American Journal of Botany
Celebrating 100 years 1914–2014

American Journal of Botany 101(1): 6–25. 2014.

AJB CENTENNIAL REVIEW

THE EVOLUTIONARY-DEVELOPMENTAL ORIGINS OF MULTICELLULARITY[1]

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Multicellularity has evolved at least once in every major eukaryotic clade (in all ploidy levels) and numerous times among the prokaryotes. According to a standard multilevel selection (MLS) model, in each case, the evolution of multicellularity required the acquisition of cell–cell adhesion, communication, cooperation, and specialization attended by a compulsory *alignment-of-fitness* phase and an *export-of-fitness* phase to eliminate cell–cell conflict and to establish a reproductively integrated phenotype. These achievements are reviewed in terms of generalized evolutionary developmental motifs (or "modules") whose overall logic constructs were mobilized and executed differently in bacteria, plants, fungi, and animals. When mapped onto a matrix of theoretically possible body plan morphologies (i.e., a morphospace), these motifs and the MLS model identify a "unicellular ⇒ colonial ⇒ multicellular" transformation series of body plans that mirrors trends observed in the majority of algae (i.e., a polyphyletic collection of photoautotrophic eukaryotes) and in the land plants, fungi, and animals. However, an alternative, more direct route to multicellularity theoretically exists, which may account for some aspects of fungal and algal evolution, i.e., a "siphonous ⇒ multicellular" transformation series. This review of multicellularity attempts to show that natural selection typically acts on functional traits rather than on the mechanisms that generate them ("Many roads lead to Rome.") and that genome sequence homologies do not invariably translate into morphological homologies ("Rome isn't what it used to be.").

Key words: animals; algae; bacteria; body plans; coenocyte; cytokinesis; dynamic patterning modules; embryophytes; evolution; fungi; multilevel selection (MLS) theory; phragmoplast; siphonous; Spitzenkörper.

This paper reviews the evolutionary origins of multicellularity and explores the developmental *bio*-logic constructs required for the fabrication of a multicellular body plan. A broad comparative approach is adopted because multicellularity has evolved multiple times in different ways in very different clades (Fig. 1) and because different criteria have been established to define individuality in the context of multicellularity (Herron et al., 2013). Estimates of the exact number of times vary, depending on how multicellularity is defined and in what phylogenetic context. When described simply as a cellular aggregation, multicellular organisms are estimated conservatively to have evolved in at least 25 lineages (Grosberg and Strathmann, 2007), making it a "minor major" evolutionary transformation. When more stringent criteria are applied, as for example a requirement for sustained cell-to-cell interconnection, communication, and cooperation, multicellularity has evolved multiple times in bacteria (e.g., Actinobacteria, Myxobacteria, and Cyanobacteria; see Bonner, 2000), but only once in the Animalia, three times in the Fungi (chytrids, ascomycetes, and basidiomycetes), and six times among the algae (twice each in the rhodophytes, stramenopiles, and chlorobionta; Niklas and Newman, 2013). Regardless of a canonical definition or an exact count of its occurrence on the tree of life, the emergence of multicellularity raises a number of important but as yet unresolved questions. For example, what if any are the selection barriers to (and the drivers toward) it? Are the motifs in the morphological transformation series that are seen in multicellular lineages the result of adaptive evolution, relaxed selection, or the inevitable consequences of generic physical laws coupled to very simple genomic processes? Is there a genomic toolkit or a set of "master genes" responsible for multicellularity, and are they shared among all eukaryotic clades? Indeed, are the multiple origins of multicellularity truly independent, given that all eukaryotes ultimately shared a last common ancestor? This last question is particularly intriguing in light of (1) multilevel selection theory that requires the evolution of an alignment-of-fitness among the cells of a multicellular progenitor and (2) the fact that all eukaryotes shared a last common unicellular ancestor that must have evolved an alignment among the various metabolic interests of its endosymbionts to integrate the activities of its proto-organelles, e.g., the TOC–TIC translocon protein-import system of the land-plant plastid envelope incorporates a cyanobacteria-like core (Jarvis and Soll, 2001).

[1]Manuscript received 28 August 2013; revision accepted 14 November 2013.

The author thanks Drs. Michael L. Christianson (University of California, Berkeley), Dennis W. Stevenson (The New York Botanical Garden), Dominick J. Paolillo, Jr. and Randy O. Wayne (Cornell University), and two anonymous reviewers for providing constructive suggestions. The author also thanks the Editor-in-Chief of the *American Journal of Botany*, Dr. Judy Jernstedt (University of California, Davis), for inviting this contribution. Support from the College of Agriculture and Life Sciences, Cornell University is gratefully acknowledged. This paper is dedicated to the memory and achievements of Professor Donald R. Kaplan (1938–2007).

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doi:10.3732/ajb.1300314

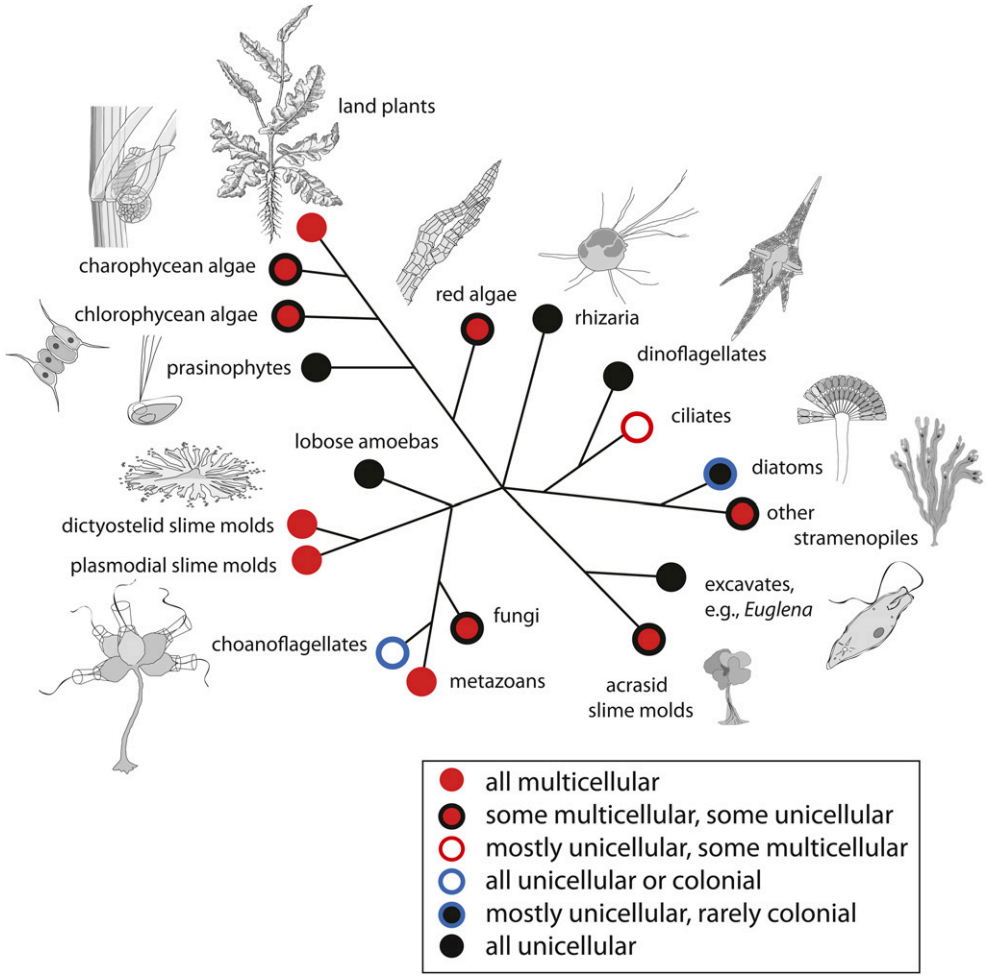

Fig. 1. Representative diverse origins of multicellularity shown on a highly redacted and unrooted phylogenetic diagram of the major eukaryotic clades (modified from a variety of sources). Although some lineages or clades are entirely unicellular or multicellular (e.g., lobose amoeba and the land plants, respectively), most contain a mixture of body plans such as the unicellular and colonial body plans (e.g., choanoflagellates) or a mixture of the unicellular, colonial, and multicellular body plans (e.g., ciliates and stramenopiles). In general, early-divergent persistent (EDP) lineages are dominated by unicellular species (e.g., prasinophytes in the chlorobiontic clade), whereas later-divergent lineages contain a mixture of body plans (e.g., chlorophycean and charophycean algae). Species-rich, late-divergent persistent (LDP) lineages tend to be exclusively multicellular (e.g., the land plants and metazoans).

Many workers have addressed these and other questions, often in very different ways that reflect their taxonomic or research focus (e.g., Bonner, 1998; Kirk, 2005; Newman and Bhat, 2009; Knoll, 2011; Niklas and Newman, 2013). The perspective taken in this review is an evolutionary-developmental perspective, which shows that a phenotypic novelty can be achieved by the acquisition of similar developmental motifs or "modules" that nevertheless differ in how they are mobilized or executed in different kinds of organisms. Consider the criteria for sustained cell–cell adhesion and communication in the construction of a multicellular organism. Intercellular adhesion in the brown algae involves phlorotannins and polymers of D-mannuronic and L-guluronic acids (Kreger, 1962); among the land plants (embryophytes), it is achieved by a middle lamella typically dominated by Ca^{2+}-rhamnogalacturonanic-rich pectins, which also participate in cell wall loosening (Pelletier et al., 2010). These compounds differ chemically from the type-1 transmembrane cadherin proteins responsible for animal cell–cell adhesion (Hulpiau and van Roy, 2009) or the glycoprotein-based "glues" produced by many fungi (Epstein and Nicholson, 2006). Likewise, intercellular communication in the embryophytes involves plasmodesmata (Cook et al., 1997; Raven, 1997), which differ significantly from the desmosomes and the tight or gap junctions of chordates (Fig. 2), the plasmodesmata-like structures seen in some brown algae (Terauchi et al., 2012), or the cytoplasmic bridges in *Volvox* (Green et al., 1981; Hoops et al., 2000) and colonial choanoflagellates (Dayel et al., 2011). Nevertheless, it is very likely that cell–cell adhesives and intercellular communication modules evolved from the co-option of mechanisms participating in the life cycles of unicellular ancestral life-forms (e.g., Suga et al., 2013). For example, molecular analyses identify a diversity of cadherins in the unicellular choanoflagellate *Monosiga brevicollis* (an early-divergent taxon from the unicellular metazoan progenitor) that appear to function in environment-responsive, intracellular signal transduction, e.g., tyrosine kinase and hedgehog signaling (Abedin and King, 2008). By the same token, ancient intercellular communication may have evolved by the co-option of prokaryotic

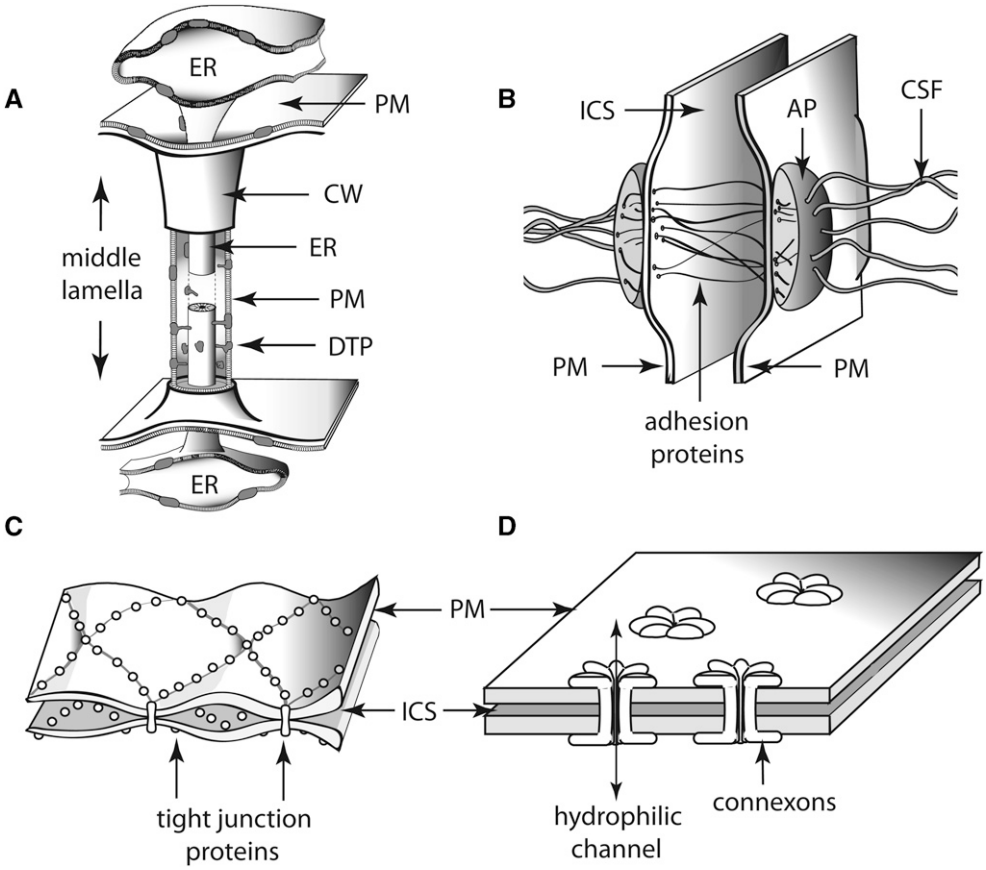

Fig. 2. Schematics of the diversity of cell-to-cell adhesion and intercellular lines of communication among adjoining (A) land plant and (B–D) animal cells. Each of these cell-to-cell linkages participates in the establishment of cell polarity as well as physiological communication among adjoining cells. Thus, each represents an analogous evolutionary innovation for two of the essential features of multicellularity. (A) Plasmodesma. (B) Desmosome. (C) Tight junctions. (D) Gap junctions. *Abbreviations*: AP = attachment plaque (plakoglobins), CSF = cytoskeletal filaments (keratin), CW = cell wall, DTP = desmotubular proteins, ER = endoplasmic reticulum, ICS = intercellular space, PM = plasma membrane.

two-component signaling pathways involving histidine kinases, response regulators, and in some cases, histidine-containing phosphotransfer proteins (see Schaller et al., 2011) that have been identified in a broad spectrum of eukaryotes including *Thalassiosira*, *Chlamydomonas*, *Dictyostelium*, a variety of fungi, and *Arabidopsis* (Anantharaman et al., 2007). Indeed, the molecular bases for cell-to-cell adhesion and communication may have evolved simultaneously in some cases, e.g., metazoan tetraspanin-enriched protein/membrane microdomains participate in cell–cell adhesion and communication as well as membrane fusion and cell migration (Bailey et al., 2011; Wang et al., 2012). Much like the functionally analogous structures collectively referred to as "leaves" (but called laminae or blades by phycologists, phyllids by bryologists, and fronds by pteridologists), multicellularity is a recurrent morphological theme in evolution that was reached in different ways by different lifeforms, i.e., in cladistic parlance, multicellularity is a character with numerous character states.

The foregoing introduces two themes that will be refined throughout this review. The first is that natural selection typically acts on functional traits and not directly on their underlying generative mechanisms. This feature enables sometimes radically different variants of a developmental motif, such as cell–cell adhesion, to achieve the same functional trait (Marks and Lechowicz, 2006). The second interrelated theme is that extensive and careful analyses are required to evaluate the hypothesis that any two developmental patterns are homologous even when they are evoked by the same molecular (genomic) sequences (for an excellent example, see Müller, 2003). This assertion also applies logically to the identification of genomic orthologies in ways that extend beyond the gene (and thus proteins or noncoding RNAs) to include the effects of domain accretion, i.e., the addition of sequences encoding extra structural domains to protein-coding genes (Koonin, 2005).

These two themes are developed by (1) presenting a brief history of evolutionary-developmental (evo-devo) biology to establish the concept of the developmental module, (2) reviewing and comparing the modules and their variants required to construct a multicellular organism, particularly plants, which are here broadly defined to include any eukaryotic photoautotroph (to include the polyphyletic algae), (3) ordering these modules in a sequence of their evolutionary occurrence that accords with a standard multilevel selection (MLS) model for multicellularity, (4) exploring an alternative route to multicellularity via the siphonous/coenocytic body plan, and (5) returning to open-ended questions such as how multistable gene expression patterns are coordinated simultaneously during the development of a multicellular life-form (by considering the genome as more than the sum of its gene expression patterns).

A BRIEF HISTORY OF EVO-DEVO

The role of the gene and gene networks—Beginning with a series of papers in the early 20th century and culminating with his book *The Genetical Theory of Natural Selection*, Ronald A. Fisher (1930) founded the field of population genetics and designated the gene as the unit of stable hereditary transmission between successive generations. This genocentric view of inheritance asserted the preeminent importance of allele frequency distributions and differential reproductive success in evolutionary processes. However, it failed to explore alternative origins of phenotypic variation. It simply assumed that all phenotypic variants result from gene mutations. Ensuing debates consequently dealt with the tempo and magnitude of mutations, but they largely ignored their causes (e.g., Lewontin, 1974). Perhaps even more restrictive was the additional assumption that the phenotype could be mapped directly onto the genotype and thus described simply by changes exclusively at the level of individual genes or sets of genes.

This outlook was challenged in the 1970s and 1980s within a field of study soon to be called evolutionary-developmental biology, or simply evo-devo, which asserted that evolutionary phenotypic transformations are the result of changes in gene expression patterns rather than the immediate products of mutations of individual genes (Laubichler, 2003; Laubichler and Maienschein, 2013; Müller, 2007). Arguably perhaps, this perspective can be traced back to a seminal paper by Roy J. Britten and Eric H. Davidson entitled *Gene Regulation for Higher Cells: A Theory* (Britten and Davidson, 1969), which focused on the connection between advances in molecular biology, gene expression patterns, and differentiation. Using *Strongylocentrotus purpuratus,* the purple sea urchin, as their model system, Britten and Davidson explored how developmental processes are regulated by the differential activities of multiple sets of genes. In their exposition, they established a clear logical connection between regulatory changes in gene expression and its consequences on phenotypic variation, which redirected attention away from the consequences of *individual* mutations to the importance of changes in the patterns of *gene network expression*.

The implications of this paradigm shift were immediately obvious to theorists—the genome became seen as an integrated regulatory system characterized by numerous possible interactions among modular components (now called *gene regulatory networks*, GRNs) affecting different aspects of differentiation and morphogenesis. An important implication of this paradigm was that GRNs were heritable units and that large-scale phenotypic changes could be the result of alterations in GRNs rather than the result of mutations affecting the code for structural proteins. In passing, it is worth noting that this perspective resonated with a much earlier and equally important conceptual shift, i.e., the transition from the *Weltanschauung* of Ernst Haeckel (1834–1919) to the world view of St. George Mivart (1827–1900). Haeckel's Biogenic Law affirmed that "Phylogenesis is the mechanical cause of Ontogenesis" (Haeckel, 1879, p. 7; see Laubichler, 2010), whereas Mivart argued that changes in ontogeny are the central cause of evolutionary change (Mivart, 1871, pp. 233–234). Like Thomas Huxley, Mivart accepted evolution as a fact, but he rejected natural selection as the primary agency of evolution and speciation.

The homology–analogy paradox—It is certainly true that many of the architects of the modern synthesis recognized that the effects of genes are interactive and that continuously varying traits generally have a polygenic basis (e.g., Dobzhansky, 1970). However, the argument that modifications of developmental mechanisms are an important route to phenotypic novelty did not seriously inform the neo-Darwinian model in which gene mutation and natural selection sensu stricto continued to dominate thinking. Indeed, this argument even failed to convince no less an illustrious embryologist as Gavin R. de Beer (1899–1972) who wrote "It is now accepted that … mutations, and recombinations of genes … are responsible for the appearance of novelties in evolution" (de Beer, 1958, p. 22). In contrast, the rapid advances in molecular biological techniques following the Britten and Davidson 1969 paper had three long-lasting effects: (1) they expanded the repertoire of organisms used to model the effects of developmental changes on evolution, (2) they allowed for increasingly broader comparative studies of GRNs, and (3) they permitted a refinement of earlier conceptualizations of the roles played by GRNs during the course of ancestor–descendant transformations.

With continued advances, researchers began to see a pecking order in GRN importance in which some elements are highly conserved and thus shared across phyletically vastly different clades, whereas other elements performed multiple functions as "switches" or "input–output" circuits (see Niklas, 2003), and still others affected specific cell fates. In so doing, the privileged position of the gene was replaced by the privileged position of highly conserved GRN control elements called "generic toolkits" by Carroll et al. (2001) and "kernels" by Davidson (2006). Perhaps the best known of these are the transcription factors that contain the more broadly distributed homeobox protein-binding DNA motif, which function in animal body axis patterning, and the MADS–box genes in fungi, plants, and animals, which have parallel functionalities. For example, the mouse and human *Pax6* genes have extensive DNA sequence similarity (Gehring, 2002) and function similarly upstream of the development of otherwise structurally very different kinds of eyes (Quiring et al., 1994) including those of squids (Tomarev et al., 1997).

The discovery of extensive homologous molecular sequences participating in the development of structures that were classically considered to be analogous as opposed to homologous, as for example compound and camera eyes, was puzzling—so much so that it was referred to as the "molecular homology–analogy paradox" (Wilkins, 2002; see also Newman, 2005, 2006). As noted by Gehring (2002, p. 69), "… there is no fundamental necessity to use a particular transcription factor for a particular function … since a transcription factor can regulate any gene". This paradox was resolved by noting that molecular homology at the level of regulatory genes guarantees neither developmental nor phenotypic homology (Müller, 2003; Niklas, 2006). Consider the ectopic expression of the normal form of *ey* in *Drosophila* and the normal *Sey* gene in mouse inserted into the fruit fly genome (Halder et al., 1995). Because *ey* and *Sey* retain their participatory function in the development of photoreceptors, the similarities of the observed phenotypes resulting from their expression indicates that these regulatory gene sequences have evolved little since the divergence of arthropods and chordates hundreds of millions of years ago. However, the gene networks targeted by *ey* and *Sey* have changed profoundly as have the morphological products resulting from their participation.

The evolution of transcription factors is further illustrated by the MADS box gene *LEAFY* (*LFY*), which is found in mosses, ferns, gymnosperms, and angiosperms. Among flowering plants, the single *LFY* gene product binds to sequences in the enhancers

of several homeotic floral genes (e.g., *APETALA1*). Among nonflowering plants, several *LFY* gene products control more general and numerous aspects of the life cycle. Thus, although the *LFY* DNA binding domain is strongly conserved across all plant taxa, the LFY protein as a whole has diverged in its functionality across taxa from mosses to angiosperms. This functional divergence is indicated by the ability of *LFY* cDNAs (isolated from mosses, ferns, and various gymnosperms linked to the *Arabidopsis* LFY promotor) to progressively recover the *lfy* mutant in *Arabidopsis* (Maizel et al., 2005), i.e., the recovery pattern mirrors the phyletic distance of the *LFY* cDNA source from angiosperms.

Two scenarios have been suggested to explain this phenomenology, both of which draw attention back to questions asked earlier in the introduction (i.e., is there a genomic toolkit or a set of "master genes" … and are they shared among all eukaryotic clades?). In the example given here, *LFY* either controls similar networks of genes that have coevolved with target genes that have themselves become modified during plant diversification, or the function of *LFY* in the different embryophyte lineages has changed completely as a result of the recruitment or intercalation of new target genes (Maizel et al., 2005). In either case, the biology *LFY* illustrates that molecular homology neither guarantees nor intrinsically reveals morphological homology and that the meaning of phrases such as "shared toolkits" and "master genes" is ambiguous at best.

DEVELOPMENTAL MODULES AND MORPHOSPACES

***Modules*—**A question asked earlier was: Are the multiple origins of multicellularity truly independent given that all eukaryotes ultimately shared a last common ancestor? We can examine this question by noting that the molecular homology–analogy paradox is easily turned on its head. That is, the developmental mobilization of very dissimilar molecular systems or processes can produce much the same phenotypic effects. This dictum has been formalized by Newman and coworkers who proposed a framework for conceptualizing the development and evolution of multicellular animals based on dynamical patterning modules (DPMs), each of which involves one or more sets of shared gene networks, their products, and physical processes common to all living things (Newman, 2005, 2006; Newman and Bhat, 2009; Newman et al., 2009). Although the importance of some of these physical processes (e.g., cohesion, viscoelasticity, diffusion, and activator–inhibitor Turing dynamics) has been known for a long time, experimental work continues to show more elaborate phenomenologies such as how the mechanical environment experienced by the extracellular matrix can alter gene expression patterns and thus cell fate by changing the physical properties of the nuclear membrane (Swift et al., 2013). Within the DPM framework, physical generic processes operating in tandem with developmental modules can act in isolation or in combination to give rise to a "pattern language" for the formation of the basic body plans of multicellular animals (Newman and Bhat, 2009; Newman et al., 2009). "Generic" in this context refers to a causal explanation predicated on mechanical forces due to the geometrical arrangements of mesoscale materials, abstract properties of network organization, symmetry breaking, or irreversibility. An explanation is generic because it can apply to living as well as nonliving phenomena. The generic nature of the physical processes associated with DPMs makes it theoretically possible for an assortment of stereotypical forms to emerge rapidly once multicellularity was achieved by metazoans, particularly since some DPMs can originate by the co-option of genes or gene regulatory networks present in unicellular or colonial organisms (Newman and Bhat, 2009; Newman et al., 2009). For instance, neuropeptide and glycosphingolipid metabolism genes previously found only in metazoans are reported for the colonial choanoflagellate *Salpingoeca rosetta* (Fairclough et al., 2013), whereas the molecular components required for the polarization of cell layers with adherens junctions are reported for the demosponge *Amphimedon queenslandica* (Fahey and Degnan, 2010).

In theory, these DPMs or their analogues can operate in plants and fungi as well as animals because of fundamental similarities among all eukaryotic cells. Consider for example cell-to-cell adhesives (see Ferris et al., 2001; Stanley et al., 2005). All eukaryotic cells have the capacity to secrete polysaccharides and structural glycoproteins that self-assemble to form extracellular matrices around animal and plant cells. Both types of matrices contain interpenetrating polymeric networks that employ hydroxyproline-rich glycoproteins (HRGPs) as major scaffolding components (collagen in animals and the HRGP extensin superfamily in various algae and in the embryophytes). These proteins generally form elongated, flexible, rod-like molecules with marked peptide periodicity (much like the modularity seen in mussel adhesives) with repeat motifs dominated by hydroxyproline in a polyproline II helical formation extensively modified by arabinosyl/galactosyl side chains. It is possible therefore that this "superfamily" of cell-to-cell adhesives evolved by the co-option of an ancestral gamete–gamete self-recognition or cell-adhesion-to-substratum toolkit. Likewise, the evolutionary expansion of pre-existing gene families encoding regulatory proteins in combination with novel physical and regulatory interactions resulting from such expansions may also have played critical roles and may even have driven the evolution of multicellular complexity (Pires and Dolan, 2012), as illustrated by the basic helix-loop-helix (bHLH) protein family involved in diverse cellular developmental processes in plants and animals (reviewed by Feller et al., 2011) and a wide array of microtubule-associated proteins in algae, embryophytes, fungi, and metazoans (Gardiner, 2013).

Nevertheless, the DPMs identified by Newman and coworkers cannot be applied directly to plant or fungal development because of substantive differences among these three major eukaryotic clades (Meyerowitz, 2002). For example, during animal development, cells are typically free to migrate and slide past one another in ways that permit differential adhesion, cortical tension, and other processes that can facilitate the sorting and assembly of some tissues, e.g., differences in CAM and P-adherin promote cell sorting during in vitro (but not in vivo) *Xenopus* gastrulation (Ninomiya et al., 2012). In contrast, plant cells are characterized as having rigid cell walls that are typically firmly fixed to one another. Plant signaling molecules can also act intercellularly as well as intracellularly as transcriptional modulators and determinants of tissue as well as cell fate (see Cui et al., 2007; Urbanus et al., 2010; Garrett et al., 2012), thereby blurring the functional separation of gene regulatory networks affecting multi- as opposed to single-cell differentiation. Although the intercellular transport of developmental transcription factors is not unknown in animal systems, it is very rare (Prochiantz, 2011). Further, cell polarity in plants involves PIN and PAN1 proteins, whereas animal cell polarity involves integrin, cadherin, and PAR or CDC42 proteins (Geldner, 2009; Dettmer and Friml, 2011; Zhang et al., 2012). Finally, cell division mechanics and the deposition of cell walls differ even among

closely species in the same lineage, as for example in different desmids and in different filamentous ascomycetes (Hall et al., 2008; Seiler and Justa-Schuch, 2010).

In light of these and other issues, Hernández-Hernández et al. (2012) proposed a preliminary set of six DPMs associated particularly with critical embryophyte developmental processes: (1) the formation and orientation of a future cell wall (FCW), (2) the production of cell-to-cell adhesives (ADH), (3) the formation of intercellular lines of communication and spatial-dependent patterns of differentiation (DIFF), (4) the establishment of axial and lateral polarity (POL), (5) the creation of lateral protrusions or buds (BUD), and (6) the construction of appendicular leaf-like structures (LLS). For the purposes of this review, only the first four of these modules (i.e., FCW, ADH, DIFF, and POL) are relevant because cell-to-cell adhesion and intercellular communication are the *condicio sine qua non* of simple multicellularity across all eukaryotic clades and because these modules operate in a pairwise manner in many multicellular algae and fungi as well as in the land plants (Fig. 3).

For example among embryophytes, the ADH and FCW modules operate in tandem because the presence of adhesive pectin polysaccharides in the middle lamella is associated with the deposition of the future primary cell walls of adjoining cells (Knox, 1992; Willats et al., 2001; Jarvis et al., 2003). The cell wall begins to be formed from cell plates during cytokinesis, such that cell adhesion is the default state (Knox, 1992; Jarvis et al., 2003) (Fig. 4A, B). Additionally, the proportion and chemical state (e.g., level of esterification) of each of the cell wall components is spatiotemporally regulated over the course of development, locally as well as globally, adjusting the mechanical properties of cells and tissues and contributing to the regulation of cell and organ growth in size, as well as to organogenesis (Jarvis et al., 2003; Peaucelle et al., 2011). A somewhat analogous system operates during the extension of fungal hyphae (Fig. 4C; see section *Cytokinesis and cell wall deposition*). The DIFF and POL modules are also functionally interconnected because both are required for cell-type specification and intercellular communication. For example, among embryophytes, DIFF and POL involve the transport of metabolites, transcription factors, and phytohormones through plasmodesmata. In some developmental systems, plasmodesmata also enable a type of generic physicochemical reaction–diffusion patterning mechanism (Pesch and Hülskamp, 2004; Jönsson et al., 2005; Benítez et al., 2011) that includes lateral inhibition mechanisms. Experimental evidence in *A. thaliana* and other model systems likewise shows that auxin flow and cell wall mechanical forces reciprocally interact during the emergence of polarity, whereas auxin promotes polar expansion by localized cell wall loosening, involving the acidification of the apoplast and the concomitant disruption of noncovalent bonds among cell wall polysaccharides (Cosgrove, 2005). The preferential localization of PINs (or their transporting vesicles) that determines auxin fluxes also targets loci for future cell wall loosening (Heisler et al., 2010). Among animals, cell polarity has been

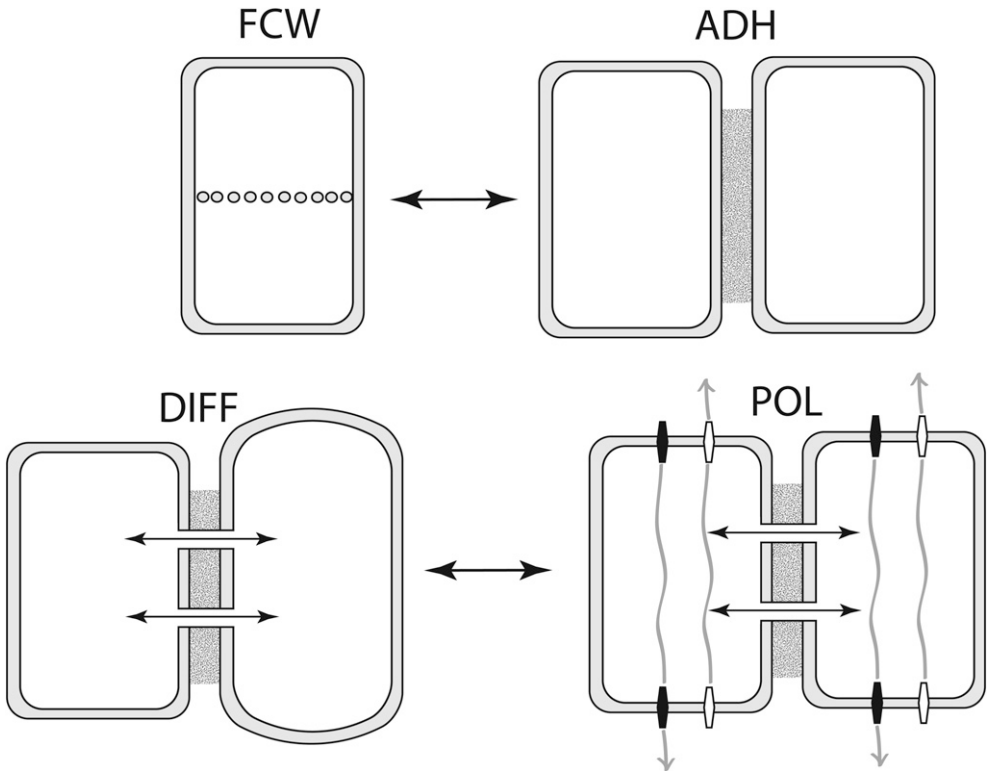

Fig. 3. Paired dynamic patterning modules (indicated by arrows) that participate in the evolution of multicellularity. The acquisition of each of these modules is required for the evolution of multicellularity. These modules operate in pairs for organisms with cell walls because cell-to-cell adhesion is related to the location of a new cell wall (see Figs. 4, 5) and because intercellular communication operates in tandem with cell polarity. *Abbreviations*: ADH, the capacity for cell-to-cell adhesion. DIFF, the establishment of intercellular communication and cellular differentiation, FCW, the future cell wall module (establishes the location and orientation of the new cell wall), POL, the capacity for polar (preferential) intercellular transport. (Adapted from Hernández-Hernández et al., 2012.)

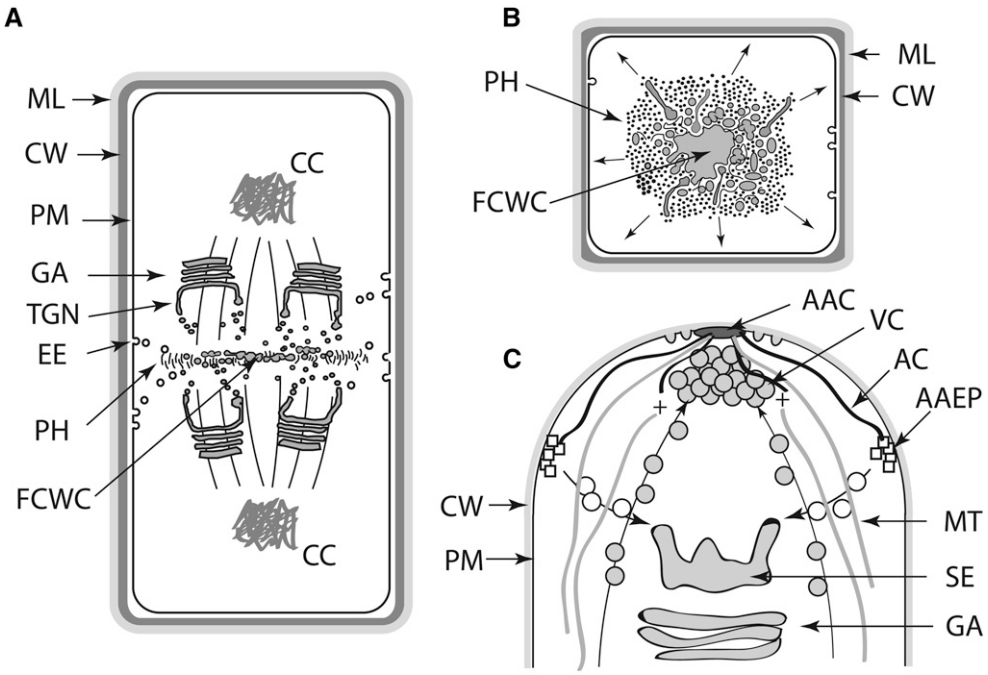

Fig. 4. Schematic of the (A, B) embryophyte phragmoplast (longitudinal and transverse views, respectively) and the (C) Spitzenkörper-polarisome of filamentous fungi. *Abbreviations*: AAC = apical actin cluster, AC = actin cable, AAEP = actin/ABPA endocytoic patches, CC = condensed chromatin, CW = cell wall, EE = endocytotic elements, FCWC = future cell wall components, GA = Golgi apparatus, ML = middle lamella, MT = microtubule, PH = phragmoplast, PM = plasma membrane, SE = post-Golgi sorting endosome, TGN = trans-Golgi network, VC = vesicle cluster.

extensively studied in the context of PAR proteins, the atypical protein kinase C (aPKC), and other proteins such as the small G protein Cdc42 as well as the roles played by the position and orientation of the mitotic spindle that in turn depend on heterotrimeric G protein signaling and the motor protein dynein (reviewed by Ahringer, 2003). For example, ASIP/PAR-3, PAR-6, and aPKC form complexes that participate in the formation of the tight junctions in mammalian epithelial cells. These junctions establish the apical and baso-lateral domains of the epithelium that limit the movement of plasma membrane proteins (Suzuki et al., 2001; see also, Goodrich and Strutt, 2011).

As predicted by Newman and coworkers, each of the FCW, ADH, DIFF, and POL modules involves the participation of generic physical mechanisms such as mechanical forces. Consider for example how the FCW module operates in embryophytes in response to mechanical stresses (see Matzke, 1946; Miller, 1980; Lintilhac, 2013). Centrifugation experiments of both haploid and diploid land plant cells show that the position of the interphase nucleus (which prefigures the preprophase band and the phragmoplast) establishes the location of the future division plane (Mineyuki and Gunning, 1990; Murata and Wada, 1991). On the basis of these and other observations, Besson and Dumais (2011; see also Pickett-Heaps et al., 1999) proposed that embryophyte cell division involves a microtubule (MT)-length-dependent force-sensing system that permits the cytoskeleton to position the nucleus (and thus the preprophase band) into a mechanically equilibrated location (Fig. 5). If the nucleus in interphase is positioned artificially off-center, the MTs radiating from it, outward to the cell cortex, will recenter the nucleus based on differences in the tensile forces generated among the MTs differing in length. Collectively shorter as opposed to longer MTs would be favored to achieve an equilibrium configuration that would axiomatically coincide with the minimal area plane. This model accords nicely with Hofmeister's (1863) and Errera's (1888) rules, both of which identify the location and orientation of the new cell wall based on simple geometric rules. Cells that are too large would have MTs that would be unable to tether the nucleus to some cell wall facets; cells that are too small would have MTs experiencing compressive rather than tensile forces. Clearly, genomic components are required for the operation of the FCW module as revealed by the persistent participation of subfamily III leucine-rich repeat-receptor-like kinases in symmetric and asymmetric cell division (Zhang et al., 2012). Thus, organisms may rely on physical forces to establish a simple default developmental condition, but they must modify their responses to these forces to achieve alternative developmental options. This is illustrated by how cell wall stresses induce the synthesis of different chitin synthase enzymes to rescue alternative septation and cytokinetic patterns in mutated yeast cells (Walker et al., 2013), or how the formation of the structures prefiguring the appearance of villi in the gut of the chick embryo relies on compressive mechanical forces generated by the differentiation of nearby smooth muscle tissue that cause the buckling of endoderm and mesenchyme (Shyer et al., 2013).

***Mapping modules into morphospaces*—**The roles of the FCW, ADH, DIFF, and POL modules played during the evolution of multicellularity are shown when their functionalities are mapped onto a morphospace identifying the major plant body plans and when this map is informed with a series of morphological transformations predicted by a simple multilevel selection model for the evolutionary appearance of multicellularity.

In general terms, a morphospace is a representation of all of the theoretically possible phenotypes within a specific group of organisms (e.g., Raup, 1966; for a review and examples, see McGhee, 1999). Each axis defining the domains within a morphospace

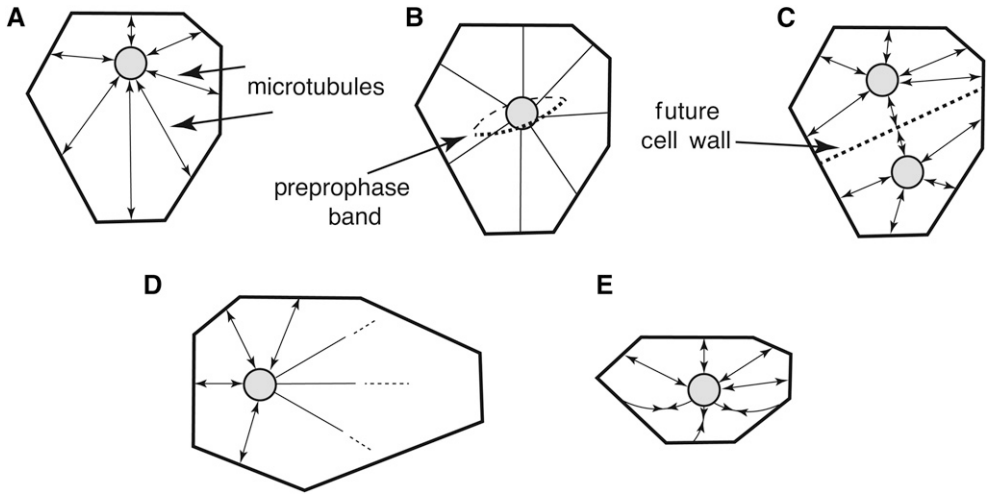

Fig. 5. Schematic of a generic mechanical model for the future cell wall (FCW) module (see Fig. 3) based on the hypothesis of Besson and Dumais (2011). The preprophase band is established around the nucleus before mitosis and prefigures the location of the phragmoplast, which facilitates the deposition the future cell wall. It is hypothesized that the location of the nucleus (and thus the preprophase band and the future cell wall) is determined by microtubules achieving equilibrium lengths, minimizing the distance between their attachments to the cell wall and the nucleus (A–C). The microtubules tethering the nucleus to the walls of cells that are (D) too large or (E) too small are unable to achieve mechanical equilibrium unless additional mechanisms are evoked.

represents a developmental variable or process that describes or obtains a phenotypic character (with one or more character states). Each intersection of two or more axes identifies a hypothetical phenotype with the character states specified by the variables or processes stipulated by the participating axes. A morphospace for plant body plans was constructed previously using four developmental axes, each with two character states (Niklas, 2000): (1) whether cytokinesis and karyokinesis are synchronous, (2) whether cells remain aggregated after they divide, (3) whether symplastic continuity or some other form of intercellular communication is maintained among neighboring cells, and (4) whether individual cells continue to grow indefinitely in size (Fig. 6). The intersections of these axes identify four major body plans, each of which can be theoretically either uninucleate or multinucleate: (1) the unicellular body plan, (2) the siphonous/coenocytic body plan, (3) the colonial body plan, and (4) the multicellular body plan. The addition of a fifth axis—the orientation(s) of cell division—distinguishes among the various tissue constructions of the multicellular plant body plan: (1) the unbranched filament, which results when cell division is confined to one plane of reference, (2) the branched filament (with or without a pseudoparenchymatous tissue construction), which requires two planes of cell division, and (3) the parenchymatous tissue construction, which requires three planes of cell division (Fig. 7).

A review of the secondary and primary literature treating the algae (Graham et al., 2009 and references therein) shows that all but two of the 14 theoretically possible phenotypes are represented by one or more species. It also reveals considerable homoplasy among various plant lineages. For example, the unicellular multinucleate variant with determinate growth is represented by the chlorophycean alga *Bracteacoccus* and the ulvophycean alga *Chlorochytridium*; the colonial multinucleate body plan is represented by the chlorophycean algae *Pediastrum* and *Hydrodictyon*; the siphonous body plan is represented by the ulvophycean alga *Caulerpa* and the xanthophycean alga *Vaucheria*; and the multicellular multinucleate (siphonocladous) branched variant is represented by the rhodophycean alga *Griffithsia* and the ulvophycean alga *Cladophora*. Among the multinucleate multicellular (siphonocladous) variants differing in tissue construction, the unbranched and branched filamentous variants are represented by the ulvophycean algae *Urospora* and *Acrosiphonia*, respectively; the siphonocladous body plan with a pseudoparenchymatous tissue construction is represented by species within the ulvophycean genus *Codium*.

The two variants that are not represented by any extant or extinct species are the uninucleate indeterminate (siphonous) body plan and the parenchymatous siphonocladous variants. The absence of the former may be the result of physiological constraints imposed by the volume of cytoplasm that a single nucleus can sustain, a hypothesis proposed by Julius Sachs (1892) and recently revisited in the context of the midblastula transition in animal ontogeny (Collart et al., 2013). A convincing explanation for the absence of a parenchymatous siphonocladous body plan remains problematic, although constraints on the construction of reproductive organs have been unconvincingly proposed in the context of the red algae (Hommersand and Fredericq, 1990). Regardless, the evolutionary significance of the ADH, FCW, DIFF, and POL modules in light of the plant body plan morphospace is obvious: ADH is required for the construction of the colonial and multicellular body plans; FCW participates in the synchronicity of cyto- and karyokinesis and participates in the tissue construction of a multicellular plant body; and DIFF and POL are required for intercellular physiological coordination and cellular specialization.

The ADH, FCW, DIFF, and POL modules also establish character polarities in the context of a multilevel selection theory for the evolution of multicellularity (Folse and Roughgarden, 2010; Niklas and Newman, 2013) (see section *Two-phased multicellular evolution*). This theory identifies the unicellular body plan as ancestral to the colonial body plan that in turn is ancestral to a truly multicellular body plan, i.e., it identifies a "unicellular ⇒ colonial ⇒ multicellular" body plan transformation series that requires ADH to establish and maintain a colonial body plan and FCW, DIFF, and POL to coordinate and

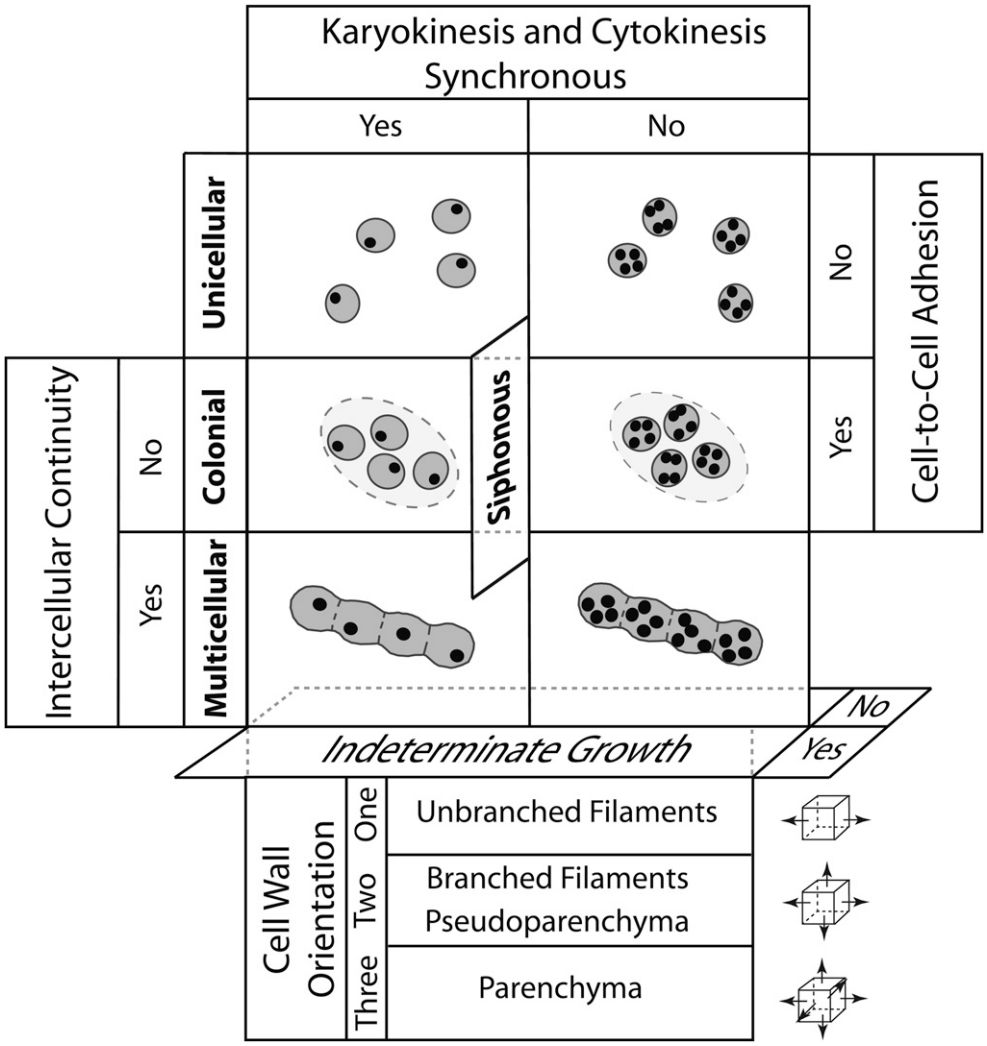

Fig. 6. A morphospace for the four major plant body plans shown in bold (unicellular, siphonous/coenocytic, colonial, and multicellular) resulting from the intersection of five developmental processes: (1) whether cytokinesis and karyokinesis are synchronous, (2) whether cells remain aggregated after they divide, (3) whether symplastic continuity or some other form of intercellular communication is maintained among neighboring cells, and (4) whether individual cells continue to grow indefinitely in size. Note that the siphonous/coenocytic body plan may evolve from a unicellular or a multicellular progenitor. The lower panels dealing with the plane of cell division, localization of cellular division, and symmetry pertain to the evolution of complex multicellular organisms. (Adapted from Niklas, 2000.)

specify intercellular activities to achieve an integrated multicellular phenotype whose complexity exceeds simple dyatic interactions among individual cells.

The volvocine green algae provide particularly valuable insights into aspects of the unicellular ⇒ colonial ⇒ multicellular transition series (Bonner, 2000; Kirk, 2005; Herron and Michod, 2008). The ancestral state in the volvocines is inferred to be a unicellular organism probably similar to extant species of *Chlamydomonas*. Transformation of the unicellular cell wall into an extracellular matrix (seen in the Tetrabaenaceae ⇒ Goniaceae ⇒ Volvocaceae transformation series), incomplete cytokinesis (seen in the Goniaceae ⇒ Volvocaceae transformation series), and the appearance of additional derived traits produce forms ranging from simple cellular aggregates (e.g., *Tetrabaena socialis*) to colonies with complex, asymmetric cell division, to quasi-multicellular organisms with full germ-soma division of labor (e.g., *Volvox carteri*) (Kirk, 2005; Herron and Michod, 2008). In the case of the latter, cytoplasmic bridges with multiple functionalities are maintained among neighboring cells, i.e., they participate in the mechanics of kinesin-driven inversion, and they serve as conduits to provide nutrients to developing gonidia (Hoops et al., 2000). In adult plants, these bridges are extensive in number and broader than plasmodesmata (~200 nm in diameter; Green et al., 1981). These bridges are developmentally severed in some volvocine taxa, which provide interesting examples of a multicellular to colonial transformation series.

The prokaryotes provide additional insights. Among the unicellular cyanobacteria, amidases hydrolyze the peptoglycan-rich septal walls of adjoin cells thereby separating them shortly after they divide. Likewise, *N*-acetyl-muramyl-L-amidase (AmiC1) hydrolyzes the septal peptoglycans adjoining *Escherichia coli* cells, whereas the *E. coli AmiABC* triple mutant results in the formation of colonies consisting of filaments (Lehner et al., 2013). However, in multicellular members of the Nostocales, Lehner et al. (2013) have shown that the amidase AmiC2 protein localizes to and drills clusters of ~20 nm pores through the

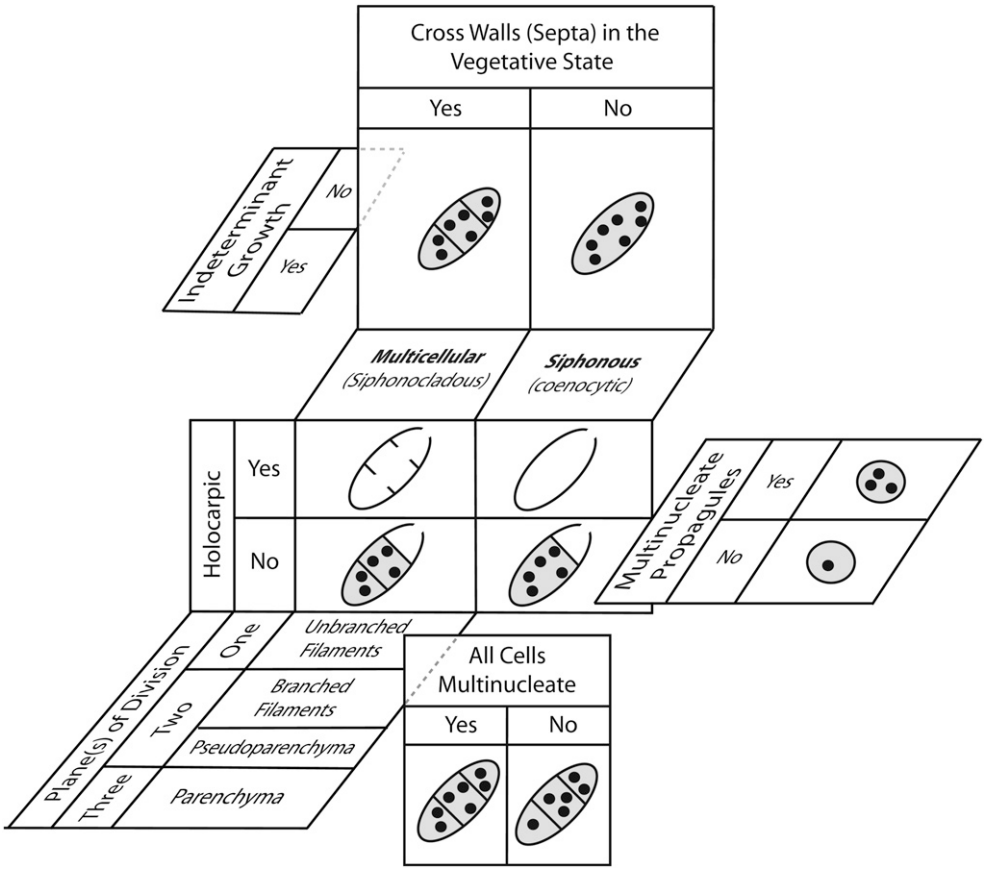

Fig. 7. An elaboration of the multinucleate condition (among the four body plans identified in Fig. 6) resulting from the intersection of three additional developmental processes: (1) whether the organism dies after reproducing (e.g., algae with a holocarpic or nonholocarpic life cycle), (2) whether it produces uninucleate or multinucleate propagules (the latter as produced by arbuscular mycorrhizal fungi, the Glomeromycota, e.g., *Glomus etunicatum*), and (3) whether all cells are multinucleate. (Adapted from Niklas et al., 2013.)

septal walls of *Nostoc punctiformae* (strain ATCC 29133), which form intercellular conduits that establish communication among cells and simple multicellularity. It is likely that these pores also participate in cellular specialization, e.g., the reaction–diffusion system responsible for the differentiation of heterocysts in *Anabaena* and *Nostoc* (described by Wilcox et al., 1973).

Evidence for a transition from colonial to multicellular lifeforms in animals and in different algal lineages is reviewed by Niklas and Newman (2013).

Two-phased multicellular evolution— But how does a colonial aggregate of cells achieve individuality? Multilevel selection theory identifies two evolutionary stages—an alignment-of-fitness phase (denoted as MLS1) in which genetic similarity among adjoining cells prevents cell–cell conflict and an export-of-fitness phase (denoted as MLS2) in which cells become interdependent and collaborate in a sustained physiological and reproductive effort (Michod and Anderson, 1979; Damuth and Heisler, 1988; Michod and Nedelcu, 2003; for a general review, see Folse and Roughgarden, 2010). Phyletic analyses of lineages in which obligate multicellularity has evolved are consistent with this MLS1 and MLS2 model. They also show that lineages characterized by species with clonal group formation are more likely to have undergone an evolutionary transition to obligate multicellularity than lineages characterized by species with nonclonal group formation (Fisher et al., 2013).

MLS1 is typically achieved by a "unicellular bottleneck", which occurs in every organism's life cycle, e.g., a spore, zygote, or uninucleate asexual propagule (see Niklas and Newman, 2013). This bottleneck establishes genetic homogeneity among subsequently formed cells (or, more precisely, among nuclei) even among asco- and basidiomycete heterokaryotic fungi for which experimental data indicate competition among genetically different nuclei sharing the same cytoplasm. For example, nuclear ratios of heterokaryons in the ascomycetes *Penicillium cyclopodium* and *Neurospora crassa* are reported to change depending on environmental conditions in ways that reflect the underlying fitness of the constituent homokaryons grown in isolation (Jinks, 1952; Davis, 1960).

James et al. (2008) report similar results for *Heterobasidion parviporum* and conclude that this basidiomycete violates the standard model of what constitutes an individual since genetically different nuclei compete among themselves to form homokaryotic hyphae. However, it must be recognized that an absence of conflict does not mean an absence of competition—and competition can be a good thing. Indeed, there is evidence to suggest that competitive–cooperative interactions have shaped form–function relationships even at the simple molecular level (Foster, 2011). Consider that many developmental processes employ lateral inhibition in which neighboring genomes

compete to adopt the same cell fate, e.g., during gonad development of *Caenorhabditis elegans*, cells compete to develop into either a terminally differentiated cell or a ventral uterine precursor cell, which is determined by the relative amounts of the LIN-12 receptor and its LAG-2 ligand (Greenwald, 1998). Likewise, during *Drosophila melanogaster* development, cell competition controls the size of wings. The ubiquitous expression of Myc abolishes cell competition, and wings become larger than normal; the addition of even a very few wild-type cells reestablishes cell competition and brings wing size back to normal (de la Cova et al., 2004).

It is equally important to recognize that mitosis does not invariably result in genetically identical derivative cells even in the absence of mutation or chromosomal aberrations. Preferential sister chromatid segregation is observed in plants, fungi, and animals (Lark, 1967; Lark et al., 1966; Rosenberger and Kessel, 1968). Further, during the early development of female mammals, one of the two X chromosomes is randomly silenced and faithfully perpetuated during subsequence cell proliferation (Lyon, 1961; Chow et al., 2005). Methylation patterns of cytosine in CpG doublets and other epigenetic changes provide additional avenues for establishing genetically different groups of cells in the same organism, each of which required the evolution of stable interdependent cell lineages sharing the same genome but expressing different gene network patterns.

Indeed, epigenetic mechanisms may be critical to *maintaining* multicellularity. Consider that the principal limitation to achieving and maintaining cooperation is the appearance of "defectors" in an evolutionary game setting (i.e., participants that consume resources but fail to confer any benefit to other players) (Hamilton, 1964). An obvious example of cellular defectors is animal neoplasms, which may have deep genetic roots in terms of the regulation of cell proliferation and de-differentiation (Coutinho et al., 2003). Numerous mechanisms to maintain cooperation and reduce or eliminate defectors have been suggested, among which the effects of group selection, direct and indirect reciprocity, network structure, and tag-based donation schemes are perhaps best known (Nowak, 2006; Celiker and Gore, 2013). However, all of these mechanisms require players to remember past proceedings or to possess some method of recognizing one another as players in the same game. Epigenetic mechanisms as well as signaling pathways that connect metabolic status with nutrient availability or other environmental factors (e.g., the TOR signaling pathway) provide one solution to dealing with defectors, while the unicellular bottleneck provides, at least initially, a homogeneous collection of cooperating cells. There are other tactics as well. Theoretical models show that resource limitations can cause the rules of a game to change in ways that foster cooperation among players with no memory and no recognition of one another (e.g., Requejo and Camacho, 2013). Likewise, zero-determinant models show that altruistic and generous strategies can sustain cooperation and reduce negative interactions (e.g., Stewart and Plotkin, 2013). It is worth noting further that cheater mutants in the social amoeba *Dictyostelium discoideum* and the mouse *Mus musculus* are reported to cooperate in ways that conform to normal developmental patterns and that do not disrupt the functionality of the collective organism (Santorelli et al., 2008; Dejosez et al., 2013), which indicates cooperation may be an emergent property of ancient and robust gene networks. Finally, theoretical considerations indicate that "cheaters" can evolve to function as asexual propagules in very ancient proto life cycles (Rainey and Kerr, 2010). In summary, cooperation among cells and nuclei can evolve along a number of routes and with different consequences, which may explain why colonial life-forms, multinucleate cells, and multicellularity are not uncommon.

Nevertheless, MLS2 requires that selection shift from the level of individual cells to the level of an emergent entity that reproduces a functionally integrated phenotype with a heritable fitness (typically followed by some degree of cellular specialization). The key difference between MLS1 and MLS2 is that the fitness of the cell-group (aka "the colony") is an additive function of the fitness of individual cells, whereas the fitness of a multicellular organism is nonadditive (Damuth and Heisler, 1988). Put differently, the evolution of a multicellular organism requires a means to guarantee the heritability of fitness at the emergent level of the multicellular entity. In some but not all multicellular organisms, this guarantee is accomplished by sequestering a "germline" e.g., animals and embryophytes, respectively (see Dickinson and Grant-Downton, 2009). (It is noteworthy that, with very few exceptions [e.g., *Volvox*], the separation of a germline from the soma does not occur in the land plants nor in any algal lineage, i.e., somatic embryogenesis is the norm.) A germ–soma separation may be an indirect consequence of the necessity to compensate for the increasing costs of evolving a progressively larger body size (see Solari et al., 2013). Body size matters because the probability of compounding a genetic error or mutation increases as a function of the number of cell divisions required to achieve the size of a mature organism. Small multicellular organisms have a lower probability of introducing errors into their reproductive cells, whereas progressively larger organisms escape Muller's ratchet (the inevitable accumulation of deleterious mutations) by ultimately sequestering cells in a germ-line. It is also worth noting that cellular specialization may evolve more easily in larger organisms than in smaller because unsuccessful attempts at specialization are more easily tolerated in larger organisms (Willensdorfer, 2008).

However, obligate sexual reproduction is not required to override the conflict between a multicellular individual and its constituent cells (Buss, 1987; Michod, 1997). As noted, in the absence of somatic mutations, the presence of a spore or similar reproductive unit assures a unicellular bottleneck regardless of the type of life cycle. Although it can be difficult for asexual organisms to escape the consequences of Muller's ratchet and its consequences on fitness (Elena and Lenski, 1997), even an asexual organism experiences an alignment-of-fitness by means of unicellular propagules, which can purge deleterious genomic changes as a consequence of the death of individual propagules. Likewise, multicellularity is not required for cellular specialization. Unicellular bacteria, algae, yeast, and amoeba exhibit alternative stable states of gene expression patterns and manifest alternative cell morphologies during their life cycles, often as a result of competing processes, e.g., motility vs. mitosis. This feature is particularly intriguing in light of the studies showing that seemingly random fluctuations in cellular dynamics may provide a simple switch for changing cell fate. For example, *Bacillus subtilis* can exist in two stable forms, called vegetative and competent, under conditions of nutrient deficiency. A simple mathematical model using a stochastic algorithm can predict how and when these two cellular conditions are decided based on the level of biological noise in the system (Ozbudak et al., 2002; Maamar et al., 2007). In addition, mathematical models indicate that cellular differentiation can emerge among genetically identical cells in response to the incompatibilities among competing physiological processes (Ispolatov

et al., 2012) or simply because of the metabolic costs of switching the tasks a cell must perform to stay alive or complete its life cycle (Goldsby et al., 2012). In more derived lineages, an alignment-of-fitness can compensate for conflicts of interest among cellular components such that a division of cellular labor becomes possible and even necessary. Even a loose "colony" of cells can have emergent biological properties that give it a collective edge in which every cell benefits (Solé and Valverde, 2013). The origin of the cellular differentiation (DIFF) module therefore may reside in the inherent multistability of complex gene regulatory networks (Laurent and Kellershohn, 1999) with somatic or reproductive functional roles for different cell-types possibly established by natural selection ad hoc.

AN ALTERNATIVE ROUTE TO MULTICELLULARITY

The conventional scenario for the evolution of multicellular organisms posits a unicellular (uninucleate) ⇒ colonial ⇒ multicellular body plan transformation series. However, an alternative series is equally plausible in the case of algae, embryophytes, fungi, and even animals—the direct developmental transition of a multinucleate cell into a multicellular life-form (Niklas et al., 2013).

The siphonous/coenocytic body plan—This alternative route is perhaps best illustrated in the context of the morphospace shown in Fig. 6 with the aid of only three additional modules, each of which has two character states: (1) whether the organism dies after reproducing (e.g., algae with a holocarpic or nonholocarpic life cycle), (2) whether it produces uninucleate or multinucleate propagules (the latter are produced by arbuscular mycorrhizal fungi, the Glomeromycota, e.g., *Glomus etunicatum*), and (3) whether all cells are multinucleate (Fig. 7). A survey of the literature shows that all theoretical possibilities within this domain exist in nature and that considerable homoplasy exists, e.g., the siphonous holocarpic variant with uninucleate propagules is represented by xanthophycean alga *Botrydium* and the chlorophycean alga *Characiosiphon*, whereas its counterpart with multinucleate propagules is represented by the xanthophycean alga *Vaucheria* and the chlorophycean alga *Protosiphon*. The literature also reveals that some siphonous lineages are morphologically diverse and complex (e.g., Pia, 1920) and that many are ancient, e.g., a multilocus time-calibrated analysis reveals that the Dasycladaceae likely originated in the Neoproterozoic (Verbruggen et al., 2009).

Perhaps more important in the context of this review is the observation that, in theory, the siphonous body plan can serve as the ancestral condition for a multicellular organism (by the developmental elaboration of cross wall formation), just as it may be derived from a multicellular organism (by the developmental suppression or elimination of cross wall formation). The "siphonous ⇒ multicellular" transformation series is not just a theoretical possibility. It occurs during the ontogeny of siphonocladean algae through a process called segregative cell division (Fig. 8), which may have evolved by co-opting a wound healing response mechanism in a siphonous ancestor (Børgesen, 1905; La Claire, 1982). It also occurs after endosperm free-nuclear development (Schnarf, 1931), during early stages in gymnosperm and animal embryogenesis (Bower, 1881; Zalokar and Erk, 1976; Foe and Alberts, 1983), chytrid zoospore differentiation (Lessie and Lovett, 1968), asco-, basidio-, and zygospore formation (Bracker, 1968; Moore et al., 2011), and during the vegetative transition from the fully coenocytic mycelial phase to the fully septate mycelial phase of Zygomycota and some Basidiomycota (e.g., Ainsworth and Rayner, 1991). Siphonous/coenocytic body plans also occur in the schizontic phase of foraminifera and giant amoebae (e.g., *Chaos chaos* aka *C. carolinensis*), whereas the ichthyosporeans *Creolimax fragrantissma* and *C. arctica* (which are members of an early-divergent lineage related to the metazoans) form multicellular colonies directly from fully grown multinucleate syncytia as a result of synchronized karyo- and cytokinesis (Suga and Ruiz-Trillo, 2013). Indeed, just as in some algae with a diplobiontic alternation between a diploid and a haploid generation, both phases in the life cycle of gymnosperms also have a free nuclear phase followed by the development of a multicellular body plan, e.g., ≈1000 free nuclei are formed during the early development of the *Cycas* megagametophyte; an equivalent number of free nuclei occur early in the development of the *Dioön* embryo (Bierhorst, 1971). Interestingly, all eusporangiate heterosporous plants have a free nuclear phase (unlike leptosporangiate ferns, which do not have it); there are no known homosporous land plants with a free nuclear phase (Dennis W. Stevenson, personal communication).

The siphonous body plan may confer a selective advantage similar to that of polyploidy (without having the disadvantages of polyvalency), i.e., it permits a single cell to achieve a large size (Kondorosi et al., 2000) and thus explore more than one microenvironment for nutrients. However, on the basis of character polarities inferred from molecular cladistic analyses of different plant clades (e.g., Lewis and McCourt 2004; Maistro et al., 2007; Verbruggen et al., 2009; Cocquyt et al., 2010), siphonous organisms may have evolved under a variety of conditions under different selection regimes (Niklas et al., 2013). For instance, based on their analyses of the Ulvophyceae, Cocquyt et al. (2010) concluded that the siphonous body plan is an evolutionary dead end emerging from a unicellular ancestor that evolved indeterminate growth under selection pressure to forage for resources from its substrate. In contrast, inspection of a phylogeny for the Tribonematales (Xanthophyceae) constructed by Maistro et al. (2007) using plastid *rbcL* and *psaA* genes shows that the unicellular uninucleate and the colonial uninucleate body plans (represented by *Chlorellidium tetrabotrys* and two species of *Heterococcus*) occur in what most workers consider to be members of an early-divergent, persistent lineage within this clade; the siphonous body plan (represented by *Asterosiphon dichotomus* and *Vaucheria terrestris*) occurs in species belonging to a lineage that diverged later; and that the multicellular uninucleate body plan (represented by species of *Tribonema* and *Xanthonema*) resides on a lineage that diverged even later within this clade. It is possible therefore that within the Tribonematales, the "unicellular (uninucleate) ⇒ siphonous ⇒ multicellular" transformation series may have evolved at least once. This hypothesis is consistent with the occurrence of siphonocladous species within this clade. It should be further noted that the "unicellular ⇒ siphonous ⇒ multicellular" transformation series is consistent with current phylogenies for fungi that show that the Zygomycota comprise an early-divergent lineage with reference to the appearance of either the Glomeromycota or the dikaryomycetes (Voigt and Wöstemeyer, 2001; Lutzoni et al., 2004).

That this transformation series is as rare as the evolution of very large siphonous/coenocytic organisms is not surprising. A siphonous organism is vulnerable to the rapid systemic spread of a pathogen if its cell membrane is breached. Likewise, although *Caulerpa* cells are among the largest cells, Einstein's equation for random walks shows that, beyond a certain size,

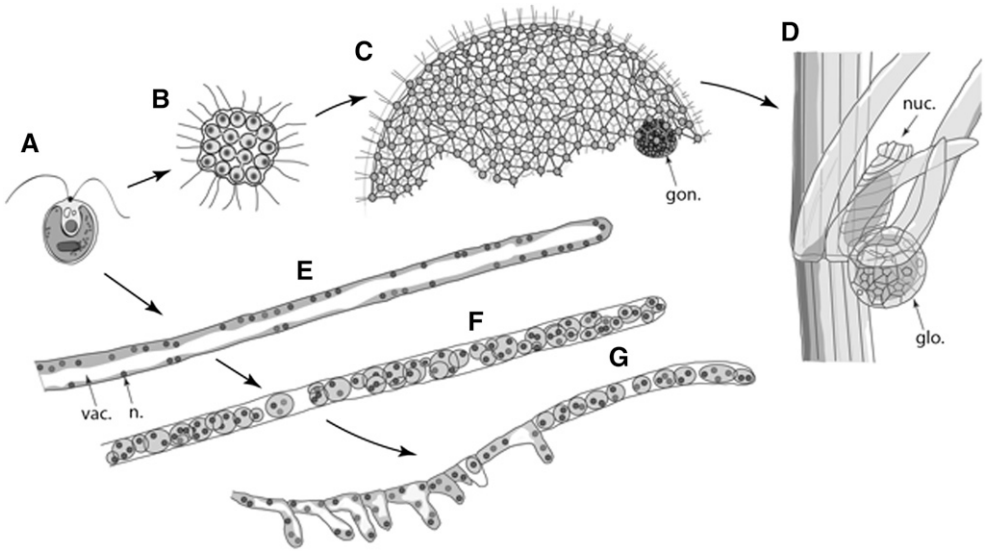

Fig. 8. Two plant body-plan transformation series, each of which is capable of achieving multicellularity. The conventional model posits a unicellular (uninucleate) ⇒ colonial (without and eventually with germ cell line) ⇒ multicellular transformation series illustrated here with *Chlamydomonas* (A) ⇒ *Gonium* (B) + *Volvox* (C) ⇒ *Chara* (D). An alternative model also starts with a unicellular *Chlamydomonas*-like ancestor (A) that evolves a siphonous/coenocytic body plan (E) that subsequently achieves multicellularity by means of a cellularization process perhaps analogous to segregative cell division (F, G), which occurs during the early development of some multicellular (multinucleate) green algae such as *Siphonocladus*. Both of these transformation series requires an alignment-of-fitness phase and an export-of-fitness phase. The taxa used to illustrate these two transformation series are not drawn to scale and are not intended to show phylogenetically legitimate ancestor–descendent relationships. *Abbreviations:* glo. = globule, gon. = gonidium, n. = nucleus, nuc. = nucule, vac. = vacuole.

selection favors cellularization because the time a message takes to travel from one side of a cell to another increases dramatically as the volume of the cytosol increases. Specifically, this equation takes the form $\bar{x}^2 = 2Dtx$, where is $\bar{x}^2$ is the square of the mean distance a molecule moves randomly from its point of origin ($x = 0$), D is molecular diffusivity, and t is time. Therefore, if $D = 10^{-5}$ cm^2/s, we see that $t = 5 \times 10^{-4}$ s when $x = 10^{-4}$ cm (about the width of a bacterium), but that $t \approx 14$ h when $x =$ 1 cm (about the width of test tube). Active transport and cytoplasmic streaming can ameliorate this generic physical limitation. However, both of these require energy and a sophisticated cytoskeletal system with diverse molecular motor proteins to target the delivery of specified molecular cargoes. Another difficulty is that coordinated development even in a siphonous organism requires positional information that gene expression levels carry, which is limited by molecular noise (Tkačik and Walczak, 2011). The ability of nuclei (whether in a single cell or in adjoining cells) to register their position is limited if the concentrations of transcription factors are low, or if the absolute copy numbers of the output proteins are small. There are physical sources of noise as well that cannot be reduced without a cell investing additional resources in making these proteins.

On the other hand, there are at least three advantages of having a siphonous body plan: (1) a buffer against deleterious mutations as a consequence of multiple genome copies (or, conversely, a platform to segregate defective nuclei during the formation of uninucleate gametes or multinucleate asexual propagules), (2) higher metabolic and growth rates due to amplified chromosomal copies of ribosomal RNA cistrons (as an analog of one of the possible effects of endoreduplication or polyploidy), and (3) a multinucleate cell has the potential to occupy diverse microenvironments when coupled with indeterminate growth (which, for a tubular organism with a large vacuole, can confer the additional advantage of increasing the effective surface area with respect to volume for mass and energy exchange).

Cytokinesis and cell wall deposition—The foregoing shows that a multicellular organism can evolve directly either from a colonial, or a siphonous/coenocytic progenitor (Fig. 8). In either case, the evolution of multicellularity requires the acquisition of a mechanism to partition cytoplasm into uni- or multinucleated cellular units. In the context of this review, this capacity is biologically trivial since the machinery for cytokinesis is present in every unicellular organism capable of binary fission and thus not unexpectedly involves ancestral molecular and physical commonalities, e.g., the endosomal protein ESCRT III participates in the final act of cytokinetic membrane constriction in virtually every eukaryotic lineage (Carlton and Martin-Serrano, 2007, 2009). Far more important, at least for plants and fungi, is that fact that cellularization also requires a mechanism for coordinating cytokinesis with cell wall deposition. Although some commonalities exist among phyletically unrelated organisms (Nacry et al., 2000; Assaad, 2001; Baluška et al., 2012), the mechanism by which this is accomplished differs among clades and even within the same clade (Mine et al., 2008; Seiler and Justa-Schuch, 2010). For example, cytokinesis among embryophytes involves a phragmoplast and the formation of a new cell plate that starts at the center of the cell and proceeds centrifugally outward toward the parental cell wall (phragmoplastic cytokinesis; see Fig. 4A, B) (Pickett-Heaps et al., 1999), a process that involves a complex sequence of cytoskeletal and membrane dynamics with a vast array of molecular players (Dhonukshe et al., 2006; McMichael and Bednarek, 2013). With the exception of the charophycean algae and the Trentepohliales, cytokinesis among the various other algal lineages is accomplished by a diaphragm-like furrowing of the plasma

membrane and associated cell wall layers that develops as a centripetally growing septum (phycoplastic cytokinesis; see Graham, 1996). A hybrid of these two forms of cytokinesis is reported for the charophycean (Zygnematales) alga *Spirogyra* in which a diaphragm-like furrow organizes the centripetal ingrowth of a furrow and a phragmoplastic-like array of microtubules organizes the centrifugal development of cell plate vesicles (Sawitzky and Grolig, 1995). In contrast, among the filamentous fungi, cell wall deposition involves a functional analog of the phragmoplast, called the Spitzenkörper-polarisome complex (Girbardt, 1957; Harris et al., 2005), which organizes the vectorial delivery of cytosolic vesicles containing cell wall and membrane constituents that are synthesized on the pre-existing plasma membrane and extruded into the cell wall space at the leading edge of extending hyphae (see Fig. 4C). Finally, structural convergences during cytokinesis and cell wall synthesis occur among the Ascomycota, uredinalian basidiomycetes (Littlefield and Bracker, 1971), Florideophycidae (Pueschel, 1977), and some green algae (Brawley and Sears, 1982), all of which form septal plugs. The convergence in their appearance can be stunning as for example in the plugs produced by the green alga *Smithsoniella* and some Florideophycidae (Sears and Brawley, 1982). In some of these taxa, septal plugs can be removed to create new cytoplasmic avenues of intercellular communication and transport much in the same fashion as secondary plasmodesmata can be used. Nevertheless, all of these structures differ in their chemical composition and ultrastructural details.

Raison d'être for multicellularity— Are the motifs in the morphological transformation series seen in multicellular lineages the result of adaptive evolution, relaxed selection, or the inevitable consequences of generic physical laws coupled to very simple genomic processes? As noted, regardless of an organism's body plan, every genome is capable of multiple gene expression patterns that can produce different cellular functionalities (Laurent and Kellershohn, 1999; Libby and Rainey, 2013). This phenomenology is seen in unicellular bacteria as well as in unicellular algae, yeast, and amoebae exhibiting alternative stable states of gene activity during their life times. It therefore predates developmental transcription factors, such as MADS box and Hox gene products (along with their *cis*-acting target sequences) that regulate multistable regulatory networks whose alternative states determine cell type identity in multicellular organisms. Given this ubiquity, it is tempting to suppose that multicellularity might "simply happen" if it conferred no disadvantage or if it provided even the slightest advantage as for example by allowing interconnected and related cells to use different resources simultaneously. Although a single cell cannot express multiple gene expression patterns simultaneously, a multicellular organism can. Nevertheless, experimental enucleation of large cells, such as those of *Acetabularia*, shows that the full repertoire of gene expression patterns can reside in a single intracellular mRNA or ribosomal pool that survives long enough for complex morphogenetic events to proceed to completion in stage-specific ways because they can be developmentally retrieved from the pool at the right time and in the right place from within a single cell (reviewed by Menzel, 1994). How this is achieved remains unknown. That it exists however indicates that multicellularity is neither required for complex patterns of morphogenesis nor for the simultaneous coexistence of different gene network products. However, as noted, there is one important difference between the co*existence* and the co*expression* of intracellular mRNA or ribosomal pools of information. A single cell is capable of the first; a group of related cells is required for the second. Indeed, mathematical models show that cellular/nuclear differentiation can emerge even among genetically identical adjoining cells in response to competing physiological processes (Ispolatov et al., 2012; see also Furusawa and Kaneko, 2002). Although the evolution of a minimum number of different cellular/nuclear functionalities is likely required before selection can act on variants of gene expression patterns to establish ancillary developmental modules that control cell differentiation (Arnellos et al., 2013), it appears that the preconditions required to attain multicellularity exist in every lineage and perhaps in every cell.

This assertion does not directly address a critical question, viz. does multicellularity provide any measureable selective advantage? Experience and selection theory show that major evolutionary innovations are not retained within a lineage if they are incompatible with survival. However, this platitude does not mean that every transition requires a large or even a measurable advantage (Grosberg and Strathmann, 2007), nor that phenotypic responses to selection must invariably align with an adaptive advantage (Bonduriansky and Day, 2009). Theoretical models for filamentous bacteria show that strains with the same fitness can produce genotypes differing in cell number as a result of differences in cell division and death rates or as a result of changes in the environmental carrying capacity. One model, which has empirical support, also shows that differences in fitness attributable to morphology are not required a priori for the evolution of life cycles with multicellular entities (Rossetti et al., 2011), although advantages may arise later (Koschwanez et al., 2011). The retention of multicellularity in some lineages therefore may reflect a simple probabilistically driven random walk from the left (unicellular) wall of life to the right (multicellular) wall (sensu Gould, 1989). To be sure, there are examples of unicellular organisms descending from multicellular ancestors (Velicer et al., 1998). Likewise smaller life-forms, such as some species of *Pleodorina*, can have larger multicellular ancestors, like *Volvox* (Kirk, 1998), and the reduction in the size of the gametophyte generation of the embryophytes over the course of land plant evolution is an obvious example of the diminution of a multicellular body plan. However, in general, once a body plan passes through the export-of-fitness phase and achieves complex multicellularity, its capacity for random evolutionary reversion to a simpler body plan is reduced for reasons that have little or nothing to do with selection on fitness.

Nevertheless, there are undeniable advantages to a multicellular body plan and even to the colonial or siphonous body plan that theoretically prefigure its evolution. For example, even the most simple colonial body plan allows different cells to use different resources as a result of physiological specialization even in the absence of morphological differentiation. Likewise, the colonial body plan (and the siphonous body plan) can also reduce the risk of predation as a consequence of achieving larger sizes (that can ratchet body size upward as a result of an "arms war"). Eight-celled colonies of *Chlorella vulgaris* are established and are sustained when steady-state cultures of this normally unicellular green alga are inoculated with the phagotrophic flagellate *Ochromonas vallescia* (Boraas et al., 1998). Likewise, the green alga *Desmodesmus* forms large colonies composed of tough-walled cells with more spiny projections when exposed to *Daphnia* (Hessen and van Donk, 1993). The induction of colony formation in response to predation appears to be a widespread phenomenon in some lineages (see for example Verschoor et al., 2004).

Indeed, one explanation for the waves of multicellular experimentation occurring at ~1500 Mya in the fossil record is the expansion of predation resulting from the evolution of phagocytosis (Stanley, 1973). Another benefit to aggregating cells into a collective is an increase in the acquisition and utilization of extracellular resources. For example, undifferentiated colonies resulting from incomplete cell separation of *Saccharomyces cerevisiae* appear spontaneously in cultures with low sucrose concentrations (Koschwanez et al., 2013). The cells in these colonies cooperate in ways that reduce starvation and provide protection. (Holmes et al. [2013] have shown that an ethanol-induced prion formed from the Mot3 transcription factor alters the expression of *FLO11* [a major factor governing cell-cell adhesion in this species] and induces multicellularity in *S. cerevisiae*. Oud et al. [2013] have also shown that genome duplication and *ACE2* mutations can also induce multicellularity in this species.) Further, growth experiments of organisms such as the coccolithophyte *Phaeocystis* show that the colonial phenotype has faster rates of cell division compared to the unicellular phenotype (Veldhuis et al., 2005) perhaps because the extracellular adhesive matrix can be used to store nutrients that can be used under conditions of environmental stress. These and other selective advantages are perpetuated in the multicellular body plan, which confers an additional benefit—the encapsulation of nuclei into separate but symplastically connected cells establishes discrete physiological boundaries that permit the isolation of different molecular regulators, trafficking routes, cytosolic recycling, transcytosis, exocytosis, and metabolite degradation. Intercellular communication within the multicellular body plan also permits the concerted coordination among these processes to establish, maintain, or switch supracellular physiological domains as well as a stratagem for segregating and isolating defective nuclei resulting from deleterious mutations (Klekowski and Kazarinova-Fukshansky, 1984), which are known to accumulate at cell- and tissue-specific rates (see for example Vijg et al., 2005).

Finally, it is worth noting that, across diverse animal and plant species, lifespans increase, albeit not one-to-one with increasing body size. For instance, Marbà et al. (2007) analyzed published data and show that lifespan scales, on average, as the 1/4 power of body size across aquatic and terrestrial, unicellular and multicellular plant species. This allometry accords with scaling relationships predicted by a generalized theory for the metabolic optimization of life-history traits (West et al., 1997; Niklas and Enquist, 2001). If multicellularity confers even a marginal increase in longevity, it might evolve as a life-history strategy for finding an optimal window of opportunity for reproduction or a strategy for reducing transcription errors in cells sequestered for reproduction. At least one theory for ageing predicts that, if the level of extrinsic mortality is low, selection can favor organisms with a greater investment in reproduction at the cost of somatic maintenance (and thus a decrease in the rate of survival of the individual) (Kirkwood, 1977). Conversely, under less optimal environmental conditions selection may favor larger organisms with a longer survival probability until conditions for reproduction become more favorable. It is therefore possible that the raison d'être of multicellularity resides in the mathematics of a generic selection theory for reproductive success (sensu the disposable soma theory of Kirkwood, 1977), although one must always entertain the possibility that increased lifespans are a consequence rather than a cause of multicellularity.

A brief digression on the alternation of generations— The linkages among body size, life-history traits, and gene expression patterns provide a logical extension of the previous line of speculation, viz., life cycles with a di- or polyphenic alternation of generations might evolve as a consequence of contentious maternal and paternal gene network expression patterns responding to unpredictable, stressful, or heterogeneous environmental conditions. It also provides a clue regarding the increase in the body size of the diploid multicellular generation across embryophyte evolution alluded to earlier in this review (also see Niklas and Kutschera, 2010).

Consider the life-history consequences of genomic conflict (i.e., parent-specific gene expression patterns; see Trivers and Burt, 1999) in the case of the maternal and paternal genomes in a multicellular dioicious species (e.g., residing in the eggs and sperm of the charophycean alga *Coleochaete scutata*) in which the latter "selfishly" induces higher levels of nutrient provisioning for its sired zygotes (to favor its perpetuation in subsequent generations perhaps in the form of either increasing the number or the vigor of the haploid zoospores in which it resides). It might also evolve the capacity to eventually delay zygotic meiosis to increase the number of subsequently formed haploid cells, which would be particularly efficacious when fertilization events are rare (Searles, 1980; Haig and Wilczek, 2006). If resources are limited, this paternal genome is in conflict with a maternal genome with access to limited resources. This genome might respond by selectively aborting zygotes (a form of postfertilization "mate-choice") and divert nutrients to only the most vigorous zygotes. It might also divert some of its resources to vegetative growth or asexual reproduction to reduce the "50% cost" of sexual reproduction (particularly in a species with zero paternal metabolic investment in the development of offspring; see Williams, 1975). In either case, a selfish paternal genome would gain ascendency in future generations.

The life cycles of unicellular organisms provide clues to some of the ancestral toolkits that could evoke genomic conflicts immediately after syngamy. For example, gamete fusion in *Chlamydomonas* induces a number of processes (linked to the short diploid phase in the life cycle including meiosis) that are controlled by a heterodimeric homeodomain transcription factor formed by Gm1 and Gsp1 carried by the − and + gamete, respectively (Lee et al., 2008). Genetic modifications of either *Gm1* or *Gsp1* could conceivably delay the onset of meiosis or affect subsequent postfertilization events. Regardless of the mechanism, it is not difficult to see how the conflicting interests of the maternal and paternal genomes of a dioicous oogamous species (and the manner in which these interests can be reconciled) might favor an evolutionary shift from a haplobiontic-haploid life cycle (as seen in *C. scutata*) to a diplobiontic life cycle in which the multicellular gametophyte still dominates (as seen in moss *Physcomitrella patens*). Indeed, it is not difficult to extend this scenario to account for the evolutionary reduction in the size of the gametophyte generation over the course of embryophyte evolution, particularly since matrotrophy is a pleisomorphic feature of this clade as well as a feature characterizing many charophycean algae (Graham and Wilcox, 2000).

CONCLUDING REMARKS

The morphological theme of multicellularity and the confluence of generic and genomic processes by which it was achieved

in different clades continue to draw attention to classical but largely unanswered questions in microbiology, botany, zoology, and mycology. Among these is the relationship between the organism and the cell and the extent to which an organism's external form (morphology) and internal structure (anatomy) are necessarily interrelated (Kaplan, 1992, 2001; Kaplan and Hagemann, 1991; Korn, 1999). An evolutionary-developmental perspective in tandem with the growth of molecular biological techniques has informed the pursuit of these and other questions, but we are still remarkably ignorant about many fundamental processes. A contributing factor to this ignorance is the assumption that patterning processes and the mechanisms accounting for them are the same in different organisms. The fact that fungal mitotic divisions are intranuclear, whereas microtubules invade the nuclear space after the dissolution of the nuclear envelop to form the division spindle in most plant and animals cells is sufficient to caution against canonical discussions about cell division. Another factor is that molecular sequence homology does not necessarily translate into morphogenetic or organographic homology. The various cases presented in this review are sufficient to show that this is not invariably true and that detailed analyses are required to determine whether two structures or processes are truly developmentally homologous. A third factor is a paucity of phylogenetically disparate model organisms to answer questions that span vastly different life-forms. A related problem is that many of the model developmental systems that are currently available are species drawn from species-rich late-divergent persistent lineages (that are often, but inappropriately called "crown" groups), which can manifest numerous derived character states, that need to be juxtaposed with data drawn from species from early-divergent persistent lineages (often, but inappropriately called "basal" groups) to assess ancestral character states. A recent example is how the absence of SMG1 in *Arabidopsis thaliana* led some workers to conclude that this core kinase in the nonsense mRNA decay pathway was generically absent in earlier-divergent plant groups (see, however, Lloyd and Davies, 2013).

The study of the evolution of multicellularity draws these and other limitations into sharp focus. By so doing, it also provides a venue in which to resolve them by synthesizing information from fields of study as diverse as paleontology and proteomics (Friedman et al., 2004; Niklas and Kutschera, 2010). What is the relationship between organismic size and shape? How do cells and tissues coordinate their activities? What accounts for the differences between the gametophyte and the sporophyte? These and other fundamental questions puzzled Leo Errera, Wilhelm Hofmeister, and many other developmental biologists, and they continue to do so today. Their resolution can only be resolved by a concerted interdisciplinary approach in which research questions are carefully but phylogenetically broadly framed. The literature reviewed here shows that this can be (and is being) done, but that much more is needed to marry the *Weltanschauungen* of evolutionary and developmental biology.

A living organism must be studied from two distinct aspects. One of these is the causal-analytic aspect which is so fruitfully applicable to ontogeny. The other is the historical descriptive aspect which is unraveling lines of phylogeny with ever-increasing precision. Each of these aspects may make suggestions concerning the possible significance of events seen under the other, but does not explain or translate them into simpler terms. — Sir Gavin R. de Beer (1938)

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