seen in adult wild-type individuals of the green alga \textit{Volvox carteri} forma \textit{nagariensis} (Starr, 1969, 1970), the clarity with which these patterns can be traced to events during embryogenesis (Starr, 1969; Green and Kirk, 1981, 1982) and the identification of a number of mutants in which these patterns are perturbed in various ways (Starr, 1970; Huskey \textit{et al.} 1979; Callahan and Huskey, 1980), all combine to make this organism an attractive model for discerning how three-dimensional patterning mechanisms may be programmed into a unidimensional genome.

A representative asexual adult of \textit{V. carteri} f. \textit{nagariensis} has two cell types arranged in a simple and highly predictable pattern. Approximately 2000 small somatic cells are rather uniformly spaced at the surface of a transparent spheroid, and 16 large, asexual reproductive cells, or 'gonidia', lie below the surface in four rhomboidal quartets (Fig. 1). These two cell types differ more than a thousand-fold in volume, and are at least as different in other aspects of their phenotype: the somatic cells are biflagellate, postmitotic cells specialized for motility and programmed to undergo senescence and death, while the gonidia are non-motile germ cells that are potentially immortal (Starr, 1969, 1970). Thus the organism presents with crystal clarity the central issue of developmental biology: how does an organism possessing two or more cell types in a predictable spatial pattern arise from a single cell?

The cellular pattern of asexual spheroids is generated by asymmetric division

The regular cellular pattern of the \textit{V. carteri} adult has its
D. L. Kirk and others

origins in a stereotyped program of embryonic cleavage divisions, plus a set of mechanisms that hold all cells in predictable relationships to their neighbors—even in the face of a major morphological rearrangement of the embryo as a whole, and a growth process that separates neighboring cells by many cell diameters.

When mature, each *Volvox carteri* gonidium initiates a set of rapid cleavage divisions that will generate all of the cells that will be present in an adult of the next generation. Although the embryos normally develop inside the parental spheroid, embryogenesis proceeds in identical fashion (and thus is more readily studied) in gonidia that have been isolated from the parent when they are nearly mature (Fig. 2). The first five cleavage divisions of a gonidium are all symmetrical, producing a hollow, spherical embryo containing 32 crescent-shaped cells only, whereas each of their larger sister cells will serve as the source of one gonidium in the next generation. At the 7th and 8th cleavage cycles, the large gonidal initials divide asymmetrically two more times, each time cutting off an additional small somatic initial in an anterior/counterclockwise direction (Fig. 2C). Then the gonidal initials withdraw from the cleavage cycle, while the somatic initials cleave symmetrically 3 or 4 more times.*

At the end of cleavage, all cells that will be present in the adult are present in the embryo, but the topology of the embryo is the reverse of that of the adult: the gonidial initials bulge toward the exterior, and the flagellar ends of the somatic cells are directed toward the interior (Fig. 2D). This predicament is corrected by a process known as inversion (Starr, 1969; Viamontes and Kirk, 1977; Viamontes et al. 1979; Green et al. 1981), in which the embryo turns completely inside out (Fig. 2E,F). Throughout this wholesale rearrangement process, the spatial pattern that was set up by programmed cleavage divisions is not perturbed: all cells retain a fixed set of nearest-neighbor relationships throughout inversion because each cell is linked to its neighbors by an average of 25 stout cytoplasmic bridges. Indeed, these bridges, which are the result of incomplete cytokinesis (Green and Kirk, 1981), appear to constitute the coherent structural framework against which the cells exert the forces that drive the inversion process (Green et al. 1981).

Some hours after inversion, the cytoplasmic bridges linking nearest neighbors are broken, permitting cells to draw away from one another as the spheroid expands by deposition of a complex extracellular matrix (Kirk et al. 1986). At the same time as the cytoplasmic bridges break down, however, new linkages between neighboring cells are generated when a series of fibrous ‘cellular compartments’ with interlocking side walls (‘CZ3’ in the terminology of Kirk et al. 1986) form around each cell as an early step in the expansion process (Fig. 3). Although the young post-inversion gonidia withdraw from the surface layer and round up as soon as the bridges that had linked them to neighboring somatic cells are broken, the compartments in which the gonidia lie are fused to the compartments of neighboring somatic cells (Fig. 3C). Because this component of the matrix holds gonidia in close proximity to the somatic cells to which they are related by descent, it is possible, at least in principle, to deduce the lineage relationships among all cells of the *V. carteri* adult from their spatial relationships within the spheroid.

*The cleavage pattern described here is the idealized one. Sometimes, fewer than 16 of the anterior blastomeres divide asymmetrically to generate gonidal initials, particularly under suboptimal culture conditions. But even then the cleavage pattern appears to be rather precisely programmed, since an ordered set of probabilities can be assigned to the sequence in which particular anterior blastomeres will fail to divide asymmetrically as the conditions undergo progressive deterioration (Gilles and Jaenicke, 1982).*
Different patterns of asymmetric division occur during sexual reproduction

Modified developmental patterns are observed when asexual *V. carteri* cultures are switched into the sexual phase of the life cycle by exposure to a powerful, species-specific pheromone (Starr, 1969, 1970; Starr and Jaenicke, 1974; Mages *et al.* 1988) that is produced both by fully-mature sexual males (Balshüsemann and Jaenicke, 1990) and by heat-shocked asexual individuals (Kirk and Kirk, 1986). Gonidia of asexual spheroids exposed to this pheromone do not develop into gametes directly; rather they initiate a modified pattern of symmetric and asymmetric divisions to produce a new generation of spheroids that contain gametes rather than gonidia. Although genetically male and female strains are indistinguishable in the asexual phase, they are dimorphic in the sexual phase (Starr, 1969, 1970).

In sexually induced female embryos, asymmetric division occurs one cycle later than in uninduced embryos, and it occurs throughout the anterior three quarters (rather than half) of the embryo. As a consequence, sexual females contain a larger number (up to 48) of somewhat smaller and denser reproductive cells than asexual spheroids (Fig. 4A). These cells mature into eggs that are capable not only of being fertilized, but also of redifferentiating as gonidia if fertilization fails to occur.

In sexually induced male embryos, asymmetric division is delayed even longer – to the 8th or 9th cleavage cycle – and it occurs in all blastomeres. Then division ceases. As a consequence, young sexual male spheroids characteristically contain reproductive cells and somatic cells in a 1:1 ratio (Fig. 4B). A day later,
Fig. 3. The structures that hold Volvox cells of common genealogy in a fixed spatial relationship. (A) During embryogenesis, cytoplasmic bridges that are produced in each cleavage furrow as the result of incomplete cytokinesis link sister cells to one another. Because of their larger size, presumptive gonidia (g) protrude from the plane in which presumptive somatic cells (s) lie; nevertheless, a portion of each embryonic gonidium is held in the somatic cell layer by cytoplasmic bridges that link it to the cells to which it is related by descent. (B) Before the cytoplasmic bridges have completely broken down in the post-embryonic period, fibrous extracellular matrix is deposited around each cell, forming a series of 'cellular compartments' that are fused to neighboring cellular compartments on all sides. (C) Because of these fused compartment walls, all cells retain their nearest-neighbor relationships even after the cytoplasmic bridges have broken down and the gonidia have rounded up and withdrawn from the plane of the somatic cells.

Some mutations recombine normal asymmetric division patterns and germ cell phenotypes in new ways

As just outlined, there is normally a close correspondence between the program of asymmetric division that a V. carteri embryo exhibits and the type of reproductive cell that is subsequently produced. However, analysis of mutants clearly indicates that there is no necessary relationship between these two aspects of phenotype. By now, virtually all possible combinations of the various cleavage patterns and reproductive cell types have been observed in mutants (Starr, 1970; Huskey et al. 1979; Callahan and Huskey, 1980; Starr and Jaenicke, 1989). Presumably, the lesions in most such mutants are in loci that are involved in determining which of the alternate asymmetric division programs will be selected for execution in different phases of the life cycle. Therefore, consideration of the phenotypes associated with these mutations should provide some insight into the ways in which global aspects of the asymmetric division process are genetically programmed.

Huskey et al. (1979) defined four mul ('multiple
Pattern formation in *Volvox* 71

Fig. 5. A selection of *V. carteri* asexual-female spheroids with mutant gonidial patterns. (A) An individual with a *mulB* mutation; gonidia are present in the pattern normally associated with the eggs of a sexual female. (B) An individual with a *mulC* mutation; gonidia are present in the pattern normally associated with the androgenidia of a sexual male. (C) An individual with a mutation of an incompletely characterized *mul* locus; gonidia of variable size are present in unpredictable numbers and locations. (D) An individual with a *gls* mutation; no 'true' gonidia are produced, but because this strain also carries a mutation of the *regA* ('somatic regenerator') locus, somatic cells redifferentiate as gonidia. Redifferentiation has already occurred in the posterior portion of the spheroid shown here, but anterior cells still retain eyespots and other features of somatic cells. (E) A 'revertant' derived from the preceding strain; because of a mutation at a *sug* (suppressor of gonidialess) locus, small numbers of 'true' gonidia are sometimes produced. (F) A 'quasi-gonidialess' mutant isolated from a wild-type strain; 0–4 gonidia are produced per spheroid.

Mutations at other loci result in a dissociation of the normal relationships between cleavage pattern and germ cell phenotype in sexually-induced embryos only. A sexual male bearing the R-1 mutation (Starr, 1970), for example, produces sperm packets in the spatial pattern normally associated with egg production in the sexual female. In contrast, a female bearing a mutation at the *megA* ('multiple egg') locus produces a 1:1 ratio of eggs to somatic cells via a cleavage pattern like that normally associated with sexual development in males (Callahan and Huskey, 1980).

The MegA phenotype (i.e. the phenotype due to a *megA* mutation) arose by mutagenesis of a standard female strain. The same phenotype (production of fertile eggs in the sexual-male pattern) has now been reported in a change-of-sex mutant that was derived by mutagenesis of a male strain (Starr and Jaenicke, 1989). At first glance, it might appear that these two strains generate the same phenotype from an entirely different combination of alleles: specifically, a mutant mating-type locus and wild-type pattern-forming alleles in the sexually transformed male, versus a mutant pattern-forming gene and a wild-type mating-type locus in the MegA female. However, on the basis of phenotype alone, there is no way of ruling out the alternative possibility that different mutations imposed on two different initial states may have caused these two strains to converge on the same genetic state: namely, one in which a portion of the mating type locus that regulates
germ cell phenotype has the female character, while a portion that regulates the cleavage program has the male character.

A very different category of mutation dissociating the normal relationship between cleavage pattern and germ cell phenotype is present in two other strains. Starr (1970) recovered a 'sterile female' mutant that activates the sexual female cleavage program – but not the egg differentiation program – in response to the sexual inducer. Thus, induced spheroids of this strain contain gonidia in the typical egg pattern and bear a superficial resemblance to uninduced MulB spheroids. The pan (‘parthenogenetic androgonidia’) mutation recovered by Callahan and Huskey (1980) results in the corresponding phenotype in males. Like the sterile female, the Pan male has the normal asexual phenotype in the absence of the sex-inducer. But in the presence of inducer, it produces gonidia in the sexual male pattern, and in this regard superficially resembles uninduced MulC or MulD strains. A reasonable working hypothesis would appear to be that in both the Pan male and the sterile female the portion of the mating-type locus that activates a sex-specific cleavage program in response to inducer functions normally, but the portion that activates the corresponding gametogenesis program in response to inducer is defective.

At least one type of mutation, mul-2, affects pattern formation in both the asexual and sexual phases. In Mul-2 strains, the cleavage and germ-cell distribution patterns that are characteristic of wild-type sexual females are seen not only in sexual females, but also in males, asexual males and asexual females (Callahan and Huskey, 1980). The mul-2 lesion would appear to be a constitutive mutation of a gene involved in establishing the sexual-female cleavage pattern, such that expression of this program no longer requires the action of an inducer-activated female mating-type locus.

Other mutations produce aberrant asymmetric division patterns

The mutants described in the preceding section all exhibit, in one or more phase of the life cycle, a pattern-forming program of asymmetric divisions that normally characterizes a different phase of the life cycle. Presumably, they define a set of loci that normally interact to match a particular global pattern of asymmetric divisions with a particular program for germ cell differentiation. However, a number of other mutants have been described that exhibit various cleavage and germ-cell distribution patterns different from any observed in the wild-type life cycle. Presumably, these mutants define additional functions that are involved in determining temporal and spatial aspects of division symmetry.

Alleles at an unknown number of incompletely defined loci result in asymmetric division patterns in the asexual phase that are randomized both spatially and temporally. Such lesions result in adults with gonidia that range appreciably in size, and are distributed without an obvious spatial pattern (Fig. 5C). Such mutations may represent lesions in steps close to the cleavage-plane determination process itself, such that asymmetric division has become partially decoupled from the influence of the control elements normally associated with global pattern regulation.

Mutation at the mulA locus (Huskey et al., 1979), defined by the ‘multi’ mutant described by Starr (1970), results in asexual spheroids that contain clusters of up to four gonidia in each of the areas where a wild-type spheroid would have a single gonidium. The sizes of the gonidia in each MulA cluster tend to be inversely related to the number of gonidia in the cluster. The initial pattern of asymmetric division at the 6th division cycle is apparently normal in this mutant, but then MulA gonidial initials often divide symmetrically in the 7th and/or 8th cycle (when wild-type gonidial initials divide asymmetrically again) to generate a cluster of two, three or four gonidia of reduced size. This phenotype suggests that the mulA locus is involved in determining division symmetry in terminal stages of the pattern-forming process only. However, this interpretation is not readily extended to include an explanation of the effect of the mulA mutation on sexual development. Although the mulA mutation has no obvious effect on pattern formation in sexual females, it diminishes the regularity that normally characterizes the sexual-male pattern: some MulA males produce excess somatic cells, while others in the same culture produce excess androgonidia – to the virtual exclusion of somatic cells (Starr, 1970). Interpretation of this effect must await a more careful examination of the cleavage patterns in MulA sexual males than has yet been reported.

Two other mutations have been described that also abolish the strikingly regular cellular pattern that normally characterizes sexual males. A sexual male bearing a radA (‘reduced androgonidia’) mutation has a 1:3 ratio of sperm packets to somatic cells, rather than the usual 1:1 ratio (Callahan and Huskey, 1980). The embryological basis of this phenotype remains to be described, but may involve an additional round of division (symmetrical in the somatic initials, and asymmetrical in the androgonidial initials) following a normal differentiative cleavage. The R-2 strain (Starr, 1970) also produces fewer androgonidia than a wild-type male, but for a quite different reason. In R-2 sexual males, relatively few cells participate in asymmetric division, while the remaining cells divide symmetrically to produce somatic cells that are considerably larger than those produced by asymmetric division. The R-2 mutation, in contrast to the radA mutation, also reduces gamete number in the sexual female. It does this by restricting asymmetric divisions to an embryonic region not much different than that where they normally occur in asexual embryos. Thus it appears that the locus that is mutant in R-2 may be involved somehow in expanding the region in which asymmetric divisions may occur following sexual induction.
The size of cells produced by asymmetric division determines their fate

In all of the *Volvox carteri* embryos in which cleavage has been carefully studied – whether asexual or sexual, wild-type or mutant – there is one common rule that is followed: whenever and wherever visibly asymmetric divisions occur, the large cells give rise to germ cells, while their smaller sister cells give rise to somatic cells only. Is it the difference in cell size, or some accompanying difference in cytoplasmic quality, that determines the difference in fate of the large and small sister cells produced by asymmetric division? Pall (1975) advocated the former, while Kochert (1975) advocated the latter hypothesis.

Currently available genetic and experimental evidence strongly supports the concept that in *V. carteri* it is the difference in size, and not some associated difference in cytoplasmic quality, that is causally important in determining the different fates of the large and small sister cells produced by asymmetric division. Briefly, some of the more important lines of evidence (which will be more thoroughly summarized elsewhere) include the following. (1) In mutants that execute substantially fewer than the normal number of embryonic cleavage divisions, the average cell size at the end of cleavage is elevated, and both the relative and the absolute abundance of gonidia per spheroid is also elevated – roughly in inverse proportion to the number of divisions completed (Pall, 1975; D. L. Kirk, unpublished observations). (2) When heat shock is used to interrupt cleavage of wild-type embryos shortly after two rounds of asymmetric division (that is, after any putative cytoplasmic determinants should have been segregated), presumptive somatic cells remain approximately 16 times larger than if they had been permitted to complete the usual number of divisions. Most, and in many cases all, of these presumptive somatic cells differentiate as gonidia (D. L. Kirk and M. M. Kirk, unpublished observations). (3) In a temperature-sensitive cleavage mutant that exhibits gross perturbations of cleavage plane orientations at elevated temperature, large cells are generated in wholly abnormal locations when the temperature is raised during cleavage, and all viable cells that remain above a certain size at the end of cleavage differentiate as gonidia (K. J. Green, D. L. Kirk, D. M. Mattson and K. A. Stamer, unpublished observations). (4) When cells from the posterior hemisphere of asexual *V. carteri* embryos (cells that would never produce gonidia normally) are surgically isolated at the 16-cell stage, they subsequently undergo unpredictable variations in cleavage behavior, and frequently execute one or more asymmetric divisions; the large cells produced by such adventitious asymmetric divisions then invariably develop as gonidia (A. Ransick, personal communication).

Observations of this type clearly indicate that in order to understand the process of germ–soma specification and pattern formation in *V. carteri* it will be essential to elucidate the genetic and cytological mechanisms by which the location and orientation of the cleavage plane is established in an individual blastomere.

**One class of mutations abolishes asymmetric division**

As summarized above, many categories of mutation modify the pattern of asymmetric divisions observed in one or more phases of the life cycle. Hence, it appears that there must be a considerable number of loci regulating temporal and spatial aspects of the global asymmetric division pattern. But one category of mutation has been identified that has a qualitatively different effect on asymmetric division than all of the pattern mutations described earlier. In a *gls* (‘gonidialess’) mutant, symmetric cleavage divisions occur with beautiful regularity, but no asymmetric divisions occur, and hence (as the name of the mutant implies) no gonidia are formed (Fig. 5D).* Although wild-type alleles at the various *mul* loci, etc. are required to establish a normal global pattern of asymmetric divisions, a functional *gls* locus is apparently required to execute any asymmetric divisions at all. Therefore, we anticipate that the *gls* mutation will turn out to be epistatic to all of the pattern forming mutations described above.

The regularity of the symmetric division process in *gls*− embryos indicates that this mutation has no discernible affect on the functioning of the cytokinetic machinery *per se*. We postulate, therefore, that the *gls* locus must encode some component that is required to shift the cytokinetic apparatus from the middle to one side of the dividing cell. (The times and places at which the *gls* function acts presumably are specified by the actions of the various pattern-forming genes.) Secondary mutations have been recovered that permit occasional asymmetric divisions, and hence permit formation of a small number of gonidia, in embryos bearing the *gls* mutation (Fig. 5E). We suspect that such secondary suppressors of *gls* may encode additional components that interact with the *gls* product to shift the location of the cytokinetic apparatus. An understanding of how the *gls* product might function in this manner obviously will require detailed understanding of the cytokinetic mechanisms of *V. carteri* blastomeres.

**Cell division in the volvocales is based on a distinctive type of cytokinetic apparatus**

In recent years it has become increasingly apparent that

* ‘Tight’ mutations of this class (which abolish asymmetric division entirely) can only be recovered and maintained on a genetic background that includes a *regA* mutation, which causes somatic cells to redifferentiate as reproductive cells (Starr, 1970; Huskey and Griffin, 1979; Kirk *et al.* 1987). However, ‘leaky’ mutations of this type (*q-gls*, or ‘quasi-gonidialees’ mutations, which permit only occasional asymmetric divisions) have been recovered on a wild-type background (Fig. 5F) (Kirk, 1990).
cell division in members of the Order Volvocales (which includes Volvox and its colonial and unicellular relatives) differs in a number of significant ways from the more familiar division processes of either higher plants or animals, because it is based on the functioning of a distinctive type of mitotic and cytokinetic apparatus, some components of which are shared with various other groups of green algae (Zimmerman, 1921; Kater, 1929; Metzner, 1945; Johnson and Porter, 1968; Deason and Darden, 1971; Pickett-Heaps and Marchant, 1972; Cavalier-Smith, 1974; Coss, 1974; Triemer and Brown, 1974; Pickett-Heaps, 1975; Stewart and Mattox, 1975; Marchant, 1977; Floyd, 1978; Birchem and Kochert, 1979; Green et al., 1981; Hoops and Floyd, 1982; Huang et al., 1982; Hoops, 1984; Mesquita and Fátima Santos, 1984; Adams et al., 1985; Wright et al., 1985; Aitchison and Brown, 1986; Harper and John, 1986; LeDizet and Piperno, 1986; Domozycz, 1987; Doonan and Grief, 1987; Gaffal, 1988; Salisbury et al., 1988; Holmes and Dutcher, 1989; Segaar et al., 1989; Segaar et al., 1990). Mitotic and cytokinetic mechanisms, and the structure of the division apparatus, have been particularly well studied in Chlamydomonas reinhardtii, a close unicellular relative of V. carteri (Johnson and Porter, 1968; Coss, 1974; Huang et al., 1982; Adams et al., 1985; Wright et al., 1985; Harper and John, 1986; LeDizet and Piperno, 1986; Doonan and Grief, 1987; Gaffal, 1988; Salisbury et al., 1988; Holmes and Dutcher, 1989). Among the earliest external signs that division is imminent in a Volvocalean cell is the formation, on the anterior end of the cell, of a shallow groove that predicts the location and orientation of the cleavage furrow to be formed later (Doonan and Grief, 1987; Holmes and Dutcher, 1989; Segaar and Gerritsen, 1989). This incipient furrow that forms in prophase (at about the time that flagella are being resorbed or detached from their basal bodies) always bisects the basal body apparatus.

The interphase Volvocalean basal body apparatus includes (at a minimum) a pair of mature basal bodies, a probasal body associated with each mature basal body, four flagellar roots composed of microtubules (MTs), and a set of striated fibers that join the basal bodies, probasal bodies and flagellar roots to one another in a taxonomically diagnostic three-dimensional array (Melkonian, 1984a). The basal body apparatus is the ‘morphogenetic center’ of both interphase and dividing Volvocalean cells. Basal bodies not only serve as the bases and MT-organizing centers (MTOCs) of the flagella during interphase, but, together with their associated roots and fibers, they form the MTOCs for the interphase cytoskeleton, and thereby play a central role in organizing the rest of the interphase cell (Stearns and Brown, 1979; Melkonian, 1984b; Aitchison and Brown, 1986) and the mitotic spindle (Doonan and Grief, 1987; Segaar, 1990). More importantly for present considerations, the basal body complexes are also the MTOCs for the cytokinetic apparatus (Johnson and Porter, 1968; Segaar et al., 1989).

The basal bodies remain firmly attached to both the plasmalemma and the nucleus throughout the cell cycle, even during mitosis when they are located near the spindle poles and appear to be acting as centrioles (Coss, 1974; Doonan and Grief, 1987). But the resorption or detachment of flagella that occurs during prophase frees the basal bodies to move laterally in the plane of the plasmalemma during cell division. Each basal body is attached to the interphase nucleus throughout the cell cycle by a discrete fiber (Kater, 1929; Wright et al., 1985) that turns out to be rich in the calcium-activated contractile protein, centrin (Salisbury et al., 1988). At the point where a connecting fiber contacts the interphase nucleus, it separates into discrete sub-fibers (or ‘fimbria’) that spread out over the nuclear envelope (Fig. 6A). In prophase, these centrin-based fibers contract into a coherent plaque-like structure, in the process drawing the nucleus close to the basal bodies and the plasmalemma (Salisbury et al., 1988). At this time, each probasal body also elongates to become a full-length basal body (Gaffal, 1988). Then, as the spindle forms and elongates, the centrin-rich plaque splits, and each half, with its associated basal body pair, moves laterally with one of the spindle poles (Salisbury et al., 1988; Holmes and Dutcher, 1989). The direction of spindle elongation and basal body separation is approximately perpendicular to the incipient cleavage furrow.

By metaphase and anaphase (Fig. 6B), the mitotic spindle is crescent-shaped, with its poles held near the basal body complexes and plasmalemma, and its equator lying deeper in the cytoplasm, below the incipient furrow (Coss, 1974; Doonan and Grief, 1987; Salisbury et al., 1988; Holmes and Dutcher, 1989). The nuclear envelope persists throughout mitosis, except at the spindle poles, where it is fenestrated; a specialized, centrosome-like cytoplasmic region, free of ribosomes and other organelles, surrounds each polar fenestra (Johnson and Porter, 1968), in the zone between the termini of the spindle MTs and the region where the basal body complex is now known to lie.

By early telophase, the basal bodies begin to move back toward the incipient cleavage furrow, drawing with them the reforming daughter nuclei to which they are still attached (Salisbury et al., 1988). As the interzonal spindle breaks down during late telophase, this centripetal movement of the basal bodies and nuclei continues, bringing sister nuclei close together (Fig. 6C), and bringing the basal bodies to locations straddling the incipient cleavage furrow (Johnson and Porter, 1968; Pickett-Heaps, 1975; Doonan and Grief, 1987; Salisbury et al., 1988).

The most distinctive feature of cell division in the
Volvocales and certain other green algae is the formation of two paired sets of MT arrays, both of which run parallel to the prospective cleavage plane in the internuclear zone; together these MT arrays form a taxonomically distinctive cytokinetic structure known as the 'phycoplast' (Pickett-Heaps, 1969, 1975). The more obvious of the phycoplast arrays are the two sets of 'cleavage' MTs (Johnson and Porter, 1968) that originate on opposite sides of the incipient furrow, in the vicinity of the sister basal bodies, and extend in parallel deep into the cytoplasm, passing between the sister nuclei en route (Fig. 6C). The second phycoplast array comprises two sets of 'internuclear' MTs that form on opposite sides of the presumptive cleavage plane in the internuclear region (Fig. 6C); these internuclear MTs are aligned in a direction that is perpendicular both to the orientation of the spindle MTs that previously traversed this region, and to the orientation of the cleavage MTs that now traverse it (Pickett-Heaps, 1969). Both sets of phycoplast MTs develop as the basal bodies complete their movement back toward the incipient furrow, and shortly after disassembly of the spindle MTs has been completed (Doonan and Grief, 1987). As the name implies, the cleavage microtubules define the plane in which cleavage will occur. The cleavage furrow now ingresses in the zone between the two parallel sets of cleavage MTs (Fig. 6C). Alignment and fusion of endomembrane vesicles along the phycoplast MTs also occurs in the internuclear region at this time, and may play some role in formation of the cleavage furrow in the central portion of the cell (Pickett-Heaps, 1975), although this has yet to be demonstrated (Domozych, 1987).

Some critical questions for understanding the control of division symmetry may be asked: How is the orientation of the incipient cleavage furrow specified at prophase? How is the axis of basal body separation and spindle elongation specified during prophase and metaphase, to insure that it is perpendicular to the incipient furrow? And how is the orientation of the cleavage MTs specified in the telophase–cytokinesis interval to insure that they will define the same plane as that defined earlier by the incipient cleavage furrow?

Recent studies indicate that these cell division axes
Volvocalean such as Chlamydomonas, as viewed from outside the anterior end of the cell (adapted from models proposed by Holmes and Dutcher, 1989 and Segaar and Gerritsen, 1989). (A) The preprophase basal body apparatus contains two ‘parental’ basal bodies (P) that are connected by a striated fiber, two ‘daughter’ basal bodies (D), two 2-membered MT rootlets (2), and two 4-membered MT rootlets (4). (Only 3 MTs are shown for the 4-membered rootlets, because the fourth MT always lies below the other three.) Each parental basal body is attached to one 4-membered rootlet and one 2-membered rootlet; each daughter basal body is attached to the same 2-membered rootlet as its parent. During preprophase, an incipient furrow forms directly above the 4-membered rootlets. (B) Early in mitosis, the striated fiber connecting the parental basal bodies breaks down, the two 4-membered rootlets are brought into contact, and they begin to slide past one another, while the 2-membered rootlets shorten. (C) Shortly beyond their contact point, the 4-membered rootlets bend sharply and follow the path originally occupied by the 2-membered rootlets. As a consequence, by metaphase (shown here) the basal bodies have separated at right angles to the incipient cleavage furrow. This results in formation of the spindle (dashed lines) in a plane perpendicular to the incipient cleavage furrow. It also results in semi-conservative distribution of basal bodies to the prospective daughter cells (i.e. one parental and one daughter basal body to each).

are defined by the MT rootlets of the basal body apparatus (Doonan and Grief, 1987; Holmes and Dutcher, 1989; Segaar and Gerritsen, 1989). The MT rootlets of a Volvocalean basal body apparatus form a cross, with alternate rootlets in the cross being invariably composed of two and four MTs, the so-called 4–2–4–2 arrangement. Thus, two rootlets of similar type lie opposite each other, and extend in opposite directions from the basal bodies. The incipient furrow that appears in preprophase is formed directly above the 4-membered MT rootlets (Fig. 7A), and its orientation is almost certainly determined by them, whereas the direction in which the basal bodies will separate (and the spindle will elongate) is defined initially by the orientation of the 2-membered rootlets (Doonan and Grief, 1987; Holmes and Dutcher, 1989; Segaar and Gerritsen, 1989). Two independently derived models propose the same mechanism for establishing the spatial coordinates for basal body separation and nuclear elongation (Holmes and Dutcher, 1989; Segaar and Gerritsen, 1989): at prophase opposite 4-membered rootlets are brought into contact at their proximal ends (Fig. 7B), and then slide past one another, each making a sharp bend near the contact point. It is postulated that it is as a result of this sliding behavior that during metaphase the two 4-membered rootlets are observed to form a cross (Fig. 7C), in which the distal portion of each lies in the plane of the incipient cleavage furrow, while the proximal portion of each lies along the spindle axis (Doonan and Grief, 1987; Holmes and Dutcher, 1989; Segaar and Gerritsen, 1989). It apparently was the distal portions of the 4-membered MT rootlets that Johnson and Porter (1968) detected lying in the cortex below the incipient furrow, and directly above the metaphase plate, and which they called the “metaphase band” of MTs. However, the term metaphase band turned out to be a misnomer, since this MT band is present in the equatorial cortex from preprophase through telophase. In fact, in at least some species, it has been shown that it is the distal portions of the 4-membered rootlets (which still lie under the incipient furrow at the completion of nuclear division), and not the basal bodies per se, that act as the MTOCs for the cleavage MTs (Segaar and Gerritsen, 1989). This would account for the perfect alignment of these MTs with the plane of the cleavage furrow, as initially defined by these same 4-membered rootlets.

It has been elegantly demonstrated that the stereotyped behaviors of various components of the basal body apparatus during cell division account for the regularity of both the symmetrical and the asymmetrical features that characterize the unicellular Volvocaleans (Holmes and Dutcher, 1989; Segaar and Gerritsen, 1989). We anticipate that it may be in subtle variations in the behavior of certain of these structural components that the differences between symmetrical and asymmetrical divisions in Volvox will be found.

The cytokinetic apparatus of Volvox exhibits many typically Volvocalean features

The studies of cell division mechanisms in Volvox embryos that have been reported to date (Zimmerman, 1921; Metzner, 1945; Deason and Darden, 1971; Birchem and Kochert, 1979; Green and Kirk, 1981;
Green et al. (1981) do not provide as comprehensive and detailed a picture as is currently available for unicellular Volvocaleans. However, such studies are adequate to indicate that the cell division apparatus and mechanisms employed by Volvox are fundamentally similar to those just described for its unicellular relatives. For example, the mitotic spindle of Volvox also develops within a fenestrated nuclear envelope and has a basal body apparatus, of only slightly modified design (Hoops, 1984), attached to the plasmalemma near each of the spindle poles, and cytoplasmic MTs run between the basal bodies at metaphase and anaphase (Deason and Darden, 1971; Birchem and Kochert, 1979). At the completion of karyokinesis, the basal bodies return to the shoulders of the incipient furrow, and a typical Volvocalean phycoplast forms, defining the plane in which the cleavage furrow subsequently ingresses from the anterior end of the cell (Birchem and Kochert, 1979; Green et al. 1981). One apparent difference is that alignment of vesicles along the phycoplast MTs in the internuclear region of the dividing cell is somewhat more obvious in Volvox than in some unicellular Volvocaleans (Birchem and Kochert, 1979; Green et al. 1981). It is postulated that such vesicles are involved in the formation of the cytoplasmic bridges that form in this region, and that persist throughout embryogenesis (Green and Kirk, 1981; Green et al. 1981); however they are formed, these cytoplasmic bridges constitute one substantial and characteristic difference between cytokinesis in the unicellular and the multicellular Volvocaleans.

From the point of view of one interested in the asymmetric division process that lies at the heart of cell determination and pattern formation in V. carteri, what has been most lacking in the previous studies has been any examination of the mechanisms by which division symmetry is controlled in Volvox embryos at the cytological level. As a first step toward understanding this, we have begun an immunocytochemical analysis of dividing Volvox embryos, employing antibodies that have proved useful for visualizing the mitotic and cytokinetic apparatus of Chlamydomonas and other unicells. Specifically, we have used antitubulin antibodies (donated by B. Huang and J. Rosenbaum) to visualize the overall distribution of MTs in the embryo, an antibody to acetylated α-tubulin (donated by G. Piperno) to visualize the location of basal bodies and basal body rootlets (LeDizet and Piperno, 1986), and antibodies to centrin (donated by J. Salisbury) to visualize the location of nuclear-basal-body connectors. Counterstaining with DAPI has been used to visualize the state of condensation and the location of chromosomal DNA. A detailed account of this work will be published elsewhere.

Such studies reveal many fundamental similarities between Volvox and its unicellular relatives with respect to the cytological features controlling the orientation of mitotic spindles. For example, triple staining with DAPI plus antibodies to acetylated α-tubulin and centrin indicates that in Volvox, as in Chlamydomonas (Salisbury et al. 1988), centrin-rich elements link the basal body apparatus to the nucleus throughout the cell cycle (see Fig. 8 for representative images). Thus, anti-centrin staining can be used to infer the location of the basal body apparatus when generalized anti-tubulin staining (which obscures the staining pattern of anti-acetylated α-tubulin) is being employed, as in Fig. 9.

In a V. carteri gonidium approaching the first division, an extensive array of cortical MTs emanate from four MT rootlets that are arranged in a cross at the anterior pole of the gonidium; the arms of this cross presage the locations of the first and second cleavage furrows (Fig. 9A). Anti-centrin staining reveals a diamond-shaped plaque at the center of the MT cross (Fig. 9A'). By analogy with Chlamydomonas (Holmes...
and Dutcher, 1989), we presume that the MT bands that presage the location of the first cleavage furrow are organized by the 4-membered MT rootlets, while those presaging the location of the second furrow are organized by the 2-membered rootlets.

During mitosis, cortical MTs largely disappear, to be
Pattern formation in Volvox
The posteriormost 4 cells of two 16-cell embryos stained by indirect immunofluorescence with anti-acetylated α-tubulin (A and B) and counterstained with DAPI (A' and B'). (A and A') Prophase. MT rootlets lie in a configuration closely resembling that predicted by the model for basal-body separation that was presented in Fig. 7C. Note the lack of overlap between the adjacent MT rootlets that were presumably sliding with respect to one another at the time of fixation. (B and B') Metaphase. The chromosomes have congressed to the spindle equators. Bright spots near the end of the rootlets indicate the locations of the basal bodies, which lie near the spindle poles. Note that the configuration of the rootlets has changed significantly since prophase: they now overlap one another extensively, in the region where they bend sharply above above the spindle equator. Note also that spindles of adjacent cells are rotated 90° with respect to one another, as would be expected for the cleavage furrows at this division stage. Bar, 5 μm.

Volvox carteri has many features to recommend it for a study of the way in which a stereotyped cellular pattern can be programmed into a genome, and the way in which such a program can then be realized at the cellular level. It possesses several cellular patterns that are normally associated with specific phases of the life cycle. Each of these patterns is simple and regular, and can be accounted for by the sequence of symmetric and asymmetric divisions that occurs during embryogenesis. A number of mutations that have been recovered indicate that both the overall pattern of asymmetric cleavage divisions, and the more proximal events determining the symmetry of individual divisions, are under close genetic regulation. Both the general architecture of the cell division apparatus in the Volvocalean algae, and the particular features of it that determine the cell division axis, are coming to be understood better than they are in any other group of organisms. Nevertheless, the challenges that still remain in converting all of these promising features into a full comprehension of the pattern-forming mechanisms of this attractive organism are far from trivial.

Technical assistance by Christine Adams and technical and editorial assistance by Marilyn Kirk are gratefully acknowledged. We are particularly grateful to the following colleagues who supplied antibodies used in the immunocytochemical analysis: Bessie Huang (monoclonal anti-α-tubulin); Joel Rosenbaum (polyclonal anti-β-tubulin), Gianni Piperno (monoclonal anti-acetylated α-tubulin) and Jeffrey Salisbury (monoclonal and polyclonal anti-centrins). This work was supported by grants from the National Institutes of Health (GM-27215) and the National Science Foundation (DMB-8615691).

References


D. L. Kirk and others


