Evolution in the Cycles of Life

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Abstract

The life cycles of eukaryotes alternate between haploid and diploid phases, which are initiated by meiosis and gamete fusion, respectively. In both ascomycete and basidiomycete fungi and chlorophyte algae, the haploid-to-diploid transition is regulated by a pair of paralogous homeodomain protein encoding genes. That a common genetic program controls the haploid-to-diploid transition in phylogenetically disparate eukaryotic lineages suggests this may be the ancestral function for homeodomain proteins. Multicellularity has evolved independently in many eukaryotic lineages in either one or both phases of the life cycle. Organisms, such as land plants, exhibiting a life cycle whereby multicellular bodies develop in both the haploid and diploid phases are often referred to as possessing an alternation of generations. We review recent progress on understanding the genetic basis for the land plant alternation of generations and highlight the roles that homeodomain-encoding genes may have played in the evolution of complex multicellularity in this lineage.

INTRODUCTION

Life Cycles of Eukaryotes

Complex multicellularity: an organismal body exhibiting tissue differentiation and three-dimensional organization

Simple multicellularity: an organismal body consisting of a group of cells originating from a progenitor cell via mitoses but with limited cellular differentiation

Although multicellular animals, plants, and fungi are the most conspicuous eukaryotes, the majority of the eukaryotic diversity consists of unicellular organisms, often referred to as protists (2). The phylogenetic distribution of protists indicates the eukaryotic common ancestor was unicellular, and the ancestral eukaryotic life cycle likely consisted of a flagellated unicellular haploid phase, with differentiation into gametes followed by gamete fusion producing a diploid zygote that would immediately undergo meiosis. Remarkably, multicellularity has evolved independently in many of the five to six major lineages of eukaryotes. It is estimated that simple multicellularity, in which differentiation is limited to soma and gametes, has evolved approximately two dozen times (70). In contrast, complex multicellularity, defined by vegetative tissue differentiation and three-dimensional organization, has evolved six times: in the metazoans, red algae, land plants, brown algae, basidiomycete fungi, and ascomycete fungi (70; **Figure 1**). It is generally assumed that complex multicellular organisms evolved from simple multicellular ancestors, implying that evolution from simple to complex multicellularity is a rarer event than evolution from unicellularity to simple multicellularity (70, 94).

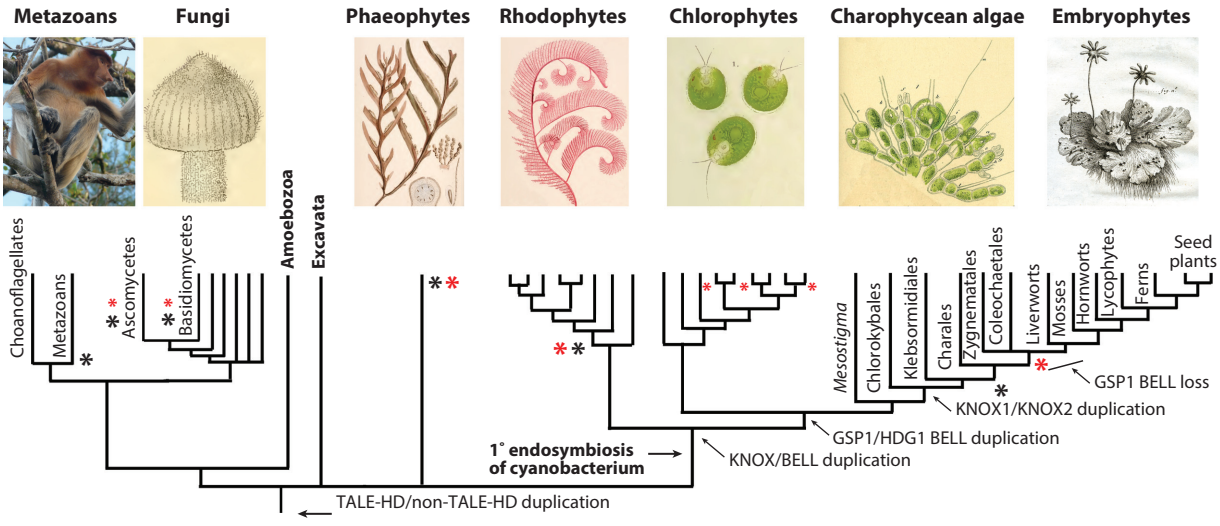

Figure 1

Eukaryotic diversity. The phylogenetic tree depicts extant eukaryotic diversity focusing on lineages in which complex multicellularity has evolved. Metazoans, fungi, phaeophytes (brown algae), rhodophytes (red algae), chlorophytes (green algae), and embryophytes (land plants) are monophyletic; charophycean algae are a paraphyletic grade. Complex multicellularity (*black asterisk*) evolved at least six independent times (70). Life cycles with alternations of generation evolved independently in several lineages (*red asterisk*; large red asterisks denote cases in which complex multicellularity occurs in both haploid and diploid generations). Fungi may have had multiple origins; two are shown (32, 114). The alternation of generations in brown algae may be isomorphic or heteromorphic, with the latter either haploid or diploid dominant (25, 114, 123), whereas those of the red algae are mostly isomorphic (73, 121, 140, 141). The alternation of generations in chlorophytes had multiple independent origins, with most species having such a life cycle being isomorphic but some are heteromorphic (113). Charophycean algae have no alternation of generations. Embryophytes have a single origin and the alternation of generations is heteromorphic. Some embryophyte species are haploid dominant (liverworts, mosses, hornworts) and others are diploid dominant (lycophytes, ferns, seed plants) (15, 58). The origins, via gene duplications, and losses of major classes of homeodomain genes described in the text are outlined. Tree adapted from Reference 1; graphics adapted from References 18, 43, 53, 87, and 102.

We are familiar with our own mammalian life cycle in which complex multicellularity occurs during the diploid phase and the haploid phase, i.e., eggs and sperm, is unicellular. However, within the diversity of eukaryotes, multicellularity has evolved in either the haploid or diploid phase, or in both phases within the same organism. Taxa exhibiting a life cycle whereby multicellular bodies develop in both the haploid and diploid phases are often referred to as possessing an alternation of generations; such life cycles have evolved repeatedly (**Figure 1**). The haploid and diploid generations of organisms whose life cycle consists of an alternation of generations can have an indistinguishable morphology (isomorphic) or the morphologies of the multicellular bodies in the two generations can differ (heteromorphic). Furthermore, in heteromorphic taxa, the dominant phase of the life cycle can be either the haploid or diploid phase, with either one dependent upon the other or the two developing independently. In species with a heteromorphic alternation of generations, a single genome directs development of two different multicellular bodies, and it is likely that multicellularity evolved first in one phase, then the other. In species with isomorphic alternations, a reasonable hypothesis is that the genetic program directing the development of the original multicellular body plan was co-opted in the other generation. A more intriguing question is whether co-option of preexisting genetic programs occurred during evolution of species with heteromorphic alternation of generations.

The foundation for our discussion is that observations in fungi (55), green algae (76), and land plants (106) suggest a common ancestral mechanism by which diploid gene expression was activated in eukaryotes. The proposed ancestral mechanism to activate diploid gene expression utilizes pairs of paralogous homeobox-containing genes. In land plants, this system diversified during the evolution of a multicellular diploid generation, i.e., the origin of an alternation of generations in this lineage. These raise the idea that the primordial role of homeobox genes was in the haploid-to-diploid transition, and that the genes were later co-opted into genetic programs patterning multicellular development. We review the genetic studies and developmental biology supporting this concept, focusing first on life cycles in the Archaeplastida, and then returning to broader comparisons across eukaryotic diversity.

Alternation of generations: a life cycle with multicellular organisms developing in both the haploid and diploid phases

Heteromorphic alternation of generations: alternation of two generations having different morphologies

Charophycean algae: a grade of freshwater and terrestrial green algae in which the embryophytes are nested

Streptophytes: monophyletic clade comprising the charophycean green algae and the land plants

Chlorophytes: a monophyletic clade of green algae primarily occupying marine habitats; includes the well-known taxa *Chlamydomonas* and *Volvox*

Evolutionary Context of Land Plants

To place comparative genetics studies spanning the green lineage in context, an understanding of the evolution of plants is required. The monophyletic Archaeplastida are the direct descendants from the primary endosymbiotic event that gave rise to the chloroplast (2). After divergence of the glaucocystophytes, little-studied unicellular microbes, the red algae (Rhodophyta) diverged from the remainder of Archaeplastida, a diversity of green algae and land plants (**Figure 1**). One clade of green algae, the monophyletic Chlorophyta, primarily inhabits marine habitats and includes the well-known taxon *Chlamydomonas* (77, 128). In contrast, charophycean green algae, inhabiting freshwater and terrestrial habitats, form a grade within which are nested the monophyletic land plants, or Embryophyta (7, 30, 77, 128). The charophycean algae plus the land plants are collectively called the Streptophyta. Multicellularity—simple, complex, or both—evolved in all three major clades: Rhodophyta, Chlorophyta, and Streptophyta (70, 114). Within the Streptophyta, an evolutionary progression from a unicellular ancestor to simple filamentous multicellularity, then complex multicellularity, and finally an alternation of generations both exhibiting complex multicellularity illustrates the diversity of life cycles (30, 77, 128).

The ancestral streptophyte was a biflagellated unicellular microbe, which if extant, might resemble *Mesostigma* (**Figure 1**), the basal-most lineage of the Streptophyta (77). Although sex has not been observed in *Mesostigma*, the ancestral streptophyte presumably had a life cycle similar to that described for the chlorophyte alga *Chlamydomonas*, in which haploid vegetative

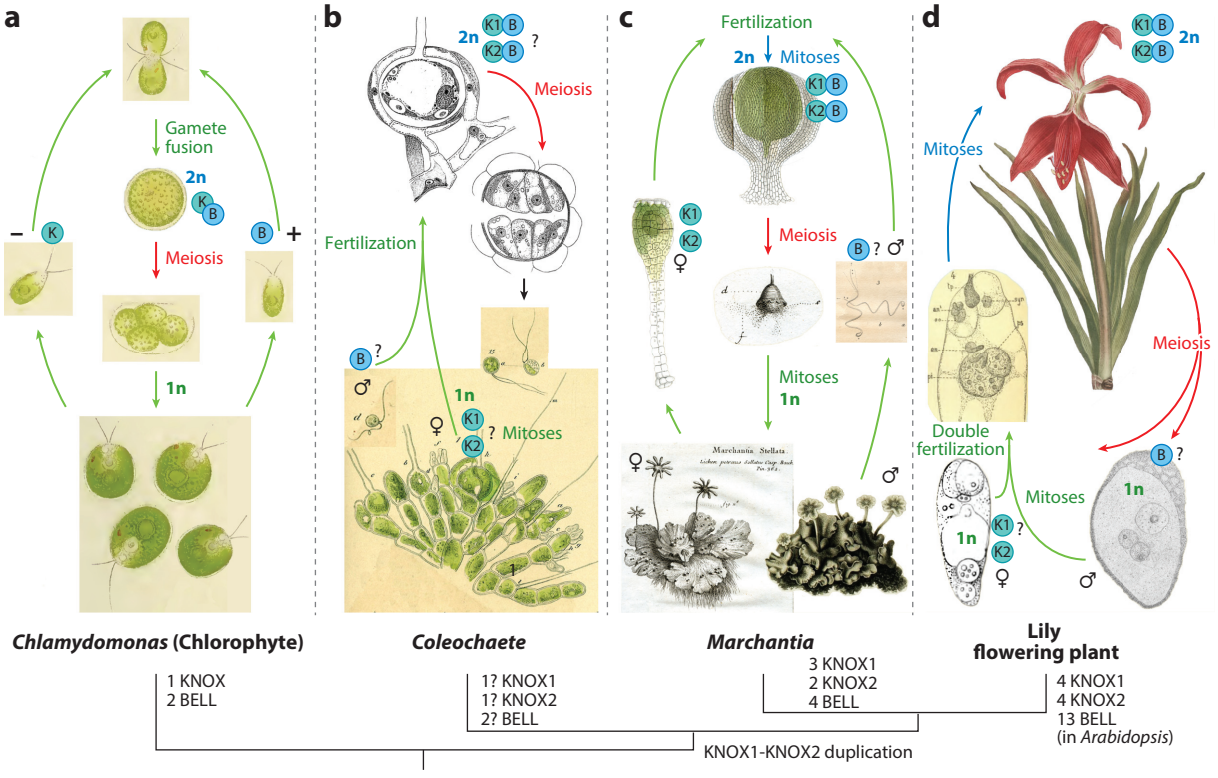

Figure 2

Life cycles and their genetic basis in Archaeplastida. Events occurring in the haploid phase and gamete fusion are noted in green, events occurring in the diploid phase are noted in blue, and meiosis is indicated in red. (*a*) *Chlamydomonas*. Vegetative cells of two mating types, + and −, differentiate into gametes. The + mating type expresses *GSP1* [a BELL gene (B)] and the − mating type expresses *GSM1* [a KNOX gene (K)]; proteins are cytoplasmic until gamete fusion, after which they heterodimerize, move into the nucleus, and activate diploid gene expression. The zygote undergoes meiosis, and motile haploid products are released. Images adapted from Reference 43. (*b*) *Coleochaete*. Gametes, motile sperm and retained egg, differentiate from multicellular haploid bodies. Following fertilization, the maternally retained zygote undergoes meiosis and subsequent rounds of mitosis to produce motile zoospores, which establish the next multicellular haploid generation. Expression and function of the three-amino-acid-length extension (TALE) homeodomain (HD) genes are unknown. Images adapted from References 98 and 102. (*c*) *Marchantia*. Gametes, motile sperm and retained egg, differentiate from the long-lived complex multicellular thalloid gametophyte. Following aquatic fertilization, the zygote is retained on the maternal gametophyte and undergoes mitotic divisions, producing a multicellular sporophyte. The sporophyte is nutritionally dependent on the gametophyte for the entirety of its life span. A proportion of sporophyte cells undergo meiosis, producing haploid spores. In the moss *Physcomitrella* (gene numbers shown), the egg cell expresses both *KNOX1* (K1) and *KNOX2* (K2) genes. It is not known whether sperm express BELL genes. Images adapted from References 87, 92, 124, and 129. (*d*) Angiosperm. The haploid generation is reduced to three cells, including two sperm cells, in the male gametophyte and seven cells, including a single egg cell, in the female gametophyte. The two sperm cells fertilize the egg and central cells of the female gametophyte, producing the zygote and endosperm, respectively. The zygote divides mitotically, producing a long-lived complex multicellular sporophyte in which only a tiny fraction of cells undergo meiosis. KNOX and BELL functions during sporophyte development have been described, but whether they function in the zygote is unknown; KNOX and BELL gene numbers from *Arabidopsis*. Images adapted from References 23, 48, 56, and 133.

cells differentiate into gametes (with two or more mating types), and gametes of differing mating type fuse to form a diploid zygote (**Figure 2**). The zygote then proceeds to meiosis, without any intervening mitoses, to produce four haploid progeny.

The next diverging lineages within the Streptophyta (**Figure 1**) are the Chlorokybales, which form small cellular aggregations and the Klebsormidiales, which develop unbranched filaments

(30, 77) and exhibit simple multicellularity in their haploid stages. In contrast, the Charales representing the next lineage has a complex multicellular haploid body and a single-celled diploid generation. The closest extant relatives of land plants, Coleochaetales and Zygnematales, have either complex or simple multicellular haploid bodies, but the diploid stage consists of a zygote that undergoes meiosis without any preceding mitotic divisions, namely, a haplontic life cycle (**Figure 2**) (35, 36, 75, 125, 126, 135, 136). The evolutionary relationships of land plants and charophycean algae indicate that haploid complex multicellularity in land plants was likely inherited from their algal ancestor but that complex diploid multicellularity evolved de novo in the common ancestor of extant land plants (45, 65, 79).

All charophycean algal lineages except *Mesostigma* and the Charales have been observed to grow in moist terrestrial habitats (30), leading to the hypothesis that charophycean algae invaded land before the evolution of embryophytes (118). Thus, many of the adaptations to land, such as cell wall construction, desiccation tolerance, and adaptation to high irradiation, may have evolved in an ancestral alga prior to the origin of land plants. The fossil record is compatible with an early land colonization by charophycean algae, with Cambrian terrestrial cryptospores of putative algal origin preceding definitive Ordovician land plant cryptospores (134). Evolution of cell wall components within the charophycean algae suggests that they were terrestrial long before the evolution of land plants and that their present habitats reflect refuges following competition with land plants (52). Consistent with a major transition in life style, the *Klebsormidium flaccidum* genome and gene content of other derived charophycean algae resemble those of land plants in many aspects (17, 59, 60, 63, 126).

Embryophytes: commonly known as land plants and characterized by a life cycle with a heteromorphic alternation of two generations

Gametophyte: a haploid organism that produces gametes (sperm and egg cells); thus, a sexual generation

Sporophyte: a diploid organism produces spores via meiosis; an asexual generation

Cryptogam: a historical term describing organisms that reproduce via spores; includes species from multiple clades of most major eukaryotic lineages

LAND PLANTS

Land plants are characterized by having a life cycle with an alternation of two generations, a haploid gametophyte and a diploid sporophyte, with each generation developing a multicellular body (58). The haploid gametophyte, the sexual generation, produces gametes (sperm and egg cells), whereas the asexual diploid sporophyte generation produces spores via meiosis. The heteromorphic alternation of generations in land plants results in the possibility that tissue differentiation in each generation is governed by different genetic programs, initiated by either fertilization (haploid to diploid) or meiosis (diploid to haploid). Two key evolutionary innovations—the archegonium, in which the egg cell differentiates and that envelops and protects the egg from the harsh terrestrial environment, and a multicellular sporophyte—contributed to land plants dominating the terrestrial flora. The early diverging land plant lineages form a grade, are collectively called the bryophytes, and have a long-lived gametophyte generation and a transient, dependent diploid generation (*Marchantia* in **Figure 2**). In contrast, vascular plants, e.g., ferns and seed plants, have a sporophyte-dominant life cycle in which the diploid generation is long-lived and the haploid generation is transient, often reduced to just a few cells, as in the pollen of seed plants (lily in **Figure 2**).

A Historical Perspective

The discovery of an alternation of generations by Hofmeister revolutionized how the plant kingdom was viewed (see sidebar, Hofmeister's Insight), uniting land plants as a monophyletic group to the exclusion of other plants. Prior to Hofmeister, plant-like organisms that reproduced via spores rather than seeds were often lumped together as cryptogams, a group that included fungi, brown algae (phaeophytes), red algae (rhodophytes), green algae, and the non-seed-producing land plants (liverworts, mosses, hornworts, lycophytes, and ferns). However, Hofmeister recognized that female gymnosperm gametophytes differentiate reduced archegonia, each containing

HOFMEISTER'S INSIGHT

> Mosses and ferns therefore exhibit remarkable instances of a regular alternation of two generations very different in their organization. The first generation—that from the spore—is destined to produce the different sexual organs, through the cooperation of which the multiplication of the primary mother-cell of the second generation, which exists in the central cell of the female organ, is brought about. By this multiplication a cellular body is produced which in the mosses forms the rudiment of the fruit, and in the vascular cryptogams, the embryo. The object of the second generation is to form numerous free reproductive cells—the spores—by the germination of which the first generation is reproduced. The leafy plant in the mosses answers therefore to the prothallium of the vascular cryptogams; the fruit in the mosses answers to the fern in the common sense of the word, with its fronds and sporangia. (58, pp. 434–35)

With this simple statement, Hofmeister united all land plants (58). Although his exhaustive treatment describing land plant life cycles is organized in a phylogenetic manner, his original publication (57) predated Darwin's *On the Origin of Species* (29), and whether Hofmeister was thinking along phylogenetic lines has been debated (42).

an egg cell, thus uniting gymnosperms with archegoniate cryptogams. Hofmeister's work clearly delineated land plants as a monophyletic group uniting one class of cryptogams (the archegoniates; bryophytes, lycophytes, and ferns) with phanerogams (gymnosperms and angiosperms) to the exclusion of other classes of cryptogams.

Despite the heteromorphic nature of the land plant alternation of generations, in the second half of the nineteenth century, two hypotheses of the evolution of the land plant alternation of generations were proposed (22; reviewed in 46, 50). In the homologous theory, the two distinct generations evolved from a common ancestor in which both the haploid and diploid generations were morphologically similar. In contrast, the antithetic theory proposed that multicellular diploid sporophyte generation of land plants evolved via a delay in zygotic meiosis, with extensive mitotic cell divisions interpolated between the formation of the zygote and the production of spores via meiosis. The proponents of the homologous theory were boosted by the discovery of apogamy and apospory. In 1874, Farlow (33) observed a fern sporophyte growing vegetatively from the gametophyte without the intervening step of sexual organ formation; i.e., a sporophyte body differentiating directly from cells of a gametophyte body without the process of fertilization, a phenomenon referred to as apogamy. Farlow's discovery stimulated Pringsheim (103) to obtain the converse, the development of a gametophyte body directly from cells of the sporophyte without the intervening step of meiosis, or apospory, a phenotype he induced by regeneration of excised moss sporophytes (**Figure 3**). A century later, Bauer closed the circle by demonstrating that aposporously produced gametophytes of the moss *Tetraphis pellucida* could be induced to form apogamous sporophytes, thus going through a morphological life cycle without either fertilization or meiosis (6). That gametophytes could be derived directly from sporophytes without meiosis (apospory) and sporophytes from gametophytes without fertilization (apogamy) boosted the standing of the proponents of the homologous theory. However, in 1893 Overton (99) reported that pollen mother cells had a reduced chromosome content relative to archesporial cells and presciently stated that it would be of interest to establish whether the alternation of generations is dependent upon a difference in chromosome number in the two generations. Immediately thereafter, Strasburger (119) reported that fern sporophytes had twice the chromosome number as gametophytes, conclusively connecting ploidy with the alternation of generations in land plants. Strasburger subsequently coined the terms haploid and diploid, corresponding to the gametophyte and sporophyte, respectively, of the land plant life cycle (120).

Apogamy: a phenomenon whereby a sporophytic body develops directly from a gametophyte without fertilization and gamete fusion

Apospory: a phenomenon in which a gametophytic body develops directly from a sporophyte without meiosis

Ploidy: a degree of multiplication of the basic number of chromosomes

Figure 3

Apospory. By severing the seta (*brown stem-like structures*) of isolated developing moss sporophytes, Pringsheim was able to induce apospory, the development of a gametophyte body (*green shoots*) directly from the sporophyte body without an intervening meiosis. Images adapted from Reference 104.

With the realization that gametophytes have half the number of chromosomes as sporophytes, the ploidy of aposporously produced gametophytes became a matter of speculation (119). Definitive evidence of ploidy increases was found in aposporously induced diploid gametophytes from 19 moss species (84–86). In some cases, tetraploid sporophytes were obtained, leading Marchal & Marchal (84–86) to speculate that rare cases of apospory in nature, as reported earlier by Brizi (19), could lead to polyploidy. Although reports of apospory in mosses and ferns are common, the frequency of aposporous hornworts (74, 109) and liverworts (89) is much lower. In the liverwort *Blasia pusilla*, apospory could be induced during only a brief temporal window, when the sporophyte is normally protected by enveloping tissues of the gametophyte, and as such was unlikely to occur in nature. As both ferns and mosses (137) are frequently polyploid in nature and there is a paucity of polyploid liverworts and hornworts (11), the frequency of apospory may be a plausible cause for ploidy increases in nature.

Bower (15, 16) and Vaizey (131) forcefully argued that the land plant sporophyte was a novel generation due to an interpolation of mitotic divisions of the zygote prior to meiosis. Bower considered that land plants likely evolved from an algal ancestor with a haplontic life cycle and considered apospory "teratological and not to be taken as evidence for evolutionary relationships between the gametophyte and sporophyte" (15, p. 368). Bower argued that land plants and their algal ancestors must be viewed in a phyletic series. Thus, he considered the gametophyte the older generation, homologous to algal haploid bodies, and the dependence of the gametophyte on water (e.g., aquatic fertilization) to stem from its algal ancestry. The invasion of land meant that sex could occur only during times of periodic flooding or sufficient dew, meaning less dependence could be placed on fertilization for reproduction. The evolution of a sporophyte, in which many cells

undergo meiosis, allows propagation and dispersion by spores in dry times. Thus, Bower argued that the origin of the alternation of generations is correlated with a change in habitat from water to land (15). The multicellular sporophyte producing thousands of meiotic progeny per fertilization event (as opposed to four in algae) would provide a selective advantage, allowing land plants to dominate the terrestrial flora and pushing extant charophycean algae to specialized niches. The subsequent evolution of the land plant sporophyte generation is one of progressive sterilization and elaboration of vegetative organs, with the sporophyte assuming the function of nutrition, a role ancestrally delegated to the gametophyte. Seed plants, whose gametes have evolved to function in an aerial environment freed from aquatic fertilization, became the dominant vegetation of the land.

Homeodomain: a DNA binding domain found in eukaryotic transcription factors and classified into two superclasses, TALE and non-TALE

BELL: a subclass of TALE homeodomain transcription factors

KNOX: a subclass of TALE homeodomain transcription factors, of which there are two further subclasses, KNOX1 and KNOX2

As land plants have a heteromorphic alternation of generations, the sporophyte genetic program must not only direct multicellular sporophyte development but also be repressed during the complex multicellular gametophyte generation, and vice versa. Although the antithetic theory for the origin of the alternation of generations in land plants is accepted today, an outstanding question is whether there was a large-scale co-option of genetic programs operating in the ancestral gametophyte into the newly evolved multicellular sporophyte. Functional studies of orthologous genes, such as those controlling rhizoid/root hair development and auxin responses, in bryophytes (*Marchantia* and *Physcomitrella*) and flowering plants have revealed similarities between genetic programs operating in bryophyte gametophytes and flowering plant sporophytes (3, 37, 66, 91, 101, 138). At least some of these are likely operating in charophycean algae gametophytes, suggesting they could be ancestral gametophyte programs (38, 122). However, little is known about developmental programs operating in bryophyte sporophytes, and no functional data are available for charophycean algal developmental genetics. Regardless, sporophyte evolution must have involved either the rewiring of existing genetic programs, the evolution of new genetic programs, or a combination of both.

THE GENETIC BASIS OF LIFE CYCLES IN ARCHAEPLASTIDA

Chlorophytes

A major breakthrough in understanding the genetic basis of life cycles in plants came through studies on *Chlamydomonas reinhardtii*, a unicellular chlorophyte alga (**Figure 2**). Environmental signals induce vegetative *Chlamydomonas* cells to differentiate into gametes. Upon gamete fusion, initial zygote gene expression is independent of protein synthesis, suggesting that gametes are preloaded with proteins directing early zygotic transcription (34). The haploid-to-diploid transition is regulated by a pair of three-amino-acid-length extension class homeodomain (TALE-HD) protein-encoding genes (76, 95, 143). The two gamete types, *plus* and *minus*, express two homeodomain genes, *GSP1* (*GAMETE SPECIFIC PLUS1*, encoding a BELL-related TALE-HD protein) and *GSM1* [*GAMETE SPECIFIC MINUS1*, encoding a KNOTTED1-LIKE HOMEOBOX (KNOX)-related TALE-HD protein], respectively (76, 143). On their own, each of the proteins is cytoplasmic, but following gamete fusion, the proteins heterodimerize and translocate into the nucleus to regulate zygotic gene expression (76). The GSP1/GSM1 heterodimer is necessary and sufficient to activate zygotic gene expression. Plus gametes lacking *GSP1* activity can fuse with minus gametes, but the resulting diploid cells fail to activate the diploid genetic program, with zygotes reentering the haploid program (95). Conversely, gain-of-function *GSP1* alleles in a minus mating-type background (or gain-of-function *GSM1* alleles in a plus mating-type background) ectopically activate the diploid program (76, 143). The zygotic program can be activated by GSP1/GSM1 heterodimers even in the absence of gamete differentiation, indicating

this complex is sufficient in any context in *Chlamydomonas* (76). Intriguingly, chlorophyte genomes encode a third TALE-HD gene, *HDG1*, a paralog of *GSP1* (**Figure 1**). In *Chlamydomonas*, *HDG1* is expressed in both types of gametes as well as the zygote, but its function is unknown (76).

Charophycean Algae

The charophycean alga *Klebsormidium* possesses two BELL paralogs, orthologous to *GSP1* and *HDG1* of *Chlamydomonas* (60). In addition, charophycean algae, except perhaps *Mesostigma*, encode two distinct subfamilies of KNOX genes, Class 1 (KNOX1) and Class 2 (KNOX2), which result from a gene duplication in an ancestral charophycean alga (**Figure 1**). The functions of these genes are not known, but development of tractable genetic systems (1, 116) may provide insights into their roles during the charophycean life cycle (**Figure 2**).

Land Plants

Land plant genomes also encode KNOX1 and KNOX2 genes, and in most genomes examined to date, multiple paralogs for each of the subclasses are present. Multiple BELL paralogs are also present; however, all appear to be orthologous to *HDG1* of *Chlamydomonas*, implying loss of the *GSP1* ortholog in the lineage leading to, or during, land plant evolution (**Figure 1**). Similar to *Chlamydomonas*, in land plants both KNOX1 and KNOX2 proteins can heterodimerize with BELL TALE-HD proteins to affect gene expression (8, 49, 115), and in planta there exists genetic evidence for strict selectivity of partners (40).

Bryophytes. The bryophyte life cycle consists of a persistent haploid gametophyte generation and a short-lived diploid sporophyte generation (*Marchantia* in **Figure 2**). KNOX function has been characterized in the moss *Physcomitrella patens*, whose life cycle is similar to that of *Marchantia*. The *Physcomitrella* genome encodes three KNOX1 genes and two KNOX2 genes. Neither class of *KNOX* gene is functional during gametophytic vegetative growth, but both are activated in the egg cell and surrounding archegonial cells and are active during sporophyte development (106, 107, 112). Both KNOX genes are expressed in the zygote, with later expression patterns diverging. Triple loss-of-function KNOX1 sporophytes exhibit defects in cell proliferation, both in early sporophyte development stages with an apical cell and at later stages during sporogenous tissue proliferation (107). Double loss-of-function KNOX2 sporophytes are aposporous, with diploid gametophyte bodies developing from isolated embryos (106), indicating that KNOX2 activity is required to repress the gametophytic genetic program during the sporophyte generation. At least three of the four BELL paralogs are primarily expressed in the antheridia, archegonial tissues, and/or the sporophyte, and gain-of-function alleles suggest at least one is involved in the haploid-to-diploid transition (61, 106). Thus, TALE-HD function in *Physcomitrella* sporophytes fulfills two critical functions—cell proliferation and repression of the gametophyte program—required for the alternation of generations.

Loss-of-function alleles in genes encoding components of the *Physcomitrella* polycomb repressive complex 2 (PRC2) result in apogamy—the development of a sporophyte body plan in a haploid gametophyte. Mutations in either *Physcomitrella CURLY LEAF* (*PpCLF*), encoding an *E*(*z*) homolog, or *Physcomitrella FERTILIZATION-INDEPENDENT ENDOSPERM* (*PpFIE*), encoding an *ESC* homolog, result in fertilization-independent sporophyte-like bodies from branches of gametophytic protonema (93, 97). The sporophyte-like bodies exhibit sporophyte gene expression (e.g., KNOX1), indicating that loss of PRC2 results in an ectopic activation of KNOX gene activity during the gametophyte generation. Thus, genome-wide polycomb-mediated repressive

Polycomb repressive complex 2 (PRC2): a multiprotein complex possessing histone methyltransferase activity that maintains repressive gene expression patterns by regulating chromatin architecture

chromatin modification is required to maintain gametophyte identity through repression of the sporophyte genetic program.

Observations in *Physcomitrella patens* suggest that at least some of the genetic machinery controlling the haploid-to-diploid transition identified in *Chlamydomonas* has been conserved in land plants. For example, in *P. patens*, KNOX functions to both direct normal sporophyte development and also repress the gametophyte developmental program during the sporophyte generation. Furthermore, ectopic KNOX activation in the gametophyte in polycomb mutants results in sporophyte-like development. These observations are consistent with the hypothesis that TALE-HD genes control the haploid-to-diploid transition throughout the Archaeplastida.

Angiosperms. An extensive literature describes the roles of KNOX1 genes in the development and differentiation of nearly every aboveground organ of the flowering plant sporophyte. *KNOX1* function has been recently reviewed (51, 54); thus, we mention only a few salient points here. The first plant homeobox encoding gene cloned was *KNOTTED-1* from *Zea mays*, for which the KNOX family was named (132). Although originally identified as a gain-of-function mutation causing knots in leaves, loss-of-function alleles of homologs result in defects in the maintenance of stem cells in the shoot apical meristem (5, 68, 83). Loss-of-function mutations in KNOX1 paralogs (four in the *Arabidopsis* genome) result in a myriad of defects in flower and stem development, including premature differentiation of lignified tissues in *BP* loss-of-function alleles (90). Gain-of-function KNOX1 alleles produce elaboration of leaflets in normally simple-leaved *Arabidopsis*, a role thought to be endogenous in many angiosperm species (4, 12, 14, 81).

In contrast, functional data of KNOX2 genes in flowering plants are sparse. In *Arabidopsis*, the four KNOX2 paralogs (*KNAT3*, *KNAT4*, *KNAT5*, and *KNAT7*) are broadly expressed in the sporophyte (40, 111, 127). One striking feature is that loss-of-function KNOX2 phenotypes often resemble gain-of-function KNOX1 phenotypes and vice versa. *knat7* loss-of-function alleles are defective in secondary wall synthesis (80, 144), a phenotype opposite to the premature differentiation of lignified tissues in *bp* mutants (90). *knat3 knat4 knat5* leaves are elaborated, similar to those induced by gain-of-function KNOX1 alleles (40). Additionally, driving both KNOX2 (*KNAT3*) and an interacting BELL partner [*SAW1* (72)] with *STM* (KNOX1) regulatory sequences phenocopies a loss-of-function *stm* mutant (40). As there is no evidence of ectopic KNOX1 activity in KNOX2 loss-of-function mutants, one hypothesis is that the two classes act in opposite manners, with at least some downstream targets in common (40). Because expression of a KNOX1 protein (STM) translationally fused with a repressor domain phenocopies KNOX1 loss-of-function, KNOX1 proteins may primarily act as transcriptional activators, whereas KNOX2 proteins would act as repressors (40). Finally, loss-of-function *knat3* alleles partially suppress phenotypes induced by ectopic BELL expression in the embryo sac (1n), indicating that *KNAT3* is expressed in wild-type embryo sacs (100), reminiscent of the egg cell expression of KNOX in *Physcomitrella* (**Figure 2**).

The 13 BELL proteins encoded in the *Arabidopsis* genome have been shown to interact with KNOX proteins in vitro and in yeast two-hybrid assays with little phylogenetic congruence between partners. However, genetic experiments in *Arabidopsis* indicate specificity in KNOX-BELL interactions in planta (40). For example, in the shoot meristem KNOX1 proteins (e.g., STM) normally partner with BELL proteins (e.g., PNY, PNF). Expression of KNOX2 alone in the meristem is inconsequential, suggesting a lack of interaction between KNOX2 and the BELL proteins already present in the meristem (40). However, coexpression of KNOX2 and an appropriate BELL partner (BELL1, SAW1, SAW2) is sufficient to induce an aberrant phenotype (40). How the in vivo specificity is accomplished is not known.

Apomixis: clonal production of seeds in angiosperms in the absence of meiosis and fertilization

Apomixis is the clonal production of seed in the absence of meiosis and fertilization whereby the maternal genotype is retained in the progeny. Some forms of apomixis resemble apospory: In

aposporous apomixis, a maternal sporophyte diploid cell differentiates with the anatomy of the gametophytic embryo sac, and the diploid egg cell develops directly into an embryo in the absence of fertilization and bypasses meiosis. In some apomictic species, fertilization of the central cell is required to produce functional endosperm, whereas in other species endosperm can develop autonomously. Although the genetic basis underlying apomixis has not been identified in any species at present, mutations that result in autonomous endosperm development have been identified in *Arabidopsis* (47, 96). The corresponding genes encode homologs of components of PRC2, of which *Arabidopsis* has three paralogs encoding the *E*(*z*) component: *CURLY LEAF*, *MEDEA*, *SWINGER* (9, 21). That autonomous endosperm development is observed in *medea* mutants suggests a possible analogy with apogamy caused by compromised PRC2 activity in *Physcomitrella*. Endosperm development, which usually requires a fertilization event, directly from gametophytic cells in the absence of fertilization could be considered apogamy. Another parallel with observation in *Physcomitrella* is the derepression of KNOX gene expression when PRC2 activity is compromised; however, in the case of *Arabidopsis* the reported derepression was observed in sporophyte tissues, e.g., leaves (67, 82, 139). The fact that *swinger curly leaf* double mutants grow as callus-like tissue spontaneously differentiating somatic embryos also suggests a resetting to an embryonic (zygotic?) program (24).

Evolution of Land Plants

Despite the voluminous literature on TALE-HD function in land plants, little is known about the possible roles of these genes in the angiosperm life cycle. Aspects of gamete-specific expression of KNOX and BELL genes to initiate zygotic gene expression, as demonstrated in *Chlamydomonas*, may be conserved more broadly throughout plants, e.g., both KNOX1 and KNOX2 expression is observed in the *Physcomitrella* egg cell, and KNOX2 activity is inferred to be in the *Arabidopsis* female gametophyte. If the land plant egg cell is equated to the minus gamete of *Chlamydomonas*, the sperm cells might be equivalent to the plus gamete, suggesting BELL might be supplied to the zygote via the sperm. The expression of three of four *Physcomitrella* BELL genes in antheridia is consistent with this hypothesis.

The KNOX1 and KNOX2 subfamilies likely arose early in the charophycean algal lineage, prior to the divergence of *Klebsormidium* (60). The evolution of a multicellular diploid generation from an ancestral single-celled diploid generation required mitotic divisions after fertilization and repression of the haploid developmental program during the diploid generation. KNOX1/KNOX2 gene duplication followed by neofunctionalization may have provided greater flexibility in sporophyte gene regulation. In *Chlamydomonas*, the KNOX-BELL heterodimer acts as an activator, with increases in target gene expression detectable 5–10 minutes after gamete fusion (34, 143). The sparse data in land plants suggest that KNOX1 could be primarily an activator, with KNOX2 acting as a repressor (40). In the land plant common ancestor, KNOX1 genes acquired functions in maintenance of sporophytic meristematic cells, as evidenced by loss-of-function phenotypes in flowering plants and moss. Likewise, in land plants *KNOX2* genes evolved to maintain diploid differentiation via suppression of the gametophytic development program. The KNOX1/KNOX2 duplication may have facilitated the evolution of more complex gene regulatory networks to perform two critical roles in the land plant sporophyte and was perhaps instrumental in the establishment of a multicellular diploid generation in land plants. However, under this scenario, why did the evolution of the multicellular sporophyte arise only after hundreds of millions of years following the KNOX1/KNOX2 gene duplication?

Unlike KNOX, BELL paralogs do not fall neatly into distinct phylogenetic classes nor are their KNOX partners easily predicted by phylogenetic relationships (40). Such a situation is expected

if in the ancestral land plant both KNOX1 and KNOX2 interacted with a single BELL partner. In the broader view, the increasing number of potential KNOX-BELL combinations could have been co-opted to pattern novel land plant sporophytic organs and contributed to the patterning of increasing sterile elaborations of the sporophyte during land plant evolution, as envisioned by Bower (16).

Isomorphic alternation of generations: alternation of two generations that have indistinguishable morphologies

Red Algae (Rhodophyta)

Red algae diverged from the chlorophyte-streptophyte clade more than a billion years ago (**Figure 1**). Traditionally, red algae were placed in two classes, the Bangiophyceae and Florideophyceae, but the former is a paraphyletic assemblage of mostly unicellular or filamentous taxa (39, 105, 114). In contrast, Florideophyceae are a monophyletic group of species with an isomorphic alternation of generations and a triphasic life cycle comprising a haploid gametophyte, a diploid carposporophyte, and a diploid tetrasporophyte (73, 121, 140, 141). The complex multicellular gametophyte produces spermatia lacking flagella and retains egg cells, which fuse to produce a carposporophyte that is dependent upon the maternal gametophyte. The carposporophyte releases diploid carpospores that develop into independent tetrasporophytes that have the same basic morphology as the gametophytes, except rather than producing gametes, tetrasporophytes develop sporangia in which cells divide meiotically, producing haploid spores. Because the gametophyte and tetrasporophyte are isomorphic, it is likely that a co-option of genetic programs from one generation to the other occurred subsequent to one generation evolving complex multicellularity—a homologous origin for an alternation of generations. The complex triphasic life cycle might have evolved in response to the lack of flagella at any stage of the life cycle (110). Given that syngamy is a rare event, selection favored a life cycle in which zygote survival was high and a multicellular sporophyte in which many cells undergo meiosis (110). As with the green Archaeplastida, red algal genomes encode KNOX and BELL orthologs, indicating the duplication producing these paralogs occurred prior to their divergence (28, 88). However, nothing is known of the function of these genes in any red algal species.

LIFE CYCLES IN OTHER EUKARYOTIC LINEAGES

Brown Algae (Phaeophyta)

Brown algae are secondary endosymbionts and obtain their chloroplasts from an ancestral red algal endosymbiosis (**Figure 1**) (2). The majority of Phaeophyta diversity exhibits an alternation of generations, and phylogenetic analyses suggest it may be the ancestral state for the group (25, 26, 114, 123). The alternation of generations may be isomorphic or heteromorphic, and can be either haploid or diploid dominant. As brown algal life cycles have been the subject of recent reviews, here we only briefly mention the *ouroboros* mutation of *Ectocarpus*, a filamentous brown alga with a heteromorphic alternation of generations (27). In *ouroboros* mutants, the life cycle continually reiterates the gametophytic generation rather than alternating between gametophyte and sporophyte generations (27). Transcriptome analysis indicates that the wild-type *OUROBOROS* gene functions to both induce the sporophyte developmental program and repress the gametophyte genetic program (27). As the *ouroboros* mutant exhibits striking parallels with KNOX/BELL mutants in *Chlamydomonas* and *Physcomitrella*, it will be intriguing to determine whether the gene is homologous as well.

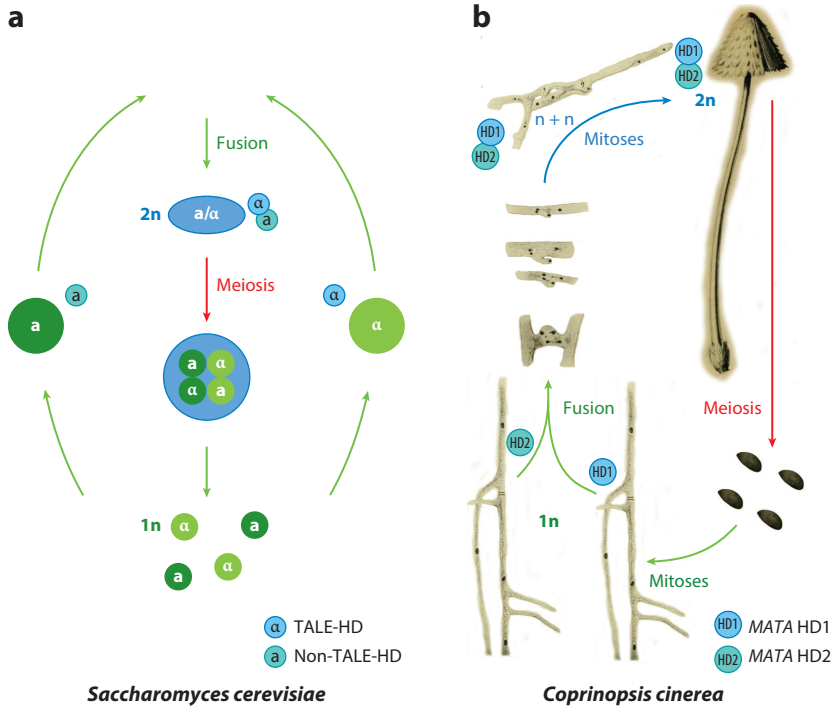

Figure 4

Fungal life cycles and their genetic basis. Events occurring in the haploid phase and gamete fusion are noted in green, events occurring in the diploid phase are noted in blue, and meiosis is indicated in red. In the ascomycete *Saccharomyces cerevisiae*, two mating types express TALE (three-amino-acid-length extension) and non-TALE homeodomain proteins, respectively, that heterodimerize in the diploid phase and repress haploid gene expression. In the basidiomycete *Coprinopsis cinerea*, a similar system functions, except that the heterodimer activates diploid gene expression and directs the initial diploid developmental program of clamp formation. Images adapted from Reference 10.

Fungi

The first genetically characterized life cycle was that of the ascomycete yeast *Saccharomyces cerevisiae* (44, 55). In *Saccharomyces*, the two mating types, a and α, express mating type–specific genes at the MAT locus: In MATα cells the *α2* gene represses a-cell–specific haploid genes and the *α1* gene activates α-specific haploid gene expression; in MATa cells, the *a1* gene is active, but it is not required in haploid cells (**Figure 4**) (44, 55). Upon fusion of a and α cells, in MATa/α diploid cells the a1 and α2 proteins heterodimerize, forming a repressor that represses haploid-specific genes, thereby resulting in the activation of the diploid zygotic program. The *α2* gene encodes a TALE-HD and *a1* encodes a non-TALE-HD. A similar situation exists in basidiomycete fungi, with the genetics elucidated in *Ustilago* (41, 62, 130) and *Coprinopsis* (71, 117). In *Coprinopsis*, haploid filamentous mycilia of different mating types fuse to form a dikaryotic mycilium, which develops structures known as clamp connections at their growing tips. The clamp connections facilitate nuclear exchange such that each daughter cell contains two nuclei, one from each of the original mating types. Stimulated by environmental factors, the dikaryotic mycilia aggregate and

differentiate into a mushroom, in which the dikaryotic nuclei finally fuse in specialized basidial cells on the mushroom underside. Meiosis occurs in basidia, producing haploid basidiospores. As with yeast, one mating type expresses a TALE-HD gene and the other mating type expresses a non-TALE-HD gene, and the two proteins heterodimerize in the dikaryon. However, in contrast to yeast, in *Coprinopsis* the heterodimer acts as an activator and at least some of its direct downstream targets include genes required for clamp connection differentiation, thus initiating the development of the complex multicellular diploid generation (64).

ANCESTRAL ROLE OF HOMEODOMAIN GENES?

The similarities in the genetic systems between fungi and *Chlamydomonas* suggest that an ancestral function of homeodomain genes was regulating the haploid-to-diploid transition in the ancestral eukaryote (76). Preloading gametes with protein products that function as heterodimers ensures a rapid switch from haploid gene expression to diploid genetic programs upon gamete fusion. The last common ancestor of eukaryotes is predicted to have possessed at least two homeodomain genes, a TALE-HD and a non-TALE-HD (13, 20, 31) (**Figure 1**). In one possible evolutionary scenario, the ancestral eukaryote used a system similar to that of extant fungi to activate diploid gene expression, one gamete expressing a TALE-HD and the other a non-TALE-HD. Examination of other independent eukaryotic lineages, e.g., the *ouroboros* mutant of *Ectocarpus* and unicellular Excavata and Amoebozoa taxa, is required to test this hypothesis (2). Gain-of-function studies, such as those performed in *Chlamydomonas*, have been suggested as a potentially fruitful approach, especially in lineages in which sex is as yet unknown (76). Of particular interest are the unicellular or colonial choanoflagellates, the sister group of metazoans, whose genomes encode either only TALE-HD or both TALE-HD and non-TALE-HD genes (69, 78). In metazoans, a homeodomain-mediated system to activate zygotic gene expression has not been described, suggesting metazoans may have lost the proposed ancestral system. Perhaps in lineages in which complex multicellularity evolved, a rapid system activating zygotic gene expression is no longer required, especially in cases in which strong maternal contributions to the egg exist or where the diploid generation is dependent upon the previous haploid generation. With respect to the latter condition, land plants, with their evolutionary shift in dominance between the haploid and diploid generations, could provide models to explore whether proposed ancestral systems have been retained in organisms with different life cycles.

If the ancestral function of homeodomain genes was regulating the haploid-to-diploid transition, their expression patterns would have them ideally placed to be co-opted to regulate developmental processes in organisms in which the diploid phase achieves complex multicellularity (76). Expansion and diversification of homeodomain gene families, especially non-TALE-HD *Hox* genes controlling development in metazoans, are consistent with this scenario. Likewise, expansion and diversification of the TALE-HD family in land plants are correlated with increasingly complex multicellular sporophyte development in this lineage. If the ancestral haploid-to-diploid transition was regulated by a TALE-HD and a non-TALE-HD, the duplication of TALE-HD into KNOX and BELL paralogs in Archaeplastida resulted in a shift such that two TALE-HD genes regulate the transition. This may have freed the non-TALE-HD gene in this lineage to evolve novel functions. In contrast to TALE-HD genes, non-TALE-HD genes are expressed in the haploid generation of charophycean algae and in both haploid and diploid generations of land plants, where they regulate development in both generations (38, 108, 142). Whether the activity of non-TALE-HD was instrumental in the evolution of complex multicellularity in the haploid generation in this lineage is a matter of speculation, but the KNOX/BELL duplication early in the Archaeplastida lineage may have at least indirectly facilitated the evolution of a heteromorphic

alternation of generations in land plants. These data raise key questions to be addressed, including whether other phylogenetically diverse eukaryotes have retained HD gene function for their diploid-to-haploid transition and whether similar co-options of HD genes have occurred to pattern diploid development in other multicellular lineages.

SUMMARY POINTS

1. Life cycles of eukaryotes are diverse, as multicellularity can occur in either the haploid or diploid phases, or both phases within the same organism, with the term "alternation of generations" applied to the latter case, and such life cycles have evolved repeatedly.
2. The morphologies of two multicellular generations can be indistinguishable (isomorphic) or can differ (heteromorphic), and in both cases, a preexisting genetic program directing body plan development in one generation may be co-opted during evolution of a multicellular body in the other generation.
3. A conceptual foundation of the land plant alternation of generations owes much to Wilhelm Hofmeister, who united land plants based on his discovery that all land plant life cycles have alternation of generations, and to Frederick Bower, who provided an adaptive rationale for its evolution.
4. Two related TALE-class homeobox genes play a key role in haploid-to-diploid transition in *Chlamydomonas*, a chlorophyte alga, and evolution of their homologs may have been instrumental in establishing alternation of generations in land plants.
5. The parallel of genetic basis among chlorophytes and two fungal lineages points to the antiquity of the genetic switch involving TALE-class homeobox genes in regulating haploid-to-diploid transition in eukaryotes.
6. The ancestral function of homeobox genes may have been to regulate the haploid-to-diploid transition in eukaryotes, where their expression in diploids could be co-opted to pattern multicellularity in this generation.
7. Although seen as anomalies, studies of apogamy, apospory, and apomixis have provided valuable insights into the alternation of generations in land plants.

FUTURE ISSUES

1. In land plants, does a complete loss of KNOX function result in the diploid zygote developing as if it were a gametophyte?
2. Is ectopic expression of KNOX and BELL in the gametophyte sufficient to activate the sporophyte developmental program in land plants?
3. Do the BELL paralogs of land plants function similarly to their *Chlamydomonas* ortholog (*HDG1*) or paralog (*GSP1*), or perhaps to both?
4. The role of KNOX-BELL activity in apomixis has not been investigated, but it seems a reasonable candidate in both autonomous embryo development—analogous with zygote activation?—and autonomous endosperm development—analogous with apogamy?

5. Is the role of BELL-KNOX genes in haploid-to-diploid transition conserved in vascular plants?
6. Do other eukaryotic lineages use TALE-HD genes, e.g., the *OUROBOROS* gene of *Ectocarpus*, to regulate the haploid-to-diploid transition?
7. If other lineages use a similar mechanism to regulate the haploid-to-diploid transition, do they use both TALE-HD and non-TALE-HD genes?
8. Is the major transition from diploid to haploid regulated by specific transcription factors, or is haploid identity the default in the absence of TALE-HD expression?

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