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SI CHLAMYDOMONAS

The *Chlamydomonas* cell cycle

Frederick R. Cross[1] and James G. Umen[2,*]
[1]*The Rockefeller University, New York, NY 10065, USA, and*
[2]*Donald Danforth Plant Science Center, St. Louis, MO 63132, USA*

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*For correspondence (e-mail: jumen@danforthcenter.org).

SUMMARY

The position of *Chlamydomonas* within the eukaryotic phylogeny makes it a unique model in at least two important ways: as a representative of the critically important, early-diverging lineage leading to plants; and as a microbe retaining important features of the last eukaryotic common ancestor (LECA) that has been lost in the highly studied yeast lineages. Its cell biology has been studied for many decades and it has well-developed experimental genetic tools, both classical (Mendelian) and molecular. Unlike land plants, it is a haploid with very few gene duplicates, making it ideal for loss-of-function genetic studies. The *Chlamydomonas* cell cycle has a striking temporal and functional separation between cell growth and rapid cell division, probably connected to the interplay between diurnal cycles that drive photosynthetic cell growth and the cell division cycle; it also exhibits a highly choreographed interaction between the cell cycle and its centriole–basal body–flagellar cycle. Here, we review the current status of studies of the *Chlamydomonas* cell cycle. We begin with an overview of cell-cycle control in the well-studied yeast and animal systems, which has yielded a canonical, well-supported model. We discuss briefly what is known about similarities and differences in plant cell-cycle control, compared with this model. We next review the cytology and cell biology of the multiple-fission cell cycle of *Chlamydomonas*. Lastly, we review recent genetic approaches and insights into *Chlamydomonas* cell-cycle regulation that have been enabled by a new generation of genomics-based tools.

Keywords: *Chlamydomonas reinhardtii*, phycoplast, multiple fission, cell-cycle mutant, mitosis, cytokinesis, Volvocine algae.

INTRODUCTION

The position of *Chlamydomonas* within the eukaryotic phylogeny makes it a unique model in at least two important ways: as a representative of the critically important, early-diverging lineage leading to plants, and as a microbe retaining important features of the last eukaryotic common ancestor (LECA) that have been lost in the highly studied yeast lineages. Its cell biology has been studied for many decades, and it has well-developed experimental genetic tools, both classical (Mendelian) and molecular. Haploidy and the relative paucity of gene duplication, compared with land plants, make it ideal for loss-of-function genetic studies, as usually a function is performed by a single copy of a unique gene. The *Chlamydomonas* cell cycle has a striking temporal and functional separation between cell growth and rapid cell divisions, probably connected with the interplay between diurnal cycles that drive photosynthesis-dependent cell growth with the cell division cycle; it also exhibits a highly choreographed interaction between the cell cycle and its centriole–basal body–flagellar cycle.

Here we review the current status of studies of the *Chlamydomonas* cell cycle. We begin with an overview of cell-cycle control in the well-studied yeast and animal systems, which has yielded a canonical, well-supported model. We discuss briefly what is known about similarities and differences in plant cell-cycle control, compared with this model. We next review the cytology and cell biology of the multiple-fission cell cycle of *Chlamydomonas*. Lastly, we review recent genetic approaches and insights into *Chlamydomonas* cell-cycle regulation that have been enabled by a new generation of genomics-based tools.

PHYLOGENY OF ANIMALS, FUNGI, PLANTS AND ALGAE

It was previously proposed that yeast could serve as a 'universal [eukaryotic] cell', such that the elucidation of cell

biology in yeast might yield insights and even direct molecular mechanisms relevant across the eukaryotic kingdom (Herskowitz, 1985). This concept was reasonable based on the phylogenetics at the time, and indeed, the concept was an extraordinarily useful one; however, the current consensus view from multiple phylogenetic approaches is that fungi and animals ('Opisthokonts') diverged from each other significantly later than plants and green algae ('Viridiplantae') diverged from Opisthokonts (Figure 1; Rogozin *et al.*, 2009). The plant lineage might even be the earliest diverging eukaryotic group from the last eukaryotic common ancestor (Rogozin *et al.*, 2009). The consensus phylogeny in Figure 1 has the unsettling implication that, in principle, features found in yeast or animals could be completely uninformative for Viridiplantae.

Sequence homology is commonly used to make inferences about shared protein function, particularly involving core cellular functions such as cell division. Whereas homology-based inferences are reasonable as starting hypotheses, the presence or absence of a homolog is insufficient as a criterion to make definitive conclusions about function. Moreover, the degree of uncertainty regarding conservation of function for a pair of protein homologs generally increases with greater sequence divergence and/or phylogenetic distance. Common examples of functional changes between homologs are the alteration of transcription factor binding site specificity, gains or losses of protein–protein interactions and changes in substrates or products of enzymes. The evolution of novel molecular pathways will naturally be even more rare, but have clearly occurred many times over the evolutionary time scales illustrated in Figure 1. With these considerations in mind, we briefly review what is known about cell-cycle control in Opisthkonts and in Viridiplantae.

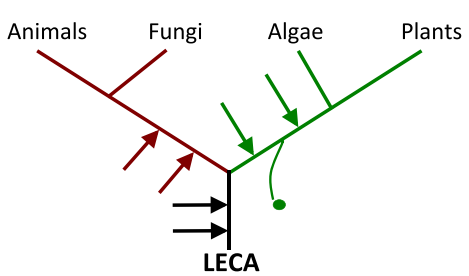

Figure 1. Eukaryotic phylogeny. The base of the tree is the last eukaryotic common ancestor (LECA). Fungi and animals (Opisthokonts, left branches) are more closely related to each other than either is to Viridiplantae (right branches). Reciprocally, green algae (e.g. *Chlamydomonas*) are much more closely related to land plants than to either yeast or animals. Other early-diverging eukaryotic lineages are not shown. The small green circle represents the unique acquisition of the photosynthetic endosymbiont that became the chloroplast. Green or brown arrows denote positions where gains or losses in the Opisthokont or Viridiplantae lineages, respectively, will result in differences between these lineages. Black arrows denote positions of innovations that are potentially eukaryotic-universal. Note that gains/losses can pertain to molecular interactions and higher-level systems, not just to gene content (see text). The branches are not drawn to scale.

Cell-cycle control in Opisthokonts

As a result of many decades of research, the molecular basis of cell-cycle control is well understood in animals and fungi (members of the Opisthokont clade of eukaryotes; brown branches in Figure 1). The controlling molecules, their interactions, dynamics and systems biology of cell-cycle control are largely conserved in these organisms (Morgan, 2007). Although there are interesting divergences, substitutions and variation in the relative importance of individual mechanisms among Opisthokonts, almost all the molecules as well as the topology and dynamics of regulatory interactions are highly conserved. Here we will briefly summarize the consensus view of this system, largely without references beyond the outstanding book by Morgan (2007).

The dominant theme in this model is intertwined, once per cell-cycle oscillations of two biochemical activities: cyclin-dependent kinase (Cdk, completely dependent on stable cyclin binding for activity); and the anaphase-promoting complex (APC), a ubiquinating enzyme leading to protein degradation of multiple targets, including many cyclins.

Figure 2 illustrates the main interactions. At the left of the diagram is illustrated the 'off' state achieved in newborn cells. This state (at least in many animal cells and in budding yeast) involves a balance between a transcriptional repressor (Rb, or Whi5 in yeast) and 'G1 cyclins' (Bertoli *et al.*, 2013). The G1 cyclins (in budding yeast, the 'CLN' cyclins; in animals, cyclins D and E) are specialized for this step.

The regulatory loop is double-negative feedback (equivalent to positive feedback), a common regulatory theme that can potentially result in bistability. The initial state of newborn cells is low cyclin, high repressor; the balance is tipped by some combination of cell growth and specific signaling pathways, at which point G1 cyclins can effectively phosphorylate and inactivate the repressor, resulting in increased gene expression of G1 cyclins (along with other genes; Bertoli *et al.*, 2013). The regulatory pathways causing the balance to tip are organism- and/or cell type-specific, as might be expected with this being a major point of control for cell proliferation.

In addition to inactivating Rb/Whi5, G1 cyclins (and probably other genes under repression by Rb/Whi5) also inactivate cyclin-dependent kinase inhibitors (Kip/CIP inhibitors in animals, Sic1 in yeast; stably binding stoichiometric inhibitors) and the Cdh1 regulator of the APC. These inhibitors are specific for a different class of cyclin-Cdk: A–type or B–type cyclins, usually bound to Cdk1. These complexes (especially cyclin B–Cdk1) are the primary activators of mitotic progression, as depicted. They are also in a double-negative feedback relationship with their inhibitors, yielding another possible bi-stable switch. This arrange-

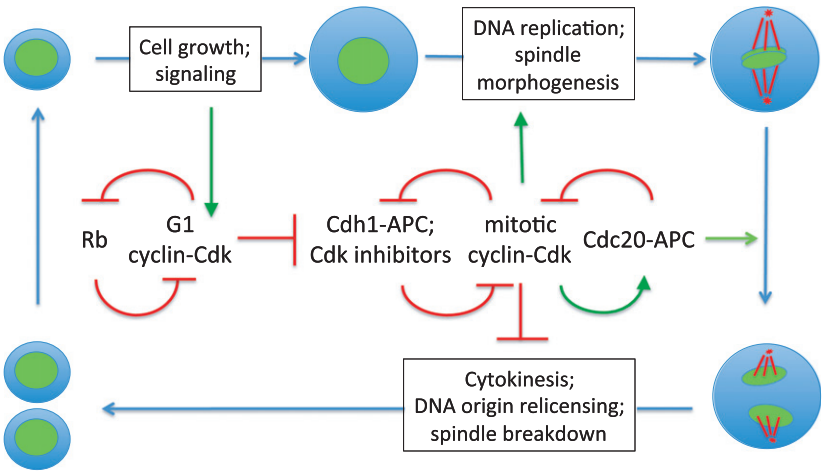

Figure 2. Consensus model for Opisthokont cell-cycle control. The figure summarizes a huge volume of work, carried out almost entirely in fungal and metazoan (Opisthokont) lineages; the reader is referred to the outstanding text of Morgan (2007) for a complete description and for primary literature references. The division cycle of a cell is illustrated on the outside; controlling machinery within; green, activation; red, inhibition. The central module involving APC and mitotic cyclin-Cdk is the most conserved; the retinoblastoma protein (Rb) is functionally replaced in yeast by the unrelated Whi5, for example (Bertoli *et al.*, 2013). Some controls are not illustrated in the figure for simplicity, such as the control of mitotic cyclin-Cdk by inhibitory phosphorylation by Wee1, and its reversal by Cdc25, accompanied by Wee1 inhibition and Cdc25 activation by mitotic cyclin-Cdk1. This architecture forms a positive (i.e. double-negative) feedback loop, as in the Rb–G1 cyclin and Cdh1–APC–mitotic cyclin interactions illustrated; these have important dynamic consequences (Pomerening *et al.*, 2003). Also not included are cell cycle-regulatory phosphatases such as Cdc14 in budding yeast, additional mitosis-regulatory kinases such as Aurora and Plk1, and cell-cycle checkpoint controls.

ment allows a 'hand-off' of control of these inhibitors: G1 cyclins are only transiently required for cell-cycle initiation. Once cyclin B–Cdk activity is established, they maintain control of these inhibitors.

In addition to promoting mitotic progression (DNA replication and spindle formation), cyclin B–Cdk1 is thought to have two other key activities: activating the Cdc20-APC complex and inhibiting the final stages of mitosis (spindle breakdown, telophase, cytokinesis and the subsequent 'licensing' of replication origins to reload the system for another replication cycle). In turn, Cdc20-APC has two central roles: first, to initiate cyclin–B ubiquitination and proteolysis, and second to promote the anaphase (chromosome segregation) by indirectly activating the protease Esp1/separase that degrade the cohesins that 'glue' replicated sisters together. The first role is central for starting the reset of the cyclin-Cdk control system: presumably by lowering cyclin B-Cdk enough that the bi-stable switch, with its inhibitors, can flip back. The second role is central to the essential cell biology of accurate chromosome segregation in mitosis. The nature of the control is such that anaphase will not be triggered until replication and spindle assembly are complete; this control is further enforced by cell-cycle 'check points' or surveillance systems (Hartwell and Weinert, 1989).

At its heart, this system constitutes a negative feedback loop (mitotic cyclin activates Cdc20, which leads to mitotic cyclin degradation); the double-negative feedback loop illustrated means that both high- and low-cyclin states are metastable. This control architecture (negative feedback loop, with positive feedback stabilizations) can result in robust oscillatory behavior (Pomerening *et al.*, 2003).

Not shown in Figure 1, but very important in many organisms, is an independent bi-stable switch involving the Cdc25 phosphatase and the Wee1 inhibitory kinase, with both acting on mitotic cyclin-Cdk; in general terms, this switch is likely to operate similarly to that illustrated (Pomerening *et al.*, 2003).

The related APC activators Cdh1 and Cdc20 are very interesting and important in this scheme. Note that although both promote cyclin B ubiquitination (and in general they have non-identical but overlapping specificity), Cdh1 is *inhibited* by cyclin-Cdk activity, whereas Cdc20 is *activated*. Cdc20 regulation is the basis for the fundamental instability of the mitotic state: 'MPF [cyclin B-Cdk1] sowing the seeds of its own destruction' (Murray and Kirschner, 1989). Cdh1 regulation is a major reason for the stability (in the absence of a trigger) of the newly born G1 state, as once Cdh1 is activated new mitotic cyclins are too unstable to accumulate to a sufficient level to turn it off again.

For specific cell-cycle pathways (DNA replication origin loading and firing; spindle morphogenesis and breakdown), mitotic cyclin-Cdk activity is thought to inhibit some steps and activate others, as indicated in Figure 2. It was noted decades ago that this arrangement allows a compact coupling of a single cycle of mitotic cyclin-Cdk activity to a single cycle of DNA replication and segregation, occurring in the correct order, thus defining and enforcing once per cell-cycle control by a 'ratchet'-like mechanism (Nasmyth, 1996). This coupling is likely to be

critical for many reasons. For example, attempted segregation before replication is complete, or cytokinesis before segregation is complete, could result in aneuploidy or broken chromosomes. These mechanisms are also considered to ensure that origins of replication can fire only once per replication cycle. All these considerations are especially critical in eukaryotes, which have multiple chromosomes, each with multiple replication origins.

A direct test of this model established 'locked' mitotic cyclin levels titrated to normal peak levels, to investigate whether, as predicted by this model, peak mitotic cyclin levels effectively blocked mitotic exit (Drapkin *et al.*, 2009). The results suggested that the model required revision to consider not just mitotic cyclin-Cdk levels, but the balance between Cdk and counteracting phosphatase (Cdc14 in budding yeast). Phosphatase PP2A-B55 may play a similar role in some animal systems (Wang *et al.*, 2011). These phosphatases are regulated to be highly active only at the time of mitotic exit, providing an additional (but organism-specific) regulatory loop (not illustrated in Figure 1).

Cell-cycle control in Viridiplantae

Much of the fundamental cell-cycle machinery defined in Opisthokonts is present in Viridiplantae at the level of conserved protein-coding sequences (Harashima *et al.*, 2013): Rb, G1 and mitotic cyclins and cyclin-dependent kinases, Kip/CIP family Cdk inhibitors, the anaphase-promoting complex, and its activators Cdh1 and Cdc20, essentially a full complement of core DNA replication machinery (origin-binding proteins, polymerases, etc.), many proteins important for spindle formation and function (tubulins, kinesins, kinetochore proteins), many proteins involved in checkpoints (for DNA replication, DNA damage, spindle assembly). This finding is fundamentally reassuring with respect to the probable global similarity of the Viridiplantae cell cycle to the well-studied Opisthokonts; however, there are some important caveats.

First, it is clear that similar or identical biochemical machinery can be 'plugged' into very different regulatory circuits. A well-characterized example is Ras in yeast (Tamanoi, 2011). Yeast and human Ras are functionally interchangeable, and both are regulated by guanine nucleotide exhange factors (GEFs) and GTPase-activating proteins (GAPs) in a biochemically near-identical manner; however, both upstream and downstream the Ras-GEF-GAP system is connected to different proteins with totally different biological inputs and outputs in yeast and humans.

Second, there are some surprising 'omissions' in the Viridiplantae cell-cycle control gene list. For example, all plants (and *Chlamydomonas*) contain likely homologs of ATM and ATR, the central kinases responsible for signaling DNA damage; however, in yeast and animals, ATM and ATR critically require the downstream kinases Chk1 and Chk2 to function. Chk1,2-deficient Opisthokonts made by genetic means are completely deficient in DNA damage responses, but plant genomes appear to lack detectable Chk1 and Chk2 orthologs. It is a reasonable speculation that some of these 'omissions' are complemented by Viridiplantae lineage-specific proteins that carry out the same function; this has been documented in a few cases. For example, Viridiplantae lack the APC inhibitor Emi1, but contain GIGAS/OSD1 and other proteins, apparently completely unrelated in sequence but carrying out a similar function in cell-cycle control (Iwata *et al.*, 2011, 2014). It is important to note that GIGAS/OSD1 was only detected by direct experimentation in plants; clearly a homology-based search will not identify such proteins that are absent from Opisthokonts.

Third, there are some Viridiplantae cell cycle gene regulatory genes that are lineage-specific, and therefore have no counterpart in Opisthokonts. For example, the CDKB family of cyclin-dependent kinases has been found in all green organisms to date, but not outside the green lineage; it is specifically expressed during mitosis, and is thought to be an important mitotic regulator (Burssens *et al.*, 1998; Mironov *et al.*, 1999; Joubès *et al.*, 2000; Bisová *et al.*, 2005; Robbens *et al.*, 2005).

Finally, direct tests have shown some surprising divergences in regulatory function between plants and Opisthokonts, even when very similar machinery is involved. For example, in Opisthokonts, Cdk1 is an essential Cdk, the main driver of mitotic progression; however, CDKA in Arabidopsis (and in *Chlamydomonas*, see below) has a much more restricted role, acting early in the cell cycle to promote cell-cycle initiation (Nowack *et al.*, 2012). Even this role is not essential, at least in Arabidopsis: cdka-null plants can be recovered. They have very substantial defects but are largely rescued by the removal of the Rb G1/S repressor. The Cdk-inhibitory kinase Wee1 is present in plants, as in animals and yeast, but has been thought to operate in very different ways (potentially independent of Cdk inhibition; Dissmeyer *et al.*, 2009).

A serious experimental challenge to genetic analysis in higher plants is the paleopolyploidization that resulted in the very high effective copy numbers of some genes (Garsmeur *et al.*, 2014). These multiple copies might have identical function, in which case loss-of-function genetics will reveal no phenotype until an *n*–tuple mutant has been constructed, or they might have overlapping but diverged roles (because of intrinsic specialization or differential timing/tissue of expression), in which case null phenotypes might be quite subtle and suggestive, for example, of tissue specificity rather than core cell-cycle function. Therefore, a great technical advantage of *Chlamydomonas* is that it diverged from land plants before this series of genome duplications. Although possessing a generally 'plant-like' genome, most (though not all) genes are present in a

single copy (Merchant *et al.*, 2007), literally so, because the organism is haploid.

Chlamydomonas and the deep roots of eukaryotic cell-cycle control

Very well-conserved proteins such as Cdk1/Cdc2/CDKA show quite similar levels of divergence between animals and yeast, compared with the levels of divergence between animals and plants. Even fission yeast and budding yeast show high divergence in this sequence despite their relatively recent divergence within the ascomycete fungi. Even more striking is the complete absence in fungal genomes of many proteins that are very important for cell-cycle regulation in animals. Remarkably, many of these proteins are found in Viridiplantae genomes. For example, Rb is present in animals, absent in all fungi (in many cases replaced functionally by the unrelated Whi5 repressor), but unambiguously present in Viridiplantae, including *Chlamydomonas*. Similarly, cyclins D and A are present in animals and plants, but are missing in yeast (replaced as a consequence of repeated gene duplication and divergence of, most likely, a single B–type cyclin in the fungal lineages; Archambault *et al.*, 2005).

Although these findings could support the idea that yeast diverged earlier than plants diverged from animals, this is almost surely not the case. The consensus topology is of early divergence of the plant lineage from the animal/fungal lineage. This conclusion is supported by unique 'only happened once' features ('rare genomic changes' in the case of molecular phylogenies), which give unambiguous topologies despite highly irregular branch lengths (Rogozin *et al.*, 2009). Therefore, it is likely that the last common ancestor of plants and animals (argued to be LECA itself; Rogozin *et al.*, 2009) had cell-cycle control of considerable complexity, including all of the machinery illustrated in Figure 2. The absence of Rb, cyclins A and D, and other regulators in fungi, which was at one point taken to imply that these were multicellular inventions, is instead most parsimoniously accounted for by the loss of these regulators from fungi (with replacement, such as Whi5 for Rb).

The consequence of these phylogenetic considerations is that *Chlamydomonas* is a highly informative genetic model in two directions that are (only seemingly) paradoxical. First, *Chlamydomonas* is a representative of the early-diverged Viridiplantae, and is by far the best-developed Viridiplantae system allowing microbial genetic analysis. Therefore, cell-cycle control features specific to Viridiplantae can be examined by the powerful methods available in microbes, without the complication of multiple gene duplicates with partially overlapping functions (Table 1; Bisová *et al.*, 2005). Second, although yeasts have been extraordinarily useful models for animal cell biology, they are not useful for studying features of animal cells that are lost or replaced in the fungal lineage. *Chlamydomonas* has retained some features, possibly derived from the LECA, that are shared with animal cells and land plants (e.g. Rb, and cyclins A and D), but that are lost in yeast. Thus *Chlamydomonas* is currently the sole microbial model for the study of these important regulators (Table 1). *Chlamydomonas* has also been a spectacular model for cilia/flagella, which surely were present in LECA but were lost in almost all fungi and in almost all land plants, and are increasingly recognized for their importance in the cell cycle as well as diverse aspects of animal cell biology and human disease (Quarmby and Parker, 2005; Pan and Snell, 2007; Christensen *et al.*, 2008; Ke and Yang, 2014).

THE *CHLAMYDOMONAS* MULTIPLE FISSION CELL CYCLE

Control points and unique features of multiple fission

Chlamydomonas and many of its green algal relatives proliferate using a modified cell cycle, termed multiple fission (also referred to as palintomy; Figure 3). Multiple-fission cell cycles are characterized by a prolonged growth phase (G1), during which cells can enlarge by more than two-fold in size. Under favorable conditions, *Chlamydomonas* cells can grow in volume by more than 10-fold during a G1 phase that lasts between 10 and 14 h. At the end of G1 *Chlamydomonas* cells undergo successive rounds of rapidly alternating S phases and mitoses (S/M) to produce 2^n daughter cells. Daughters (also termed zoospores) then hatch out of the mother cell to begin the cycle again. One round of S/M takes around 30–40 min to complete, so a typical time range for a cell to spend in S/M is between 30 min and 2 h (Harper *et al.*, 1995). The number of S/M cycles that each mother cell undergoes is dictated by cell size: large mother cells divide more times than small mother cells, so that daughters of a uniform size distribution are always produced (Craigie and Cavalier-Smith, 1982; Donnan and John, 1983). Depending on growth conditions a mother cell undergoes between one and five S/M cycles to produce 2, 4, 8, 16 or 32 daughters (Lien and Knutsen, 1979). Under a typical diurnal cycle (e.g. 12 h of light/12 h of dark) the cell cycle becomes synchronized such that growth occurs during the light phase and cell division (S/M) occurs in the dark. Multiple fission is likely to be an adaptation of motile green algae that must resorb or remove their flagella prior to division, in order to use their basal bodies to coordinate mitosis and cytokinesis: the so-called flagellation constraint (Koufopanou, 1994). Teleologically, this could be understood because when light is available flagella-dependent phototaxis is used to optimize growth, and cell division is delayed until dark, when phototaxis is not required. It is worth noting that a variant of multiple fission observed in some green algae, including the genus *Scenedesmus*, involves successive S phases and endomitoses occurring during the growth

Table 1 *Chlamydomonas* cell-cycle regulatory genes and homologs in Arabidopsis, budding yeast and humans

Cell-cycle regulatory genes[a]	Classification	Phytozome 10 gene ID[b]	*Chlamydomonas* mutant?	*Arabidopsis thaliana* homolog(s)	*Saccharomyces cerevisiae* homolog(s)	Human homolog(s)[c]
Cyclin-dependent kinases						
CDKA1	CDK1	Cre10.g465900	yes[d]	*CDKA;1*	*CDC28*	*CDK1*
CDKB1	B–type CDK	Cre08.g372550	yes[d]	*CDKB1;1, CDKB1;2, CDKB2;1, CDKB2;2*	n.a.	n.a.
CDKD1	CAK (CDK activating kinase)	Cre09.g388000	no	*CDKD;1, CDKD;2, CDKD;3*	*KIN28*	*CDK7*
CDKD2	CAK (CDK activating kinase)	Cre05.g233600	yes[d]	*CDKD;1, CDKD;2, CDKD;3*	*KIN28*	*CDK7*
Cyclins						
CYCA1	A–type cyclin	Cre03.g207900	no	*CYCA1;2, CYCA2;1, CYCA2;2, CYCA2;3, CYCA2;4, CYCA3;1, CYCA3;2, CYCA3;3, CYCA3;4,*	n.a.	*CCNA1, CCNA2*
CYCB1	B–type cyclin	Cre08.g370401	no	*CYCB1;1, CYCB1;2, CYCB1;3, CYCB1;4, CYCB2;1, CYCB2;2, CYCB2;3, CYCB2;4, CYCB3;1*	*CLB1, CLB2, CLB3, CLB4, CLB5, CLB6*	*CCNB1, CCNB2, CCNB3*
CYCAB1	n.a.	Cre10.g466200	no	n.a.	n.a.	n.a.
CYCD1	D–type cyclin	Cre11.g467772	no	*CYCD1;1, CYCD2;1, CYCD3;1, CYCD3;2, CYCD3;3, CYCD4;1, CYCD4;2, CYCD5;1, CYCD6;1, CYCD7;1,*		*CCND1, CCND2, CCND3*
CYCD2	D–type cyclin	Cre06.g289750	no	*CYCD1;1, CYCD2;1, CYCD3;1, CYCD3;2, CYCD3;3, CYCD4;1, CYCD4;2, CYCD5;1, CYCD6;1, CYCD7;1,*		*CCND1, CCND2, CCND3*
CYCD3	D–type cyclin	Cre06.g284350	no	*CYCD1;1, CYCD2;1, CYCD3;1, CYCD3;2, CYCD3;3, CYCD4;1, CYCD4;2, CYCD5;1, CYCD6;1, CYCD7;1,*		*CCND1, CCND2, CCND3*
CYCD4	D–type cyclin	Cre09.g414416	no	*CYCD1;1, CYCD2;1, CYCD3;1, CYCD3;2, CYCD3;3, CYCD4;1, CYCD4;2, CYCD5;1, CYCD6;1, CYCD7;1,*		*CCND1, CCND2, CCND3*
Retinoblastoma (RB) tumor-suppressor pathway						
DP1	DP	Cre07.g323000	yes[e]	*Dpa, DPb*	n.a.	*TFDP1, TFDP2, TFDP3*
E2F1	E2F	Cre01.g052300	yes[e]	*E2Fa, E2Fb, E2Fc*	n.a.	*E2F1, E2F2, E2F3, E2F4, E2F5, E2F6*
E2FR1	E2F	Cre13.g572950	no	*E2Fa, E2Fb, E2Fc*	n.a.	*E2F1, E2F2, E2F3, E2F4, E2F5, E2F6*
MAT3	retinoblastoma (RB)	Cre06.g255450	yes[f]	*RBR1*	n.a.	*RB1, RBL1, RBL2*
Anaphase promoting complex/cyclosome (APC/C)						
APC1	APC subunit	Cre13.g579100	no	*APC1*	*APC1*	*ANAPC1*
APC2	APC subunit	Cre10.g460532	no	*APC2*	*APC2*	*ANAPC2*
APC3/CDC27	APC subunit	Cre17.g740510	yes[d]	*CDC27A, CDC27B*	*CDC27*	*CDC27*
APC4	APC subunit	Cre12.g556650	no	*APC4*	*APC4*	*ANAPC4*
APC6	APC subunit	Cre13.g562950	yes[d]	*APC6*	*CDC16*	*CDC16*
APC8	APC subunit	Cre16.g681578	no	*APC8*	*CDC23*	*CDC23*
APC10	APC subunit	Cre13.g571850	no	*APC10*	*DOC1*	*ANAPC10*
APC11	APC subunit	Cre13.g590900	no	*APC11*	*APC11*	*ANAPC11*

(continued)

Table 1. (continued)

Cell-cycle regulatory genes[a]	Classification	Phytozome 10 gene ID[b]	*Chlamydomonas* mutant?	*Arabidopsis thaliana* homolog(s)	*Saccharomyces cerevisiae* homolog(s)	Human homolog(s)[c]
APC13	APC subunit	Cre03.g186900	no	*APC13*	*SWM1*	*ANAPC13*
CDC20	APC regulator	Cre09.g399911	no	*CDC20.1, CDC20.2, CDC20.3, CDC20.4, CDC20.5*	*CDC20*	*CDC20, CDC20B*
CDH1	APC regulator	Cre09.g406851	no	*FZR1, FZR2, FZR3*	*CDH1*	*FZR1*
Others						
CKS1	CKS	Cre03.g180350	no	*CKS1, CKS2*	*CKS1*	*CKS1B, CKS2*
WEE1	WEE kinase	Cre07.g355250	no	*WEE1*	*SWE1*	*WEE1, WEE2*
ESP1	ESP/separase	Cre01.g029200	yes[c]	*ESP/AESP*	*ESP1*	*ESPL1*
PLK1	Polo-domain kinase	Cre03.g190050	no	n.a.	*CDC5*	*PLK1, PLK2, PLK3, PLK4, PLK5*

[a]Conserved genes predicted or shown to be involved in *Chlamydomonas* cell-cycle control.
[b]*Chlamydomonas reinhardtii* gene IDs, based on the most recent release of Phytozome (http://phytozome.jgi.doe.gov).
[c]Human gene nomenclature taken from Gray *et al.* (2015).
[d]From Tulin and Cross (2014).
[e]From Fang *et al.* (2006).
[f]From Umen and Goodenough (2001).
n.a., no homolog.

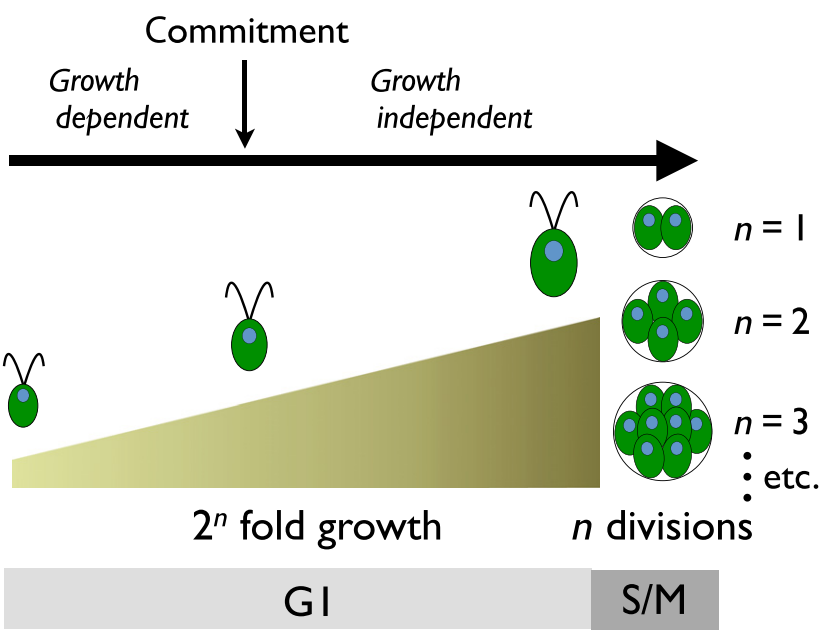

Figure 3. *Chlamydomonas* multiple-fission cell cycle. Schematic from left to right of one multiple-fission cycle. Daughter cells grow during G1 and can reach sizes many-fold larger than their starting size. At the end of G1 a series of rapid alternating S phases and mitoses (S/M) produces 2^n daughters. The cell division number (n) is determined by the cell size of the mother, and typically ranges from one to three divisions, to produce two, four or eight daughters. G1 is divided into two periods separated by the commitment point. Cell-cycle progression before commitment is dependent on growth and on cells reaching a minimum size. Cell-cycle progression after commitment is not dependent on continued growth. After S/M, daughters hatch and re–enter the G1 phase.

phase so that at the end of the light period a cell will be a multinucleate syncytium. A succession of cytokinetic events then partitions the mother cell into daughters that each contain a single haploid nucleus (Bisová and Zachleder, 2014).

Multiple fission is found in diverse protistan taxa, including heterotrophs, and may have more general adaptive utility in some environments where both cell size and the dispersal of progeny are under selection (Cavalier-Smith, 2002). Multiple fission also has parallels with developmentally regulated cell cycles of animals and land plants. For example, oocyte development in many metazoan taxa involves massive cell growth with no division, followed, upon fertilization, by rapid cycles of zygotic cell division in the absence of growth (O'Farrell, 2004). In land plants early endosperm cell cycles involve successive endomitoses, leading to a multinucleate syncytium that subsequently undergoes cellularization (Sabelli and Larkins, 2009), a process that is analogous to the multiple fission mechanism used by *Scenedesmus*.

Investigations into how the multiple fission cell cycle is controlled have produced a rough consensus on its control points, although there are somewhat conflicting reports and interpretations about control points and the relative roles of timer and sizer mechanisms, as well as contributions of circadian oscillators. Based on the overall cell cycle for *Chlamydomonas* it can be easily inferred that cell division is not triggered upon an approximate doubling of cell mass. To maintain size homeostasis in a growing population, the number of cell divisions must be equal to the number of mass doublings over some time. This requirement has been called 'coordination of growth and division'; it is an average relationship, and how it is achieved is not entirely clear in any organism (Jorgensen and Tyers, 2004). In budding yeast, the initiation of the cycle is conditional on reaching a minimum cell size, and this control is sufficient for size homeostasis. In fission yeast, the initiation of mitosis is instead the primary site of size control and the coordination of growth and division. Interestingly, in both organisms it is likely that size controls exist at both cycle initiation and mitosis; however, different controls dominate in the two organisms. (Johnston *et al.*, 1977; Fantes and Nurse, 1978; Harvey and Kellogg, 2003; Di Talia *et al.*, 2007.)

Size-dependent 'decisions' in yeasts are made one binary cell division cycle at a time. In *Chlamydomonas* a size-dependent step ('commitment') allows multiple binary divisions to occur within one multiple fission 'cycle'. It is then argued that distinct size-dependent step(s) control the number of subsequent division cycles that occur during S/M, either as a single 'memory'-based step or as a series of size-dependent individual decisions. The molecular basis of these regulatory events is largely unknown, except for the involvement of the Rb tumor suppressor pathway (see below). A molecular model for size control in *Chlamydomonas* must ultimately account for: (1) variability in cell division number (i.e. the n in 2^n), leading to a constant daughter cell size; (2) commitment to one or more divisions in G1; and (3) rules governing the length of G1 (which extends considerably beyond the time of commitment) and the timing of transition into the S/M phase. There is little or no observable G2 phase (interval between S and M phases) in *Chlamydomonas*, nor an observable gap between successive S and M phases (Jones, 1970; Harper *et al.*, 1995), although there is chromatin decondensation after each cytokinesis (Johnson and Porter, 1968). The length of S/M is therefore controlled by how many division cycles a mother cell undergoes, and as described above requires about 30 min per cycle.

Control of cell division number

Size control at S/M involves coupling between the cell size of the mother and division number. Experimental evidence for this form of size control has been obtained in several ways, including the separation of mother cells into different size classes (Craigie and Cavalier-Smith, 1982), or by manipulating growth rates or duration of growth to achieve different mother cell sizes (Donnan and John, 1983). In both types of experiments the daughter cell size is constant, regardless of the initial mother cell size. These experiments establish that *Chlamydomonas* cells have a mitotic sizing mechanism that couples mother cell size with cell division number, thus ensuring uniformity in the daughter cell size.

There are at least two interpretations or models for how mitotic size control operates in *Chlamydomonas*. The first model involves some form of cellular memory that tracks the numbers of cell doublings. John and colleagues express this idea in terms of cells attaining serial commitment points during G1 as they grow (Donnan and John, 1983; McAteer *et al.*, 1985), and this idea has also been formalized in the cell-cycle models of Zachleder and colleagues (reviewed in Bisová and Zachleder, 2014). Although the counting or memory model is plausible, it would require some form of verification or molecular markers for these additional commitments to distinguish it from less complicated alternatives. A simpler model originally proposed by Craigie and Cavalier-Smith requires no memory or counting, but instead posits that once the division phase is triggered, the mother cells continue to divide until the daughters fall below a specific threshold size (Craigie and Cavalier-Smith, 1982). The appeal of this second model is that it does not require any form of cellular memory or counting, but only requires the ability of cells to assess a single size threshold (Umen and Goodenough, 2001; Umen, 2005).

The commitment point

Published experiments on the cell cycle are in agreement that there is a cut-off point in the G1 phase after which completion of at least one S/M cycle becomes independent of subsequent growth. This cut-off point is referred to as commitment (Spudich and Sager, 1980; Craigie and Cavalier-Smith, 1982; Donnan and John, 1983). The term 'commitment point' used in this review is a single control point that is experimentally and operationally defined, and is assessed by moving cells into the dark or withdrawing nutrients, and then determining whether individual cells have completed the cell cycle (i.e. were committed) or instead were arrested in G1 without dividing (i.e. were non-committed). Typically, commitment is assayed by plating cells on agar and placing them in the dark for later microscopic examination (Umen and Goodenough, 2001), or sometimes by placing an aliquot of liquid culture in the dark and counting divided cell clusters before daughters hatch (McAteer *et al.*, 1985). The number of divisions that a committed cell undergoes is not part of the definition of commitment described here, although it can be relevant

for assessing other aspects of the cell cycle such as cell growth and cell size control. For those who are not familiar with the *Chlamydomonas* cell cycle literature, the use of the term commitment can be confusing because some models (i.e. those of John *et al.* and Zachleder *et al.* described above) invoke the idea of multiple commitment points. As described below, there is strong evidence and agreement across the literature that a definable commitment point exists in G1, but there is no agreement on or direct evidence for subsequent commitment points.

In principle, the multiple fission cell cycle could operate without a G1 commitment point if cell division were to follow a timer that started at the beginning of G1 and triggered cell division when it expired, or reset itself if the cells were too small to divide; however, a single timer model for the duration of G1 does not explain experiments where growth of cells is temporarily interrupted or slowed early in G1, before cells have reached their minimum division size (Figure 4). If a single G1 timer that began at the commencement of daughter cell growth controlled cell division, then a short interruption of growth for a period of several hours in early G1, followed by the resumption of cell growth to allow at least one mass doubling before the putative timer expired, should result in S/M starting at the same time as in a control culture where cell growth was uninterrupted. Instead, the interruption of growth early in G1 (before commitment) results in a delay in subsequent cell division (Figure 4; McAteer *et al.*, 1985; Matsumura *et al.*, 2003; Vítová *et al.*, 2011b).

John and co-workers modeled progression to commitment as being strictly timer dependent (Donnan and John, 1983), but later revised their model with a cryptic sizing mechanism that blocks commitment unless cells have reached a minimum size (John, 1987). Other researchers have also concluded that a minimum size is required for commitment, but there is no consensus on whether reaching this size is sufficient for commitment or whether a timer mechanism is also involved (Matsumura *et al.*, 2003, 2010; Fang *et al.*, 2006; Oldenhof *et al.*, 2007; Vítová *et al.*, 2011a,b). These models are not in complete agreement, but we believe that the overall cell-cycle framework that they describe is correct, and that by tying classical cell physiological studies such as these to a molecular framework a coherent model for the multiple fission cell-cycle control mechanisms will emerge.

Delaying division: the key to multiple fission

For multiple fission division, cells must be able to undergo more than one mass doubling during the G1 phase, and therefore temporarily suppress division, even after they have grown large enough to divide. The prolonged time period between commitment and S/M is a distinct portion of the G1 phase that is governed by different parameters than early G1, where cell-cycle progression is dependent on growth. During the post-commitment delay period cells may or may not continue to grow, and will divide in either case. After passing commitment, cells will typically remain in G1 for 5–8 h before dividing. Although there is general

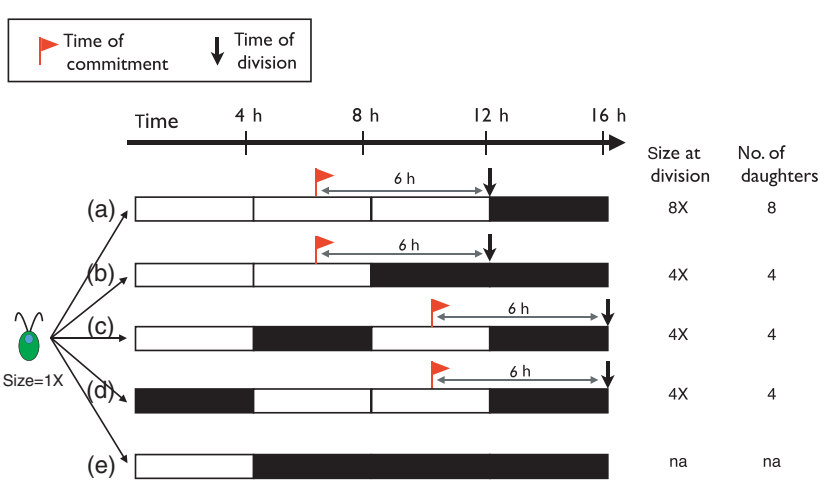

Figure 4. Experiment showing post-commitment timer. Diagram of growth-interruption experiments, showing timing of commitment and cell division. Newborn daughter cells in minimal media are placed in one of five growth regimes (a–e) consisting of light periods (open boxes) and dark periods (filled boxes). After approximately doubling in size, the cells pass commitment (red flag) and then divide about 6 h later (dark arrow). (a) Cells remain under light for 12 h and grow approximately eightfold in size before dividing at 12 h. (b) The light period lasts only 8 h, but the total cycle time remains the same, with division at around 12 h, and with the production of just four daughters. (c) The light period is interrupted, with a 4–h dark period prior to commitment. Growth resumes at 8 h, but passage through commitment and cell division are both delayed by 4 h. Note that at 12 h the cells in experiments (b) and (c) have spent the same length of time under light and are the same size, but that they do not divide at the same time. In (d), growth is delayed by 4 h at the beginning of the experiment, resulting in a 4–h delay in passing commitment and in cell division. In (e), the cells do not grow enough in size to pass commitment and therefore do not divide.

agreement on the existence and overall long duration of the post-commitment G1 period, relative to the total time in G1, there is no consensus on how its length is controlled. John and co-workers described this period as timer controlled, an observation that is generally supported by experiments where division occurs at approximately the same time across a range of post-commitment growth rates (McAteer *et al.*, 1985); however, other researchers have reported variability in the post-commitment interval (Oldenhof *et al.*, 2007; Vítová *et al.*, 2011a,b). Despite some experimental variability, the overall timing of cell-cycle events in *Chlamydomonas* when grown under a variety of diurnal regimes (e.g. 12 h light/12 h dark) involves growth during the light phase and cell division around the end of the light period or at the beginning of the dark period. This proliferation strategy is consistent with the idea that multiple fission evolved as an adaptation to ensure motility during the day, in order to optimize exposure to light, and to postpone cell division until dark, when the temporary loss of flagella and phototaxis impose less of a fitness penalty (Koufopanou, 1994).

Environmental and circadian influences on the cell cycle

Most of the experiments described in the preceding section used variable white light intensity and temperature to control the growth rate as a means of manipulating the cell cycle. Additional work has investigated environmental signaling in cell-cycle control. Experiments using acetate supplementation and the photosystem II inhibitor DCMU were performed in an attempt to uncouple the growth-promoting effects of light from potential light signaling (Voigt and Münzner, 1987). These experiments showed a modest but reproducible delay of S/M phase caused by continued illumination in late G1 phase cultures, compared with cultures that remained in the dark. DCMU and darkness block the overall cell growth equivalently, so the growth rate per se can be ruled out as an indirect mediator of light-induced division delay. The authors conclude that light directly suppresses division. DCMU and darkness are not completely equivalent, however: light-induced cyclic electron flow and the generation of ATP still occurs in the presence of DCMU.

Light quality has been further investigated by testing the effects of blue and red light on cell-cycle kinetics. It was found that blue light but not red or far-red light could induce the delay in cell division found in previous studies with white light (Munzner and Voigt, 1992). The same study identified a bimodal action spectrum for blue light, with peaks at 400 and 500 nm. Blue and red light were further investigated for effects on the commitment point. Blue light was found to cause a shift to a larger commitment cell size, and to extend the total length of the cell cycle, compared with red light at matched growth rates for both wavelengths (Oldenhof *et al.*, 2004a,b). Interestingly, the inhibitory effect of blue light on commitment as not affected by DCMU treatment and could be reversed by transferring cultures into red light, but not darkness (Oldenhof *et al.*, 2006). *Chlamydomonas* and other green algae have photoreceptors that might mediate blue and red light-dependent effects on the cell cycle. These include a single blue light-responsive phototropin gene and at least four cryptochrome-related proteins (Hegemann and Berthold, 2009). Interestingly, one of the crytoptochromes, aCRY1, absorbs both blue and red light, and a mutant with reduced aCRY1 levels has reduced expression of several genes, including circadian-expressed genes and at least one cell-cycle gene, *CDKB1* (Beel *et al.*, 2012). To date no published data show a connection between a specific photoreceptor and a cell-cycle phenotype, but this is a promising direction for future studies.

Chlamydomonas cells undergo circadian clock-controlled oscillations in a number of behaviors and metabolic functions (Matsuo and Ishiura, 2010; Schulze *et al.*, 2010), including reports of circadian controlled cell division (Bruce, 1970; Straley and Bruce, 1979). Circadian behaviors are those that continue to oscillate with a period of ~24 h under 'free-running' conditions where cells are maintained in a constant environment. The question of whether the cell cycle in *Chlamydomonas* is circadian-clock controlled is controversial. John and co-workers reduced the growth rates of cells (to greater than 24 h doubling time) to determine whether the clock controlled division (McAteer *et al.*, 1985; John, 1987), and concluded that it did not. These studies were extended more recently by the use of varied light and temperature regimes to vary growth rates and achieve a wide range of interdivision times that did not center on a 24–h period (Vítová *et al.*, 2011a,b), and reached the same conclusion. Goto and Johnson performed a set of experiments that showed circadian rhythms of daughter cell liberation (a proxy for cell division) and phototaxis under a range of free-running clock conditions, and also showed a period length change for these events in a circadian mutant, *per1* (Goto and Johnson, 1995). They further argued that circadian oscillators can be forced to entrain to non-circadian periods, so the observation of non-circadian cell division intervals by itself does not rule out the involvement of a circadian clock in gating or influencing the *Chlamydomonas* cell cycle. One possibility for disagreement on circadian clock involvement in cell division is that laboratory strains may lose clock function, or that conditions in which cells are typically synchronized for cell-cycle studies override the clock and obscure its potential input into division control. Indeed, Matsuo and co-workers reported testing progeny from several outcrosses with a circadian clock reporter strain before finding one with a robust circadian rhythm (Matsuo *et al.*, 2008). As it is clear that strains can lose robust clock

behavior (probably through long-term passage in culture with no selection), any rigorous test of the relationship between the circadian clock and cell cycle must be performed in a strain with a validated clock.

Mutational studies of multiple fission

Mutations advancing the cell cycle, so that cells divide when abnormally small, are likely to identify key regulatory elements; indeed, in fission yeast this strategy identified all elements of the highly conserved Cdc2/Wee1/Cdc25 regulatory module (Fantes, 1979; Russell and Nurse, 1986, 1987). *Chlamydomonas mat3* mutations (linked to the mating-type locus) were originally reported as having a defect in uniparental chloroplast DNA inheritance (Gillham *et al.*, 1987). Subsequent analysis revealed that the *MAT3* gene product is required for maintaining normal cell size; in its absence, the size of cells at commitment is greatly reduced. In addition, more divisions at S/M are carried out in cells of a given size. Thus *MAT3* is required for both aspects of cell-size control (see above; Umen and Goodenough, 2001). The primary small-cell-size defect in *mat3* leads to reduced levels of chloroplast DNA, a condition that is known to disrupt uniparental inheritance (Armbrust *et al.*, 1995). Cloning of the *MAT3* locus revealed it to encode a retinoblastoma-related protein (RBR), which is a conserved family of cell-cycle repressors found in most eukaryotes, but lost from yeasts and other fungi (Umen and Goodenough, 2001), probably as a result of replacement by the functionally equivalent Whi5 repressor (Cross *et al.*, 2011). In animals and plants, Rb binds to and represses the heteromeric DNA-binding complex DP/E2F (Burkhart and Sage, 2008). A similar pathway is likely to exist in *Chlamydomonas*, as loss-of-function mutations in *DP1* and *E2F1* (genes encoding E2F- and DP-related proteins, respectively) lead to large-cell-size phenotypes that are epistatic to *mat3* small-cell-size phenotypes, and MAT3 protein directly binds DP1-E2F1 (Fang *et al.*, 2006; Olson *et al.*, 2010). Additional suppressor loci with less severe phenotypes than *dp1* and *e2f1* were also identified, encoding proteins with specific functions in the multiple fission cell cycle that remain to be elucidated (Fang and Umen, 2008; Fang *et al.*, 2014). FA2 is a NIMA-related protein kinase that is required for flagellar maintenance (Mahjoub *et al.*, 2002; Fry *et al.*, 2012). FA2 promotes S/M phase entry, as evidenced by a delayed S/M entry phenotype in a *fa2* mutant, but is not required for mitotic cell-size control. Another NIMA family member, CNK2, was implicated in flagellar length regulation as well as in the mitotic sizing mechanism (Bradley and Quarmby, 2005). The relationship between these NIMA kinases and the RBR complex in controlling cell division remains unclear, but could reveal insights into how the flagella/cilia biogenesis is coordinated with the cell cycle (Quarmby and Parker, 2005; Goto *et al.*, 2013).

CYTOLOGY OF CELL DIVISION IN *CHLAMYDOMONAS*

Interphase cell architecture

An interphase *Chlamydomonas* cell has a well-defined architecture (Holmes and Dutcher, 1989; Harris, 2001). At its apical end are two basal bodies that serve as organizing centers for the internal microtubule cytoskeleton, and that nucleate a pair of flagella. Associated with each basal body is an immature pro-basal body that will mature into a new daughter basal body during the prophase of mitosis (see below; Gould, 1975). Positioned just below the basal bodies is the nucleus. The 111–Mb *Chlamydomonas* nuclear genome (Merchant *et al.*, 2007) is divided into 17 chromosomes that are packaged with histones into typical eukaryotic chromatin (Robreau and Le Gal, 1975; Woodcock *et al.*, 1976; Morris *et al.*, 1990). A cruciate set of four-member and two-member microtubule rootlets, marked by acetylated tubulin, is associated with basal bodies and extends basally along the cell periphery, providing a convenient set of polarity landmarks (Holmes and Dutcher, 1989). The basal bodies are connected to each other and to the nucleus by a system of contractile centrin-containing fibers (nuclear basal body connectors or NBBC) that serve critical roles in the spatial coordination of cytokinesis and karyokinesis. Centrin is a conserved protein found in the spindle poles of fungi and centrioles of animal cells (Salisbury, 2007). A second system of striated fibers is formed by the algal-specific protein SF-assemblin and is thought to maintain stable connections between the basal bodies and the rootlet microtubules (Lechtreck and Silflow, 1997; Lechtreck *et al.*, 2002). At the opposite (posterior) end of each cell is a single large cup-shaped chloroplast that occupies around half of the total cell volume (Gaffal *et al.*, 1995). Mitochondria are found in a large reticulate network that extends throughout the cytoplasm and around the chloroplast (Osafune *et al.*, 1972; Ehara *et al.*, 1995; Rasala *et al.*, 2014). A single eyespot formed by the juxtaposition of channelrhodopsin photosensory ion channels in the plasma membrane and pigment globules in the underlying chloroplast is positioned medially, and always adjacent to the four-member microtubule rootlet extending from the younger of the two basal bodies (Holmes and Dutcher, 1989; Dieckmann, 2003). The eyespot association with one of the two rootlets underscores the asymmetry of the mother and daughter basal bodies that are not functionally equivalent (Holmes and Dutcher, 1989). This asymmetry is also reflected in the mechanism of phototaxis, where each of the flagella beat with different forms in response to light signaling mediated by the eyespot, thereby allowing a swimming cell to turn towards light. Maintaining this asymmetry through division imposes specific constraints on the division process that are elaborated below. *Chlamydomonas* has a single conventional actin gene, and at interphase F–actin is found in the cytoplasm and surrounds

the nucleus (Harper *et al.*, 1992; Kato-Minoura *et al.*, 1997). During G1, cells enlarge but retain essentially the same architecture during their growth phase.

Overview of cell division

The process of cell division requires the duplication and spatial segregation of nuclear DNA and organelles, including the basal bodies, chloroplast and mitochondria, followed by cytokinesis to partition the segregated constituents equally into two daughter cells (Figure 5). The following is a brief description of events that occur during cell division in *Chlamydomonas*. Additional detailed descriptions of cell division can be found in Harper (1999), Marshall (2009) and Kirk (1998).

Early events

The first clear morphological change that signals imminent cell division is the shortening and eventual retraction of flagella, which takes ~30 min (Harper *et al.*, 1995). In some instances division proceeds before flagella have completely retracted (Piasecki *et al.*, 2008; Rasi *et al.*, 2009), but in either case the connections between the basal bodies and the flagella are severed prior to division (Johnson and Porter, 1968; Rasi *et al.*, 2009; Parker *et al.*, 2010), freeing the basal bodies and microtubule rootlets to coordinate subsequent mitotic events. The microtubule-severing protein katanin may be required for freeing basal bodies from cilia before mitosis, and this function may be essential for

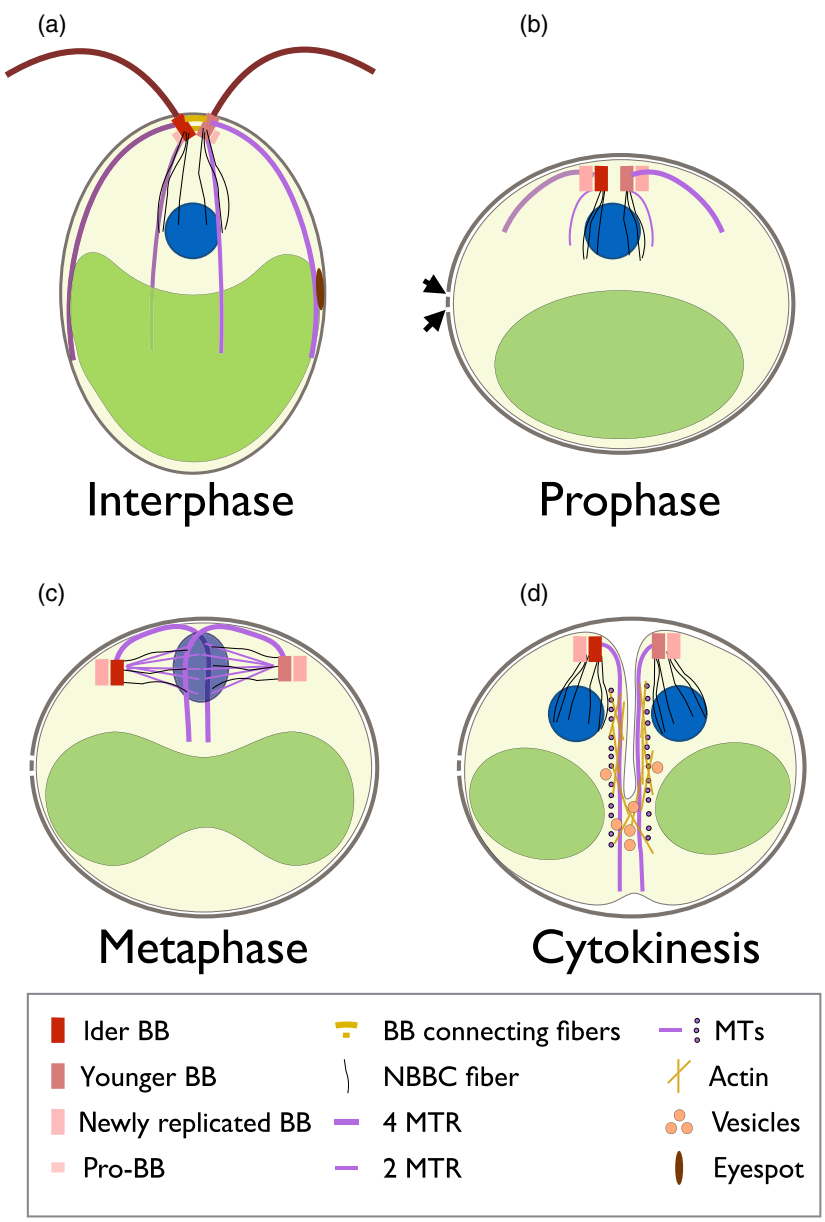

Figure 5. Cell division in *Chlamydomonas*. Schematic of four key phases of the cell cycle: interphase, prophase, metaphase and cytokinesis. Each schematic depicts a simplified cell with cell wall (gray outer border), apical flagella and eyespot in brown (interphase only), basal bodies (BBs; dark-red, light-red and pink rectangles), pro-basal bodies (small pink boxes), nucleus (blue circle) and chloroplast (green area at cell posterior). Also depicted are nucleus-basal body connectors or NBBCs (black lines), basal body connecting fibers (dark yellow lines in interphase cell), and selected microtubule structures in purple. Thickest purple lines, four microtubule rootlets (4 MTRs); intermediate purple lines, two microtubule rootlets (2 MTRs); thinnest purple lines, spindle microtubules; purple dots, phycoplast microtubules seen end-on in cross section. Actin (yellow lines) and membrane vesicles (dark-yellow circles) are only shown at cytokinesis. (a) Typical interphase cell. Note that for the sake of simplicity only rootlet microtubules are shown here. (b) Prophase cell in which flagella have been resorbed and the protoplast has rotated 90° within the mother cell wall. Arrows mark the former site of basal bodies and flagella, with respect to the mother cell wall. NBBC has contracted, drawing the nucleus towards the cell anterior, parental BBs are no longer connected and pro-BBs have elongated. Chromosomes begin condensation (not shown). (c) Metaphase cell in which the chloroplast has begun constricting and newly replicated BB pairs are present at spindle poles. Spindle microtubules enter the nucleus through polar fenestrae and attach to chromosomes. A metaphase band of microtubules passes over the future plane of cytokinesis. The nuclear envelope remains intact throughout cell division. (d) Cell undergoing cytokinesis. The chloroplast has divided. Post-mitotic nuclei and basal bodies have moved towards the center of the cell. The mitotic spindle has been replaced with the phycoplast: The 4 MTRs and cleavage microtubules extend down into the cell along the plane of division, whereas additional phycoplast MTs lie roughly perpendicular to the cleavage microtubules, also along the plane of cleavage. Actin and membrane vesicles are abundant around the cleavage furrow that starts at the apical end of the cell, but is later joined by a basally initiated furrow. Shortly after cytokinesis cells re-grow flagella and assume an interphase configuration.

mitosis to occur (Rasi *et al.*, 2009). Supporting this idea is the observation that the katanin requirement is reduced or eliminated by mutations that eliminate cilia (Rasi *et al.*, 2009).

Once the flagella are retracted or severed, the protoplast rotates 90° with respect to the mother cell wall, within which all cell divisions occur (Buffaloe, 1958; Johnson and Porter, 1968). This rotation does not appear to be essential because any alteration of its extent by changing light intensity does not affect subsequent division events (Holmes and Dutcher, 1989). Around the beginning of the first mitosis the eyespot disappears and will not reform again until the end of all divisions, when new daughter cells are formed (Holmes and Dutcher, 1989).

DNA replication and mitosis

Although the precise timing of the first S phase has not been established, it probably occurs around the time of flagellar shortening. Quantitative fluorescence measurements show that each round of nuclear DNA replication is followed rapidly with mitosis and cytokinesis (Coleman, 1982), a process that takes about 30 min per cycle (Harper *et al.*, 1995), meaning that a typical S/M phase will last between 30 min and 2 h (with between one and four divisions). DNA replication genes and other cell cycle-related genes are expressed just prior to S/M and appear to remain expressed at high levels until cells exit from S/M (Bisová *et al.*, 2005; Fang *et al.*, 2006). During prophase the 17 chromosomes of *Chlamydomonas* condense (Buffaloe, 1958), possibly as a consequence of histone modifications such as the phosphorylation of histone H3 (Keller *et al.*, 2010; Olson *et al.*, 2010).

Chlamydomonas cells undergo closed mitosis without nuclear envelope breakdown, but polar fenestrae or openings at opposite sides of the nuclear envelope become apparent at metaphase, and provide nuclear access for spindle microtubules (Johnson and Porter, 1968; Coss, 1974). During the prophase, basal bodies replicate and separate towards opposite sides of the cell, but stay associated with the plasma membrane. The NBBC undergoes a dynamic cycle of contractions and extensions during the process of mitosis and cytokinesis (Salisbury *et al.*, 1988). During pre-prophase, around the time of flagellar retraction/excision, the NBBC contracts briefly, drawing the nucleus closer to the cell anterior, and then re-extends during the prophase when the replicated basal body pairs separate to opposite poles. A second NBBC contraction occurs at the beginning of the anaphase, as chromosomes begin to separate, and perhaps functions to stretch the nuclear envelope apart towards the poles. At telophase the NBBC again relaxes around the two newly formed nuclei, and by cytokinesis has assumed its interphase appearance (Salisbury *et al.*, 1988). The metaphase spindle is nucleated from a pair of polar organizing centers that were initially reported to be separate from basal bodies (Johnson and Porter, 1968), but are now thought to contain them, where they may act as centrioles (Coss, 1974; Keller *et al.*, 2010). Immunofluorescence studies also locate the conserved basal body/centriolar proteins gamma tubulin, BLD10/CEP135 and POC1 at the spindle poles (Dibbayawan *et al.*, 1995; Silflow *et al.*, 1999; Matsuura, 2004; Keller *et al.*, 2010). Wild-type cytology strongly suggests a primary role for basal bodies in nucleating the mitotic spindle poles, yet mutants without detectable basal bodies nevertheless produce functional spindles, although spatial control is severely disrupted (Ehler *et al.*, 1995; Matsuura, 2004). On the other hand, in mutants lacking basal bodies, or with abnormal numbers of basal bodies, various defects are observed in spindle morphology, indicating either direct or indirect involvement of this structure in spindle assembly and function (Koblenz *et al.*, 2003; Keller *et al.*, 2010). During anaphase the half spindles shorten and draw the chromosomes and nuclear envelope apart, whereas a spindle-like array of microtubules appears to remain in between the separating chromatids (Doonan and Grief, 1987). The spindle disappears shortly after anaphase, and the nuclear membrane appears to remodel itself by closing or expanding around each daughter nucleus, while remaining connected through endoplasmic reticulum (ER) -like membranes in the internuclear space (Johnson and Porter, 1968). The mechanism by which the parental nuclear membrane is physically divided between daughters is unclear. During telophase chromatin decondenses, and structures such as the nucleolus reappear, but only briefly if cell undergoes additional rounds of S/M (Johnson and Porter, 1968).

Basal body replication and microtubule rootlets

Basal bodies and associated rootlet microtubules have a special function in *Chlamydomonas*, and its relatives, in coordinating spatial events during cell division (Pickett-Heaps, 1976; Holmes and Dutcher, 1989). During prophase flagella shorten and/or are severed, and the connective fibers between the two basal bodies disassemble. Each basal body has a pro-basal body associated with it, and during prophase the pro-basal bodies elongate, thus forming a set of two parent–daughter basal body pairs (Johnson and Porter, 1968; Gould, 1975; O'Toole and Dutcher, 2013). It is also during this time that the NBBC contracts, drawing the nucleus towards the newly formed basal body pairs, which are still juxtaposed and mark the future site of cleavage-furrow formation (Salisbury *et al.*, 1988). The *vfl1* mutant has defects in basal body replication and forms ectopic basal bodies and flagella during the G1 phase (Adams *et al.*, 1985). VFL1 protein localizes to mature and pro-basal bodies, but its specific function in controlling basal body replication is not known (Silflow *et al.*, 2001). The mammalian VFL1 homolog, CLERC, is a centrosomal

protein, the depletion of which by siRNA treatment leads to centriole separation and spindle defects during mitosis, but no interphase defects in centriole number, as seen in *Chlamydomonas* (Muto *et al.*, 2008).

Prior to metaphase, the basal body pairs move apart from each other towards opposite sides of the cell with their microtubule rootlets still attached. The rootlets are oriented towards each other up to the cell center. At the cell center, they bend 90° and run in opposite directions, just below the anterior plasma membrane along the axis of the future cleavage furrow, to form what has been termed the metaphase band of microtubules (Johnson and Porter, 1968; Doonan and Grief, 1987). The metaphase band may be analogous to the pre-prophase band in plants that predicts the future site of division (Doonan and Grief, 1987; Mineyuki, 1999), but the evolutionary relationship between these structures is unclear at present.

Cytokinesis

An important aspect of cytokinesis is the specification of the cell division plane, a process that varies in different taxa (Guertin *et al.*, 2002). The microtubule rootlets have been proposed to function analogously to astral microtubules in animal cells by providing positional cues for cleavage-furrow formation, and this idea is supported by the formation of ectopic or misplaced cleavage furrows at the position of rootlets in basal body mutants and in other cytokinesis-defective mutants (Adams *et al.*, 1985; Ehler *et al.*, 1995; Ehler and Dutcher, 1998). The process of cytokinesis in *Chlamydomonas* and many related green algae is associated with a special set of microtubules, termed the phycoplast (Johnson and Porter, 1968; Pickett-Heaps, 1976). During telophase the replicated basal bodies and nuclei move back towards each other, near the center of the cell, and the phycoplast microtubules begin to form between them in the plane of division. The phycoplast contains cleavage microtubules emanating from the rootlets and oriented in the direction of cleavage, which starts at the anterior end of the cell and proceeds downwards. It also contains microtubules that are roughly perpendicular to the cleavage microtubules, but also parallel with the cleavage plane (Johnson and Porter, 1968). Treatment of *Chlamydomonas* cells with microtubule inhibitors causes aberrant spindle formation and blocks cytokinesis (Ehler and Dutcher, 1998). In inhibitor-treated cells that had completed mitosis but not cytokinesis the phycoplast microtubules were partially disrupted and disorganized, suggesting that they are also important for cytokinesis.

In animal cytokinesis a contractile actomyosin ring constricts the cell membrane between the two daughter nuclei to physically divide the cell into daughters, whereas in land plants and some charophycean green algae a microtubule structure called the phragmoplast mediates cytokinesis by building a new membrane and cell wall between daughter nuclei via transport and fusion of membrane vesicles at the plane of division (Pickett-Heaps, 1976; Guertin *et al.*, 2002). There is a growing body of evidence that similar mechanisms underlie cytokinesis in these apparently divergent systems (Otegui *et al.*, 2005), and that the phycoplast may have features of both plant and animal division mechanisms. Vesicles can be seen accumulating in the vicinity of the cleavage plane near the phycoplast in *Chlamydomonas* cells (Johnson and Porter, 1968), and their transport and fusion may contribute to cleavage furrow formation, although to date no study has been performed to show a role for vesicle fusion during *Chlamydomonas* cytokinesis. Actin shows a dynamic localization pattern during *Chlamydomonas* cell division with strong staining at the cleavage furrow during cytokinesis (Harper *et al.*, 1992; Ehler and Dutcher, 1998). Myosin has also been localized to the cleavage furrow and rootlet microtubules in a pattern similar to that of actin (Ehler and Dutcher, 1998). The actin staining pattern is disrupted in cells treated with microtubule inhibitors, indicating that actin localization at the cleavage furrow requires the microtubule cytoskeleton (Ehler *et al.*, 1995); however, the role of actin at the cleavage furrow is unclear. The actin cytoskeleton during mitosis was found to be resistant to inhibitors of F–actin (Harper *et al.*, 1992), and an actin null mutant had no obvious cell growth or division defects (Kato-Minoura *et al.*, 1997). Complicating the interpretation of these findings is the discovery of an unconventional actin, NAP, that may substitute for the function of conventional actin in *Chlamydomonas* cytokinesis (Kato-Minoura *et al.*, 2003). A *Chlamydomonas* kinesin protein, CrKCBP, shows dynamic localization during division, including the spindle poles during mitosis and the phycoplast microtubules during cytokinesis (Dymek *et al.*, 2006). CrKCBP is part of a plant and green algal-specific kinsesin subfamily, kinsesin–14, the Arabidopsis homolog of which localizes to the pre-prophase band, spindle and phragmoplast, and appears to have roles in both cell division and other microtubule-related processes during interphase (Bowser and Reddy, 1997; Oppenheimer *et al.*, 1997; Vos *et al.*, 2000; Lazzaro *et al.*, 2013). Intraflagellar transport (IFT) proteins are involved in flagellar and cilia biogenesis as cargo carriers for materials that are added and removed at the flagellar tip (Pedersen and Rosenbaum, 2008), but they may also have additional roles in non-flagellar processes (Baldari and Rosenbaum, 2010). *Chlamydomonas* cells with reduced levels of IFT27, a conserved small G protein, arrested growth and showed cytokinesis defects, although the terminal phenotype was difficult to study because the cells were not viable (or escaped from RNAi-induced silencing with restored levels of IFT27; Qin *et al.*, 2007). Subsequent studies localized IFT27 and several other IFT proteins to the cleavage furrow, where they may play a role in cytokinesis that has yet to be defined (Wood *et al.*,

2012). Generally, IFT proteins in *Chlamydomonas* are non-essential and do not have obvious cell division defects; however, it should be noted that IFT mutants do not form flagella, have hatching defects and tend to form large clumps in culture, so that their growth and division cycles are difficult to study. It is possible that other IFT mutants have subtle cell-cycle defects that would only be detectable with careful single-cell studies. Supporting this idea, the dominant alleles of the anterograde IFT kinesin motor protein FLA10 show subtle chromosome loss defects, but no reported cytokinesis defects, and mutations in the centrin gene, *VFL2*, also show slightly elevated chromosome loss rates (Miller *et al.*, 2005; Zamora and Marshall, 2005).

As described in the preceding section, mechanisms for partitioning basal bodies and nuclei into separate halves of the cell during mitosis and cytokinesis are integral to the process of cell division, whereas smaller organelles and membranes may be divided equally at cytokinesis because of their relatively uniform distribution. The chloroplast is an interesting exception, as unlike the case in land plants there is only one per cell. Moreover, whereas land plant chloroplast division is relatively uncoupled from the mitotic cell cycle, in *Chlamydomonas* and other algae with single chloroplasts it is completely coupled (Miyagishima *et al.*, 2011). Careful studies of chloroplast structure during the cell cycle demonstrate that it undergoes its own cycle of dynamic shape changes and internal reorganization that is coordinated with the mitotic division cycle (Buffaloe, 1958). Electron microscopy (EM) studies and three-dimensional reconstructions of the chloroplast from mitotic cells showed chloroplast furrowing and division prior to the initiation of cleavage furrow formation (Goodenough, 1970; Gaffal *et al.*, 1995). Supporting the idea that chloroplast division is an actively regulated process, *Chlamydomonas* encodes plant-like homologs of several chloroplast division proteins (e.g. MIND, MINE, and FTSZ1 and FTSZ2), the expression of which is elevated at the time of cell division (Wang *et al.*, 2003; Adams *et al.*, 2008; Hu *et al.*, 2008; Yang *et al.*, 2008; Miyagishima *et al.*, 2012). An unexplored question is how these two division systems are linked. The chloroplast genome is multicopy and packaged into around a dozen nucleoids that are distributed throughout the stroma and replicated independently of the nuclear genome during the growth phase (Turmel *et al.*, 1980; Kuroiwa *et al.*, 1981). Chloroplast nucleoids undergo dynamic changes in structure and number during the cell cycle (Ehara *et al.*, 1990; Hiramatsu *et al.*, 2006), and appear to fragment and disperse during cell division. A mutant that makes one single large nucleoid was isolated and found to segregate chloroplast DNA in a highly asymmetric manner, with most of the DNA ending up in one daughter cell, yet all daughters appeared to get some chloroplast DNA, suggesting the possibility of active segregation mechanisms, the nature of which remain unclear (Misumi *et al.*, 1999).

Mitochondria in *Chlamydomonas* form an extensive tubular network similar in appearance to mitochondria in other eukaryotes, and are presumed to attain this morphology in a similar manner through a dynamic balance between fusion and fission (Berman *et al.*, 2008). Like the chloroplast, *Chlamydomonas* mitochondria have a multicopy genome that is packaged into nucleoids distributed throughout the network (Nishimura *et al.*, 1998; Hiramatsu *et al.*, 2006). Studies of mitochondrial membrane and nucleoid morphology during the *Chlamydomonas* cell cycle show dynamic shape changes with increased network branching, and nucleoid fragmentation and dispersal during cell division (Osafune *et al.*, 1972; Ehara *et al.*, 1995; Hiramatsu *et al.*, 2006). These changes are similar to those found during the cell cycle in animal mitochondria (Mishra and Chan, 2014), but their significance for mitochondrial function and inheritance are poorly understood.

At the completion of cytokinesis, cells will either repeat the process with another round of DNA replication, mitosis and cell division, or exit the division phase if daughter cells reach the appropriate size. For cells that continue with two or more rounds of cell division the process is similar to that described above, and the subsequent division plane will be orthogonal to the first one in either longitudinal or lateral orientation (Buffaloe, 1958; Johnson and Porter, 1968). When cells finish division they are still within the mother cell wall but very rapidly synthesize their own new cell wall. One of the first postmitotic events is the reformation of an interphase microtubule cytoskeleton, including flagella, eyespots and cruciate rootlet microtubules. The process of reflagellation is also accompanied by the upregulation of IFT and flagellar genes (Wood *et al.*, 2012). Flagella are important not only for motility, but also for hatching, as they are the site of secretion of the vegetative lytic enzyme (VLE), the expression of which is upregulated post-mitotically, and which acts to digest the mother cell wall, allowing daughter release (Kubo *et al.*, 2009).

Mutants that affect the spatial coordination of cell division

The highly ordered and interconnected nucleus-basal body-microtubule rootlet-system in *Chlamydomonas* (also found in many other green algae) is critical for ensuring that cytokinesis produces daughter cells of equal size and apportionment of organelles. As described above, critical events during division in a wild-type *Chlamydomonas* cell are physically coupled through these interorganellar connections that maintain fixed relationships between nuclei, basal bodies and microtubule rootlets throughout mitosis and cytokinesis. The importance of these connections is highlighted by the phenotypes that occur when they are defective. Cells without basal bodies (e.g. *bld2* and *bld10* mutants) or with defective basal bodies can organize a functional mitotic spindle and carry out cytokinesis, but mitosis and cytokinesis are not spatially coordinated, so

that daughters are produced with different sizes and with different numbers of nuclei (Ehler *et al.*, 1995; Dutcher and Trabuco, 1998; Dutcher, 2003; Matsuura, 2004; Keller *et al.*, 2010). Mitotic spindles formed in the absence of centrioles/basal bodies or with defective basal bodies also tend to be misoriented (Feldman and Marshall, 2009). Mutations in the centrin encoding gene, *VFL2*, have defects in the NBBC that lead to basal body segregation defects (Wright *et al.*, 1985, 1989; Salisbury *et al.*, 1988; Taillon *et al.*, 1992). *vfl2* mutants or cells with RNAi-induced knock-downs of *VFL2* have increased frequencies of cytokinetic failures and multinucleate cells, indicating misplaced cleavage furrows (Koblenz *et al.*, 2003; Keller *et al.*, 2010). Centrin also plays a role in tethering the basal bodies/centrioles to mitotic spindle poles, as they appeared to be separated in *VFL2* knock-down strains (Koblenz *et al.*, 2003). A protein of unknown function with homologs in flagellated or ciliated eukaryotes, but not elsewhere, DIP13, was found associated primarily with basal bodies, but also with flagellar axonemes and cytoplasmic microtubules (Pfannenschmid, 2003; Schoppmeier *et al.*, 2005). RNA-mediated knock-down of DIP13 resulted in a phenotype of multiflagellated and multinucleated cells, suggesting a cytokinesis defect. The mammalian DIP13 homolog, NA14, is a centrosomal protein the knockdown phenotype of which also results in cytokinesis defects (Goyal *et al.*, 2014). The specific relationship between DIP13/NA14 and centrosomal function remains to be determined.

Three mutants isolated in screens to identify cytokinesis defects have been described in *Chlamydomonas*, *cyt1*, *oca1* and *oca2* (Warr, 1968; Hirono and Yoda, 1997). *cyt1* cells are multinucleate and multiflagellate, much like *vfl2* and *vfl3* (Warr, 1968). More detailed studies of *cyt1* lead to the finding of both incomplete cytokinesis and spindle defects, suggesting a possible role in basal body function, although the mutated gene has not been identified (Ehler and Dutcher, 1998). *oca1* and *oca2* were characterized based on the morphology of division and nuclear number. At moderate frequency they form large, multinucleate and multiflagellate cells, and often show slow or arrested cleavage-furrow formation (Hirono and Yoda, 1997). The *oca1* and *oca2* mutations have not been further characterized or cloned. A recently reported cytokinesis defect was found for RNAi knock-downs of a gene, *VMP1*, encoding a vacuole membrane protein, which has homologs in animals with diverse functions and localization to various membranes (Tenenboim *et al.*, 2014). It is not clear whether the morphological defects observed in *VMP1* knock-down lines are caused by direct effects on cytokinesis or by indirect influences on cell shape, membrane biogenesis or other processes, and its phenotypes may be compounded by the fact that the knock-down was perfomed in a cell-wall-less strain background, where cell morphology is likely to be susceptible to many types of stress.

IDENTIFICATION OF ESSENTIAL CELL-CYCLE REGULATORS IN *CHLAMYDOMONAS*

A foundational method in understanding cell-cycle control in Opisthokonts was the identification and analysis of cell cycle-specific 'cdc' mutants, as a subset of temperature-sensitive lethal mutations. The advantage of this method is that it is unbiased, in the sense that no prior knowledge is needed. Disadvantages include: redundant or otherwise non-essential (but important) components cannot be detected (although they frequently can be found in secondary screens); different genes have wildly different rates of mutation to inactivation by high temperature, so genetic saturation is nearly impossible to achieve; and one requires a prior hypothesis as to what a cell-cycle control mutant would 'look like' (because mutant isolation is easy but characterization is hard, an early filter is essential). In budding yeast, this filter was uniform terminal arrest (Hartwell *et al.*, 1974); in fission yeast, it was cell growth without cell division (Nurse *et al.*, 1976).

An important consideration about these criteria is that they only work on specific biological models. 'Uniform terminal arrest' is now understood to come about primarily because of cell-cycle checkpoints, not so much because of an intrinsic requirement for the mutated gene product to move from one stage to another (Hartwell and Weinert, 1989). 'Cell growth without cell division' is essentially a subtractive criterion, which nevertheless has remarkable specificity for cell-cycle functions; however, it is entirely predicated on the hypothesis that cell growth is limiting for division, and further that there is zero regulatory feedback between the cell cycle and cell growth. The latter idea could actually seem somewhat unlikely a priori, and now some instances of cell growth–cell cycle inter-regulation have been documented (e.g. Bernstein *et al.*, 2007).

It is perhaps instructive to consider that neither criterion would be effective at discovering that microtubule depolymerization, or the blockage of DNA replication, caused a specific cell-cycle block in most animal cells, as the terminal phenotype is likely to be apoptotic cell death rather than arrest.

If one considers an entirely new unicellular organism, it is, therefore, difficult to feel robust confidence that any given criterion for initial phenotypic characterization will really capture all and only the 'cell cycle-specific' mutants. More broadly, essentially any lethal mutation will certainly result in the failure of cell proliferation, at least eventually – why then is *every* lethal mutation not a 'cell-cycle' mutation?

In *Chlamydomonas*, Howell and Naliboff (1973) isolated a set of temperature-sensitive mutants and characterized them with respect to whether they had a unique block point relative to a normal cell cycle, such that cells beyond but not before this point could divide at restrictive

temperatures. The mutants were indeed shown to have unique block points (essentially equivalent to the 'execution point' of Hartwell) in this analysis (Howell and Naliboff, 1973).

Harper *et al.* (1995) isolated 11 candidate cell-cycle mutants, based on a growth-without-division criterion. These mutants exhibited varying degrees of execution of cell-cycle functions when blocked. Although the mutated genes were not identified, study of these mutants, combined with studies with inhibitors, yielded some important insights. First, blockage of DNA replication did not block at least the initial stages of cytokinesis, and the reverse was also true; this led to the idea of independent functional sequences conditional on commitment, leading to DNA replication, nuclear division and cytokinesis. It was unremarked in this early work that this concept, although coherently explaining the data, was odd with respect to the developing checkpoint hypothesis in yeast and animals; according to the checkpoint hypothesis, for example, a failure of DNA replication would be caught by a surveillance system that would then block attempted cell division. Teleologically this is a sensible idea, as division before replication is complete will commonly result in one or zero viable daughter cells.

Recent work has revived this approach (Tulin and Cross, 2014). There are two key technical advantages, unavailable at the time when Harper *et al.* (1995) performed their study, that make this approach much more productive. First, mutant gene identification by next-generation sequencing of bulked segregants is a generic, algorithmic procedure (i.e. works identically for every mutant studied) with a high probability of success. Second, once the causative mutation is identified, there is a rich body of information about the biochemical or systems-level function of homologous proteins in other organisms that provides clear hypotheses to test. Although testing is required, this is hugely easier than trying to discover the function of a gene from scratch. As a trivial example, a mutation in a gene that is highly similar to a DNA polymerase catalytic subunit is extremely likely to exert its effects by inhibiting DNA replication; if such a mutant is, indeed, demonstrably defective in DNA replication, then it is likely that the phenotype is already largely explained.

The candidate cell-cycle mutations identified in this work were essentially based on a 'growth without division' criterion, although this required care as many cell-cycle mutants first initiated cytokinetic events, as described by Harper *et al.* (1995), and subsequently lost cell integrity and were effectively lysed. Howell and Naliboff (1973) also noted a rapid loss of viability in some of their mutants. Other mutants that retained cell integrity but still grew to approximately the normal division size, or larger, without ever dividing, were found on close examination to have relatively subtle cell growth rate defects. The distinction between these two classes (called 'div' and 'gex' for 'division' and 'G1 exit') is an empirical one, and the division between the classes is not really binary; still, it seems to be a useful initial sorting device. The 'gex' class failed in 'G1 exit', as none of the mutants tested replicated DNA when blocked, and also failed to exhibit the cytological signatures of initiation of cell division. *div* mutants all carried out one or both of these steps, suggesting the initiation but failure of completion of the division process.

What relationship the 'gex' defect has to the classical 'commitment' step is an interesting question (see above). Although traditionally considered to be related to the achievement of a specific cell size and/or passage of sufficient time since division, in wild-type cells both size and time will also correlate directly with growth rate; perhaps growth rate is in fact the key feature that is sensed, and this relationship could be uncoupled in mutants. For example, perhaps *gex* mutants grow slowly, never achieving a critical growth rate for commitment, and therefore reaching and surpassing normal division size. We do not yet know whether any gene involved in cell growth will give this phenotype, or if there is something special about the functions of the *GEX* genes. A number of the *GEX* genes are identified by multiple alleles, which at least suggests that the full *GEX* target set is unlikely to include simply any gene needed for cell growth. Classical experiments on commitment (see above) also indicate that there is no simple relationship between growth and division, as post-commitment cells will divide even when cell growth is highly restricted.

Almost all of the *DIV* and *GEX* genes had highly similar homologs in land plants, which is consistent with them being required for a fundamental cell biological process. Additionally, most of the *DIV* and *GEX* genes are broadly conserved in eukaryotes outside the plant lineage. In particular, the *DIV* genes included a number of genes with known cell-cycle roles in yeast and animals (examples: DNA polymerase subunits; APC subunits; tubulin-folding chaperones TFC–E and TFC–B; and regulatory kinases Mps1 and AurB). In general, phenotypic analysis supported the expected roles of these proteins: mutations in genes encoding replication proteins limited DNA replication; mutations in genes with products that are required to produce tubulin or microtubules resulted in failure to produce a mitotic spindle.

The anaphase-promoting complex in yeast and animals has multiple key cell-cycle roles (Zachariae and Nasmyth, 1999; Figure 1). The APC is required for the activation of separase/Esp1, the protease that cleaves cohesins attaching replicated sister chromatids (by APC-mediated degradation of the securin inhibitor of separase), and the APC is required *independently* for degradation of mitotic B–type cyclins, which have the potential to block mitotic exit if not removed. In yeast and animals, therefore, APC defects result in a metaphase block (unseparated sisters) with an

assembled spindle, high B–type cyclin-Cdk activity, and once-replicated DNA. DNA does not re-replicate in these mutants primarily because mitotic cyclin-Cdk inhibits the relicensing of DNA replication origins. Our results with *Chlamydomonas* mutants are broadly consistent with all of these APC roles being conserved: APC mutants arrest with assembled spindles, but fail to divide nuclei; they also fail to re-replicate DNA, and appear to have high levels of CDK kinase activity. The APC mutant phenotype contrasts with the phenotype of separase deficiency. These cells also fail to separate sisters, arresting with a single nucleus; however, this nucleus is polyploid, indicating that extra cycle(s) of DNA replication occurred, despite the failure of nuclear division. Strikingly, unlike the APC mutants, the separase mutant arrests with multiple cell bodies (apparently having completed several rounds of cytokinesis), only one of which contains the nucleus. This is essentially similar to the yeast separase-deficient phenotype: the interpretation could be that without separase, APC-mediated cyclin degradation still occurs, allowing multiple fission to proceed, despite the failure of nuclear division. If correct, this phenotype closely matches that observed for separate deficiencies in Opisthokonts (fungi, animals and related protozoans).

In Opisthokonts, defects in spindle assembly cause activation of the spindle assembly checkpoint (SAC), leading to the inhibition of APC activation and mitotic arrest (Musacchio and Salmon, 2007). In *Chlamydomonas*, the failure of spindle assembly (caused by mutations in tubulin-folding chaperones or in the gamma-tubulin ring complex) results in the failure of nuclear division and cytokinesis, as expected, yet DNA is re-replicated with relatively normal timing in these mutants, suggesting that cycles of mitotic CDK activation and inactivation may still be occurring. The same phenotype as these spindle mutants can be obtained by treating *Chlamydomonas* with an anti-microtubule drug APM. Taken together the data indicate that *Chlamydomonas* does not have a robust way to coordinate spindle assembly with subsequent cell-cycle events. The *Chlamydomonas* genome, like other plants, does contain clear homologs of at least some components of the SAC, such as Mad2, but there is no information as to their functional roles. DNA re-replication has been observed upon treatment of land plant tissues with microtubule-depolymerizing drugs, suggesting that this phenomenon may be widespread in Viridiplantae (discussed in Tulin and Cross, 2014).

As discussed above, Cdc2/Cdk1 is the main mitotic kinase in Opisthokonts. All Viridiplantae genomes contain clear orthologs to Cdk1, called CDKA; however, in Arabidopsis CDKA probably functions specifically in G1/S and not in mitosis (Nowack). The main Arabidopsis mitotic Cdk activity is suspected to be CDKB, a Viridiplantae-specific CDK relative. It has not been possible to test this idea directly because Arabidopsis has three CDKB genes that are likely to be at least partially redundant, and one of them has no available null allele. In *Chlamydomonas*, mutations were identified in the sole *CDKA* and *CDKB* genes (Tulin and Cross, 2014). The CDKA mutation caused delayed cell cycle entry, but mutant cells ultimately completed successful cell divisions and were fully viable, formally quite similar to the situation in Arabidopsis. Although the CDKA mutant on its own was viable at restrictive temperature, it was isolated as a double mutant with a mutation in MED6, encoding a component of the Mediator general transcription factor. The *cdka med6* double mutant combination exhibited specific arrest before cell-cycle initiation, but with essentially unimpeded cell growth. Further screening for mutations that are synthetic-lethal with the CDKA mutation (C. Atkins and F. Cross, unpublished data) has yielded a number of genes sharing MED6 connections with basal growth properties, such as general transcription or translation, although the mutants do not have any readily detectable cell-growth defects. This could imply that CDKA acts at a junction between cell growth regulation or detection and CDK-dependent cell-cycle initiation; other interpretations are possible.

In sharp contrast to the CDKA single mutant, the CDKB mutation completely blocked the first nuclear division: cells arrested after a single replication of DNA, in the early stages of the first cytokinesis, without a mitotic spindle and with apparently decondensed chromatin. This result leads to the prediction that the complete removal or inactivation of CDKB in Arabidopsis would similarly cause a block in the early stages of mitosis. Cyclin partners for CDKB in this system are unknown, and to date no cyclins have been identified in the mutant screen. This may simply be a result of the incompleteness of the screen (see below); alternatively, cyclin redundancy may prevent detection [in a rare departure from single-gene representation, *Chlamydomonas* has four cyclin D genes and three cyclin A/B genes (classed A, B and hybrid A/B), based on relatedness to homologs in plants and Opisthokonts; (Bisová *et al.*, 2005)].

Most *DIV* genes had characterized homologs in Opisthokonts, and in many cases phenotypic testing was consistent with a conserved role in *Chlamydomonas*.

A minority of *DIV* genes had easily identifiable land plant homologs, however, but less clear or no Opisthokont homologs. An interesting example is BSL1, a member of a Viridiplantae-specific phosphatase family that functions in the brassinosteroid signaling pathway. *Chlamydomonas* has only one gene in this family, and mutation of the gene results in a failure of mitosis; the phenotype is somewhat complex and requires further analysis. Interestingly, it has been proposed that in land plants, the BSL family has an essential role distinct from brassinosteroid signaling (Maselli *et al.*, 2014); the *Chlamydomonas* example leads to the testable hypothesis that this role is involved with mitotic

progression. It is hoped that this will be a pattern that can be repeated: the use of the simpler *Chlamydomonas* system to generate hypotheses to be tested in higher plants.

The cell-cycle screen of Tulin and Cross (2014) is clearly incomplete, as many genes are identified by single alleles (it is not possible to have a random collection of mutations that mostly fall in unique genes, without the existence of a substantially larger number of unidentified genes). A rough estimate would suggest around 20% completion, based on the representation of strongly expected genes (Table 1): for example, there are 12 subunits of replicative DNA polymerases, and the screen includes alleles from six of these; there are 13 subunits of the anaphase-promoting complex, and the screen includes two of these; at the start of replication there is a loading requirement that includes six minichromosome maintenance (MCM) subunits, six origin recognition complex (ORC) subunits, Cdc6 and Cdt1, and the screen has one ORC subunit and one candidate CDC6 mutation; etc. The examples above suggest a recovery of 10 out of 49 expected genes. (This is obviously a very rough estimate, but confirms that the bulk of cell-cycle genes remain to be discovered.) If the screen can be brought closer to completion, there should be significantly more Viridiplantae-specific genes like BSL1; also, a more complete toolkit for cell-cycle analysis. As is discussed above, it is clear that even highly similar orthologous genes are by no means guaranteed to have the same biological function, and a broader representation of mutations in conserved genes is required to address the question of functional divergence.

The screen is labor-intensive because it is non-selective; however, selective schemes can be devised for specific phenotypic classes, and these classes might well be saturable, depending on target complexity.

It is also likely that many genes and pathways that are important for wild-type cell-cycle control are nevertheless non-essential. Known examples include the Rb-DP-E2F pathway discussed above, and the basal body-flagellar system, the integration of which with the mitotic and spindle cycles is discussed above. Such genes and pathways will connect up in interesting ways to the set identified by ts–lethals as being essential to the cell cycle. An approach to non-essential genes regulating the cell cycle could involve the integration of the essential gene set identified by ts–lethals with the insertional library approach of Jonikas and collaborators (Zhang *et al.*, 2014); these two approaches should identify almost entirely non-intersecting sets of genes, and should be highly complementary. For example, if *Chlamydomonas* cyclins are functionally redundant, and supposing for illustration that the cyclin D1–D2 pair are jointly required, then a search for ts–lethals in a background in which cyclin D1 is inactivated might yield inactivating mutations in cyclin D2. There are considerable technical issues in setting up this kind of synthetic-lethal screen: the budding yeast 'SGA' system to do this makes use of many genetic methodologies not presently available in *Chlamydomonas*. The examples of well-studied yeast systems show that it is critical to move beyond the set of genes that are singly essential (and identifiable by ts–lethals), however. Cyclins in budding yeast provide a prominent example, as cyclin functions are parsed among nine different cyclins with varying degrees of functional overlap (Bloom and Cross, 2007); all single mutations and most multiple mutations are therefore fully viable. More challenging, redundancy can also come about at the level of complete pathways rather than detectable sequence homologs [for example, two (or perhaps three or four) partially redundant intrinsically oscillatory mechanisms regulating the budding yeast cell cycle; (Cross, 2003; Orlando *et al.*, 2008; Lu and Cross, 2010)]. Systems of this level of complexity are extremely difficult to disentangle outside of a microbial system, because of the requirements for complex strain construction. *Chlamydomonas* at present lacks some technical tools that are needed for such analysis, such as tightly regulated promoters, targeted gene inactivation and gene replacement, and appropriate fluorescent markers to detect cell-cycle events in single-cell time-lapse studies. In our view, however, many will be solvable in the near future (for example, by CRISPR technologies), and the unique phylogenetic position of *Chlamydomonas* as the most genetically amenable microbe in Viridiplantae (as well as the only well-developed microbial genetic system with homologs of animal genes such as Rb, cyclin A, etc.; see above) makes the work to overcome these technical challenges worthwhile.

FUTURE PERSPECTIVES

The results reviewed above lead to many questions; we hope that new methods, approaches and resources will continue to develop to provide answers to these questions.

- What is the molecular nature of commitment? Genetic experiments clearly implicate Rb, but it is unknown what is upstream of Rb. Results in Arabidopsis would suggest CdkA as a candidate regulator. Cell size clearly regulates commitment – what is actually 'measured' by the cell (volume, protein content, chloroplast size, number of ribosomes?), how is this measurement made and how is this information transduced to Rb? Downstream of Rb, genetic and biochemical results implicate DP/E2F. Which genes are transcriptionally regulated by DP/E2F/Rb, and does this regulation account for commitment? Do *gex* mutants, which in many cases grow considerably bigger than wild-type dividing cells, without ever initiating DNA replication or division, pass the commitment event?
- What obligate molecular events might occur during the long time delay between commitment and the first initiation of DNA replication? And once rapid division cycles

initiate, is the molecular event corresponding to commitment repeated within each cycle, or is one commitment truly sufficient for *n* divisions?

- How is cytokinesis carried out? What is the role of phycoplast microtubules, and are other cytoskeletal elements also involved? How are the phycoplast, phragmoplast and midbody related, with respect to both evolution and function?
- How is the flagellar cycle coordinated with the cell cycle?
- Is there circadian and/or direct light regulation of the cell cycle, and if so, how does it work?
- After commitment, what is the network architecture and control dynamics for the cell division cycle? How much does it differ from the Opisthokont model (Figure 2), and what Viridiplantae-specific features (both genes and regulatory loops) are present? Are oscillations of mitotic cyclin-Cdk and APC activity required for the sequential cycles in multiple fission?
- Do checkpoints exist, and if so, why are they so hard to see, and how do they work? If not, how does *Chlamydomonas* ensure faithful chromosome segregation?
- What is the nature of the involvement of transcriptional regulation in the cell cycle? Many cell-cycle genes are tightly regulated transcriptionally; is this regulation itself under cell-cycle control, or is it under the control of timers, light, cell size, or other exogenous stimuli, such that transcriptional induction is a one-way input into cell-cycle progression?

The phylogenetic considerations we have advanced lead us to hope that the answers to these questions will be illustrative not only for green algae but for the plant superkingdom as a whole, and may even shed light on cell-cycle control in the last common eukaryotic ancestor.

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REFERENCES

Adams, G.M., Wright, R.L. and Jarvik, J.W. (1985) Defective temporal and spatial control of flagellar assembly in a mutant of *Chlamydomonas reinhardtii* with variable flagellar number. *J. Cell Biol.* **100**, 955–964.

Adams, S., Maple, J. and Møller, S.G. (2008) Functional conservation of the MIN plastid division homologues of *Chlamydomonas reinhardtii*. *Planta*, **227**, 1199–1211.

Archambault, V., Buchler, N.E., Wilmes, G.M., Jacobson, M.D. and Cross, F.R. (2005) Two-faced cyclins with eyes on the targets. *Cell Cycle*, **4**, 125–130.

Armbrust, E.V., Ibrahim, A. and Goodenough, U.W. (1995) A mating type-linked mutation that disrupts the uniparental inheritance of chloroplast DNA also disrupts cell-size control in Chlamydomonas. *Mol. Biol. Cell* **6**, 1807–1818.

Baldari, C.T. and Rosenbaum, J. (2010) Intraflagellar transport: it's not just for cilia anymore. *Curr. Opin. Cell Biol.* **22**, 75–80.

Beel, B., Prager, K., Spexard, M. *et al.* (2012) A flavin binding cryptochrome photoreceptor responds to both blue and red light in *Chlamydomonas reinhardtii*. *Plant Cell*, **24**, 2992–3008.

Berman, S.B., Pineda, F.J. and Hardwick, J.M. (2008) Mitochondrial fission and fusion dynamics: the long and short of it. *Cell Death Differ.* **15**, 1147–1152.

Bernstein, K.A., Bleichert, F., Bean, J.M., Cross, F.R. and Baserga, S.J. (2007) Ribosome biogenesis is sensed at the Start cell cycle checkpoint. *Mol. Biol. Cell* **18**, 953–964.

Bertoli, C., Skotheim, J.M. and de Bruin, R.A.M. (2013) Control of cell cycle transcription during G1 and S phases. *Nat. Rev. Mol. Cell Biol.* **14**, 518–528.

Bisová, K. and Zachleder, V. (2014) Cell-cycle regulation in green algae dividing by multiple fission. *J. Exp. Bot.* **65**, 2585–2602.

Bisová, K., Krylov, D.M. and Umen, J.G. (2005) Genome-wide annotation and expression profiling of cell cycle regulatory genes in *Chlamydomonas reinhardtii*. *Plant Physiol.* **137**, 475–491.

Bloom, J. and Cross, F.R. (2007) Multiple levels of cyclin specificity in cell-cycle control. *Nat. Rev. Mol. Cell Biol.* **8**, 149–160.

Bowser, J. and Reddy, A.S. (1997) Localization of a kinesin-like calmodulin-binding protein in dividing cells of Arabidopsis and tobacco. *Plant J.* **12**, 1429–1437.

Bradley, B.A. and Quarmby, L.M. (2005) A NIMA-related kinase, Cnk2p, regulates both flagellar length and cell size in Chlamydomonas. *J. Cell Sci.* **118**, 3317–3326.

Bruce, V.G. (1970) The biological clock in *Chlamydomonas reinhardi*. *J. Protozool.* **17**, 328–334.

Buffaloe, N.D. (1958) A comparative cytological study of four species of *Chlamydomonas*. *Bull. Torrey Bot. Club* **85**, 157–178.

Burkhart, D.L. and Sage, J. (2008) Cellular mechanisms of tumour suppression by the retinoblastoma gene. *Nat. Rev. Cancer* **8**, 671–682.

Burssens, S., Van Montagu, M. and Inzé, D. (1998) The cell cycle in Arabidopsis. *Plant Physiol. Biochem.* **36**, 9–19.

Cavalier-Smith, T. (2002) r- and K-tactics in the evolution of protist developmental systems: cell and genome size, phenotype diversifying selection, and cell cycle patterns. *BioSystems*, **12**, 43–59.

Christensen, S., Pedersen, S., Satir, P., Veland, I. and Schneider, L. (2008) The primary cilium coordinates signaling pathways in cell cycle control and migration during development and tissue repair. *Curr. Top. Dev. Biol.* **85**, 261–301.

Coleman, A.W. (1982) The nuclear cell cycle in *Chlamydomonas* (Chlorophyceae). *J. Phycol.* **18**, 192–195.

Coss, R.A. (1974) Mitosis in *Chlamydomonas reinhardtii* basal bodies and the mitotic apparatus. *J. Cell Biol.* **63**, 325–329.

Craigie, R.A. and Cavalier-Smith, T. (1982) Cell volume and the control of the Chlamydomonas cell cycle. *J. Cell Sci.* **54**, 173–191.

Cross, F.R. (2003) Two redundant oscillatory mechanisms in the yeast cell cycle. *Dev. Cell* **4**, 741–752.

Cross, F.R., Buchler, N.E. and Skotheim, J.M. (2011) Evolution of networks and sequences in eukaryotic cell cycle control. *Philos. Trans. R Soc. B: Biol. Sci.* **366**, 3532–3544.

Di Talia, S., Skotheim, J.M., Bean, J.M., Siggia, E.D. and Cross, F.R. (2007) The effects of molecular noise and size control on variability in the budding yeast cell cycle. *Nature*, **448**, 947–951.

Dibbayawan, T.P., Harper, J.D., Elliott, J.E., Gunning, B.E. and Marc, J. (1995) A gamma-tubulin that associates specifically with centrioles in HeLa cells and the basal body complex in Chlamydomonas. *Cell Biol. Int.* **19**, 559–567.

Dieckmann, C.L. (2003) Eyespot placement and assembly in the green alga *Chlamydomonas*. *BioEssays*, **25**, 410–416.

Dissmeyer, N., Weimer, A.K., Pusch, S. *et al.* (2009) Control of cell proliferation, organ growth, and DNA damage response operate independently of dephosphorylation of the Arabidopsis Cdk1 homolog CDKA;1. *Plant Cell* **21**, 3641–3654.

Donnan, L. and John, P.C. (1983) Cell cycle control by timer and sizer in Chlamydomonas. *Nature*, **304**, 630–633.

Doonan, J.H. and Grief, C. (1987) Microtubule cycle in *Chlamydomonas reinhardtii*: an immunofluorescence study. *Cell Motil. Cytoskeleton* **7**, 381–392.

Drapkin, B.J., Lu, Y., Procko, A.L., Timney, B.L. and Cross, F.R. (2009) Analysis of the mitotic exit control system using locked levels of stable mitotic cyclin. *Mol. Syst. Biol.* **5**, 1–13.

Dutcher, S.K. (2003) Elucidation of basal body and centriole functions in *Chlamydomonas reinhardtii*. *Traffic*, **4**, 443–451.

Dutcher, S.K. and Trabuco, E.C. (1998) The UNI3 gene is required for assembly of basal bodies of Chlamydomonas and encodes delta-tubulin, a new member of the tubulin superfamily. *Mol. Biol. Cell* **9**, 1293–1308.

Dymek, E.E., Goduti, D., Kramer, T. and Smith, E.F. (2006) A kinesin-like calmodulin-binding protein in Chlamydomonas: evidence for a role in cell division and flagellar functions. *J. Cell Sci.* **119**, 3107–3116.

Ehara, T., Ogasawara, Y., Osafune, T. and Hase, E. (1990) Behavior of chloroplast nucleoids during the cell cycle of *Chlamydomonas reinhardtii* (Chlorophyta) in synchronized culture. *J. Phycol.* **26**, 317–323.

Ehara, T., Osafune, T. and Hase, E. (1995) Behavior of mitochondria in synchronized cells of *Chlamydomonas reinhardtii* (Chlorophyta). *J. Cell Sci.* **108**(Pt 2), 499–507.

Ehler, L.L. and Dutcher, S.K. (1998) Pharmacological and genetic evidence for a role of rootlet and phycoplast microtubules in the positioning and assembly of cleavage furrows in *Chlamydomonas reinhardtii*. *Cell Motil. Cytoskeleton* **40**, 193–207.

Ehler, L.L., Holmes, J.A. and Dutcher, S.K. (1995) Loss of spatial control of the mitotic spindle apparatus in a *Chlamydomonas reinhardtii* mutant strain lacking basal bodies. *Genetics*, **141**, 945–960.

Fang, S.-C. and Umen, J.G. (2008) A suppressor screen in Chlamydomonas identifies novel components of the retinoblastoma tumor suppressor pathway. *Genetics*, **178**, 1295–1310.

Fang, S.-C., de los Reyes, C. and Umen, J.G. (2006) Cell size checkpoint control by the retinoblastoma tumor suppressor pathway. *PLoS Genet.* **2**, e167.

Fang, S.-C., Chung, C.-L., Chen, C.-H., Lopez-Paz, C. and Umen, J.G. (2014) Defects in a new class of sulfate/anion transporter link sulfur acclimation responses to intracellular glutathione levels and cell cycle control. *Plant Physiol.* **166**, 1852–1868.

Fantes, P. (1979) Epistatic gene interactions in the control of division in fission yeast. *Nature*, **279**, 428–430.

Fantes, P.A. and Nurse, P. (1978) Control of the timing of cell division in fission yeast. Cell size mutants reveal a second control pathway. *Exp. Cell Res.* **115**, 317–329.

Feldman, J.L. and Marshall, W.F. (2009) ASQ2 encodes a TBCC-like protein required for mother-daughter centriole linkage and mitotic spindle orientation. *Curr. Biol.* **19**, 1238–1243.

Fry, A.M., O'Regan, L., Sabir, S.R. and Bayliss, R. (2012) Cell cycle regulation by the NEK family of protein kinases. *J. Cell Sci.* **125**, 4423–4433.

Gaffal, K.P., Arnold, C.G., Friedrichs, G.J. and Gemple, W. (1995) Morphodynamical changes of the chloroplast of *Chlamydomonas reinhardtii* during the 1st round of division. *Arch. Protistenkd.* **145**, 10–23.

Garsmeur, O., Schnable, J.C., Almeida, A., Jourda, C., D'Hont, A. and Freeling, M. (2014) Two evolutionarily distinct classes of paleopolyploidy. *Mol. Biol. Evol.* **31**, 448–454.

Gillham, N.W., Boynton, J.E., Johnson, A.M. and Burkhart, B.D. (1987) Mating type linked mutations which disrupt the uniparental transmission of chloroplast genes in Chlamydomonas. *Genetics*, **115**, 677–684.

Goodenough, U.W. (1970) Chloroplast division and pyrenoid formation in *Chlamydomonas reinhardi*. *J. Phycol.* **6**, 1–6.

Goto, K. and Johnson, C.H. (1995) Is the cell division cycle gated by a circadian clock? The case of *Chlamydomonas reinhardtii*. *J. Cell Biol.* **129**, 1061–1069.

Goto, H., Inoko, A. and Inagaki, M. (2013) Cell cycle progression by the repression of primary cilia formation in proliferating cells. *Cell. Mol. Life Sci.* **70**, 3893–3905.

Gould, R.R. (1975) The basal bodies of *Chlamydomonas reinhardtii*. Formation from probasal bodies, isolation, and partial characterization. *J. Cell Biol.* **65**, 65–74.

Goyal, U., Renvoisé, B., Chang, J. and Blackstone, C. (2014) Spastin-interacting protein NA14/SSNA1 functions in Cytokinesis and Axon development. *PLoS ONE*, **9**, e112428.

Gray, K.A., Yates, B., Seal, R.L., Wright, M.W. and Bruford, E.A. (2015) Genenames.org: the HGNC resources in 2015. *Nucleic Acids Res.* **43**, D1079–D1085.

Guertin, D.A., Trautmann, S. and McCollum, D. (2002) Cytokinesis in eukaryotes. *Microbiol. Mol. Biol. Rev.* **66**, 155–178.

Harashima, H., Dissmeyer, N. and Schnittger, A. (2013) Cell cycle control across the eukaryotic kingdom. *Trends Cell Biol.* **23**, 345–356.

Harper, J.D. (1999) Chlamydomonas cell cycle mutants. *Int. Rev. Cytol.* **189**, 131–176.

Harper, J.D., McCurdy, D.W., Sanders, M.A., Salisbury, J.L. and John, P.C. (1992) Actin dynamics during the cell cycle in *Chlamydomonas reinhardtii*. *Cell Motil. Cytoskeleton* **22**, 117–126.

Harper, J., Wu, L., Sakuanrungsirikul, S. and John, P. (1995) Isolation and partial characterization of conditional cell division cycle mutants in Chlamydomonas. *Protoplasma*, **186**, 149–162.

Harris, E. (2001) Chlamydomonas as a model organism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 363–406.

Hartwell, L.H. and Weinert, T.A. (1989) Checkpoints: controls that ensure the order of cell cycle events. *Science*, **246**, 629–634.

Hartwell, L.H., Culotti, J., Pringle, J.R. and Reid, B.J. (1974) Genetic control of the cell division cycle in yeast. *Science*, **183**, 46–51.

Harvey, S.L. and Kellogg, D.R. (2003) Conservation of mechanisms controlling entry into mitosis: budding yeast wee1 delays entry into mitosis and is required for cell size control. *Curr. Biol.* **13**, 264–275.

Hegemann, P. and Berthold, P. (2009) Sensory photoreceptors and light control of flagellar activity. In *The Chlamydomonas Sourcebook*, 2nd edn. (Witman, G.B. and Harris, E.H., eds). London: Academic Press, pp. 395–429.

Herskowitz, I. (1985) Yeast as the universal cell. *Nature*, **316**, 678–679.

Hiramatsu, T., Nakamura, S., Misumi, O. and Kuroiwa, T. (2006) Morphological changes in mitochondrial and chloroplast nucleoids and mitochondria during the *Chlamydomonas reinhardtii* (Chlorophyceae) cell cycle. *J. Phycol.* **42**, 1048–1058.

Hirono, M. and Yoda, A. (1997) Isolation and phenotypic characterization of Chlamydomonas mutants defective in cytokinesis. *Cell Struct. Funct.* **22**, 1–5.

Holmes, J.A. and Dutcher, S.K. (1989) Cellular asymmetry in *Chlamydomonas reinhardtii*. *J. Cell Sci.* **94**(Pt 2), 273–285.

Howell, S.H. and Naliboff, J.A. (1973) Conditional mutants in *Chlamydomonas reinhardtii* blocked in the vegetative cell cycle. I. An analysis of cell cycle block points. *J. Cell Biol.* **57**, 760–772.

Hu, Y., Chen, Z.-W., Liu, W.-Z., Liu, X.-L. and He, Y.-K. (2008) Chloroplast division is regulated by the circadian expression of FTSZ and MIN genes in *Chlamydomonas reinhardtii*. *Eur. J. Phycol.* **43**, 207–215.

Iwata, E., Ikeda, S., Matsunaga, S., Kurata, M., Yoshioka, Y., Criqui, M.-C., Genschik, P. and Ito, M. (2011) GIGAS CELL1, a novel negative regulator of the anaphase-promoting complex/cyclosome, is required for proper mitotic progression and cell fate determination in Arabidopsis. *Plant Cell* **23**, 4382–4393.

Iwata, E., Ikeda, S., Abe, N. *et al.* (2014) Roles of GIG1 and UVI4 in genome duplication in *Arabidopsis thaliana*. *Plant Signal. Behav.* **7**, 1079–1081.

John, P.C.L. (1987) Control points in the Chlamydomonas cell cycle. In *Algal Development (Molecular and Cellular Aspects)*. (Wiessner, W., Robinson, D.G. and Starr, R.C., eds). Berlin: Springer-Verlag, pp. 9–16.

Johnson, U.G. and Porter, K.R. (1968) Fine structure of cell division in *Chlamydomonas reinhardi*. Basal bodies and microtubules. *J. Cell Biol.* **38**, 403–425.

Johnston, G.C., Pringle, J.R. and Hartwell, L.H. (1977) Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. *Exp. Cell Res.* **105**, 79–98.

Jones, R.F. (1970) Physiological and biochemical aspects of growth and gametogenesis in *Chlamydomonas reinhardtii*. *Ann. N. Y. Acad. Sci.* **175**, 648–659.

Jorgensen, P. and Tyers, M. (2004) How cells coordinate growth and division. *Curr. Biol.* **14**, R1014–R1027.

Joubès, J., Chevalier, C., Dudits, D., Heberle-Bors, E., Inzé, D., Umeda, M. and Renaudin, J.P. (2000) CDK-related protein kinases in plants. *Plant Mol. Biol.* **43**, 607–620.

Kato-Minoura, T., Hirono, M. and Kamiya, R. (1997) Chlamydomonas inner-arm dynein mutant, ida5, has a mutation in an actin-encoding gene. *J. Cell Biol.* **137**, 649–656.

Kato-Minoura, T., Okumura, M., Hirono, M. and Kamiya, R. (2003) A novel family of unconventional actins in volvocalean algae. *J. Mol. Evol.* **57**, 555–561.

Ke, Y.-N. and Yang, W.-X. (2014) Primary cilium: an elaborate structure that blocks cell division? *Gene*, **547**, 175–185.

Keller, L.C., Wemmer, K.A. and Marshall, W.F. (2010) Influence of centriole number on mitotic spindle length and symmetry. *Cytoskeleton (Hoboken)*, **67**, 504–518.

Kirk, D.L. (1998) *Volvox*. Cambridge: Cambridge University Press.
Koblenz, B., Schoppmeier, J., Grunow, A. and Lechtreck, K. (2003) Centrin deficiency in Chlamydomonas causes defects in basal body replication, segregation and maturation. *J. Cell Sci.* **116**, 2635.
Koufopanou, V. (1994) The evolution of soma in the Volvocales. *Am. Nat.* **143**, 907–931.
Kubo, T., Kaida, S., Abe, J., Saito, T., Fukuzawa, H. and Matsuda, Y. (2009) The Chlamydomonas hatching enzyme, sporangin, is expressed in specific phases of the cell cycle and is localized to the flagella of daughter cells within the sporangial cell wall. *Plant Cell Physiol.* **50**, 572–583.
Kuroiwa, T., Suzuki, T., Ogawa, K. and Kawano, S. (1981) The chloroplast nucleus: distribution, number, size, and shape, and a model for the multiplication of the chloroplast genome during chloroplast development. *Plant Cell Physiol.* **22**, 381–396.
Lazzaro, M.D., Marom, E.Y. and Reddy, A.S.N. (2013) Polarized cell growth, organelle motility, and cytoskeletal organization in conifer pollen tube tips are regulated by KCBP, the calmodulin-binding kinesin. *Planta*, **238**, 587–597.
Lechtreck, K.F. and Silflow, C.D. (1997) SF-assemblin in Chlamydomonas: sequence conservation and localization during the cell cycle. *Cell Motil. Cytoskeleton* **36**, 190–201.
Lechtreck, K.-F., Rostmann, J. and Grunow, A. (2002) Analysis of Chlamydomonas SF-assemblin by GFP tagging and expression of antisense constructs. *J. Cell Sci.* **115**, 1511–1522.
Lien, T. and Knutsen, G. (1979) Synchronous growth of *Chlamydomonas reinhardtii* (Chlorophyceae): a review of optimal conditions. *J. Phycol.* **15**, 191–200.
Lu, Y. and Cross, F.R. (2010) Periodic Cyclin-Cdk activity entrains an autonomous Cdc14 release oscillator. *Cell*, **141**, 268–279.
Mahjoub, M.R., Montpetit, B., Zhao, L., Finst, R.J., Goh, B., Kim, A.C. and Quarmby, L.M. (2002) The FA2 gene of Chlamydomonas encodes a NIMA family kinase with roles in cell cycle progression and microtubule severing during deflagellation. *J. Cell Sci.* **115**, 1759–1768.
Marshall, W.F. (2009) Mitosis and cytokinesis. In *The Chlamydomonas Sourcebook*, 2nd edn. (Witman, G.B. and Harris, E.H., eds). London: Academic Press, pp. 431–443.
Maselli, G.A., Slamovits, C.H., Bianchi, J.I., Vilarrasa-Blasi, J., Cano-Delgado, A.I. and Mora-Garcia, S. (2014) Revisiting the evolutionary history and roles of protein phosphatases with Kelch-like domains in plants. *Plant Physiol.* **164**, 1527–1541.
Matsumura, K., Yagi, T. and Yasuda, K. (2003) Role of timer and sizer in regulation of Chlamydomonas cell cycle. *Biochem. Biophys. Res. Commun.* **306**, 1042–1049.
Matsumura, K., Yagi, T., Hattori, A., Soloviev, M. and Yasuda, K. (2010) Using single cell cultivation system for on-chip monitoring of the interdivision timer in *Chlamydomonas reinhardtii* cell cycle. *J. Nanobiotechnol.* **8**, 23.
Matsuo, T. and Ishiura, M. (2010) New insights into the circadian clock in Chlamydomonas. *Int. Rev. Cell Mol. Biol.* **280**, 281–314.
Matsuo, T., Okamoto, K., Onai, K., Niwa, Y., Shimogawara, K. and Ishiura, M. (2008) A systematic forward genetic analysis identified components of the Chlamydomonas circadian system. *Genes Dev.* **22**, 918–930.
Matsuura, K. (2004) Bld10p, a novel protein essential for basal body assembly in Chlamydomonas: localization to the cartwheel, the first ninefold symmetrical structure appearing during assembly. *J. Cell Biol.* **165**, 663–671.
McAteer, M., Donnan, L. and John, P.C. (1985) The timing of division in Chlamydomonas. *New Phytol.* **99**, 41–56.
Merchant, S.S., Prochnik, S.E., Vallon, O. *et al.* (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. *Science*, **318**, 245–250.
Miller, M.S., Esparza, J.M., Lippa, A.M., Lux, F.G., Cole, D.G. and Dutcher, S.K. (2005) Mutant kinesin-2 motor subunits increase chromosome loss. *Mol. Biol. Cell* **16**, 3810–3820.
Mineyuki, Y. (1999) The preprophase band of microtubules: its function as a cytokinetic apparatus in higher plants. *Int. Rev. Cytol.* **187**, 1–49.
Mironov, V., De Veylder, L., Van Montagu, M. and Inze, D. (1999) Cyclin-dependent kinases and cell division in plants- the nexus. *Plant Cell* **11**, 509–522.
Mishra, P. and Chan, D.C. (2014) Mitochondrial dynamics and inheritance during cell division, development and disease. *Nat. Rev. Mol. Cell Biol.* **15**, 634–646.
Misumi, O., Suzuki, L., Nishimura, Y., Sakai, A., Kawano, S., Kuroiwa, H. and Kuroiwa, T. (1999) Isolation and phenotypic characterization of *Chlamydomonas reinhardtii* mutants defective in chloroplast DNA segregation. *Protoplasma*, **209**, 273–282.
Miyagishima, S.-Y., Nakanishi, H. and Kabeya, Y. (2011) Structure, regulation, and evolution of the plastid division machinery. *Int. Rev. Cell Mol. Biol.* **291**, 115–153.
Miyagishima, S.Y., Suzuki, K., Okazaki, K. and Kabeya, Y. (2012) Expression of the nucleus-encoded chloroplast division genes and proteins regulated by the algal cell cycle. *Mol. Biol. Evol.* **29**, 2957–2970.
Morgan, D.O. (2007) *The Cell Cycle*. London: New Science Press.
Morris, R.L., Keller, L.R., Zweidler, A. and Rizzo, P.J. (1990) Analysis of *Chlamydomonas reinhardtii* histones and chromatin. *J. Protozool.* **37**, 117–123.
Munzner, P. and Voigt, J. (1992) Blue light regulation of cell division in *Chlamydomonas reinhardtii*. *Plant Physiol.* **99**, 1370–1375.
Murray, A.W. and Kirschner, M.W. (1989) Dominoes and clocks: the union of two views of the cell cycle. *Science*, **246**, 614–621.
Musacchio, A. and Salmon, E.D. (2007) The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* **8**, 379–393.
Muto, Y., Yoshioka, T., Kimura, M., Matsunami, M., Saya, H. and Okano, Y. (2008) An evolutionarily conserved leucine-rich repeat protein CLERC is a centrosomal protein required for spindle pole integrity. *Cell Cycle* **7**, 2738–2748.
Nasmyth, K. (1996) Viewpoint: putting the cell cycle in order. *Science*, **274**, 1643–1645.
Nishimura, Y., Higashiyama, T., Suzuki, L., Misumi, O. and Kuroiwa, T. (1998) The biparental transmission of the mitochondrial genome in *Chlamydomonas reinhardtii* visualized in living cells. *Eur. J. Cell Biol.* **77**, 124–133.
Nowack, M.K., Harashima, H., Dissmeyer, N., Zhao, X., Bouyer, D., Weimer, A.K., De Winter, F., Yang, F. and Schnittger, A. (2012) Genetic framework of cyclin-dependent kinase function in Arabidopsis. *Dev. Cell* **22**, 1030–1040.
Nurse, P., Thuriaux, P. and Nasmyth, K. (1976) Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* **146**, 167–178.
O'Farrell, P.H. (2004) How metazoans reach their full size: the natural history of bigness. In *Cell Growth: Control of Cell Size*. (Hall, M.N., Raff, M. and Thomas, G., eds). Cold Spring Harbor Laboratory Press: Cold Spring Harbor, pp. 1–22.
Oldenhof, H., Bisová, K., Van Den Ende, H. and Zachleder, V. (2004a) Effect of red and blue light on the timing of cyclin-dependent kinase activity and the timing of cell division in *Chlamydomonas reinhardtii*. *Plant Physiol. Biochem.* **42**, 341–348.
Oldenhof, H., Zachleder, V. and van den Ende, H. (2004b) Blue light delays commitment to cell division in *Chlamydomonas reinhardtii*. *Plant Biol (Stuttg)* **6**, 689–695.
Oldenhof, H., Zachleder, V. and Van Den Ende, H. (2006) Blue- and red-light regulation of the cell cycle in *Chlamydomonas reinhardtii* (Chlorophyta). *Eur. J. Phycol.* **41**, 313–320.
Oldenhof, H., Zachleder, V. and van den Ende, H. (2007) The cell cycle of *chlamydomonas reinhardtii*: the role of the commitment point. *Folia Microbiol.* **52**, 53–60.
Olson, B.J.S.C., Oberholzer, M., Li, Y. *et al.* (2010) Regulation of the Chlamydomonas cell cycle by a stable, chromatin-associated retinoblastoma tumor suppressor complex. *Plant Cell* **22**, 3331–3347.
Oppenheimer, D.G., Pollock, M.A., Vacik, J., Szymanski, D.B., Ericson, B., Feldmann, K. and Marks, M.D. (1997) Essential role of a kinesin-like protein in Arabidopsis trichome morphogenesis. *Proc. Natl Acad. Sci. USA* **94**, 6261–6266.
Orlando, D.A., Lin, C.Y., Bernard, A., Wang, J.Y., Socolar, J.E.S., Iversen, E.S., Hartemink, A.J. and Haase, S.B. (2008) Global control of cell-cycle transcription by coupled CDK and network oscillators. *Nature*, **453**, 944–947.
Osafune, T., Mihara, S., Hase, E. and Ohkuro, I. (1972) Electron microscope studies on the vegetative cellular life cycle of *Chlamydomonas reinhardi* Dangeard in synchronous culture I. Some characteristics of changes in

subcellular structures during the cell cycle, especially in formation of giant mitochondria. *Plant Cell Physiol.* **13**, 211–227.

Otegui, M.S., Verbrugghe, K.J. and Skop, A.R. (2005) Midbodies and phragmoplasts: analogous structures involved in cytokinesis. *Trends Cell Biol.* **15**, 404–413.

O'Toole, E.T. and Dutcher, S.K. (2013) Site-specific basal body duplication in Chlamydomonas. *Cytoskeleton*, **71**, 108–118.

Pan, J. and Snell, W. (2007) The primary cilium: keeper of the key to cell division. *Cell*, **129**, 1255–1257.

Parker, J.D.K., Hilton, L.K., Diener, D.R., Rasi, M.Q., Mahjoub, M.R., Rosenbaum, J.L. and Quarmby, L.M. (2010) Centrioles are freed from cilia by severing prior to mitosis. *Cytoskeleton (Hoboken)*, **67**, 425–430.

Pedersen, L. and Rosenbaum, J.L. (2008) Intraflagellar transport (IFT): role in ciliary assembly, resorption and signalling. *Curr. Top. Dev. Biol.* **85**, 23–61.

Pfannenschmid, F. (2003) Chlamydomonas DIP13 and human NA14: a new class of proteins associated with microtubule structures is involved in cell division. *J. Cell Sci.* **116**, 1449–1462.

Piasecki, B.P., LaVoie, M., Tam, L.-W., Lefebvre, P.A. and Silflow, C.D. (2008) The Uni2 phosphoprotein is a cell cycle regulated component of the basal body maturation pathway in *Chlamydomonas reinhardtii*. *Mol. Biol. Cell* **19**, 262–273.

Pickett-Heaps, J. (1976) Cell division in eucaryotic algae. *Bioscience*, **26**, 445–450.

Pomerening, J.R., Sontag, E.D. and Ferrell, J.E. (2003) Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nat. Cell Biol.* **5**, 346–351.

Qin, H., Wang, Z., Diener, D. and Rosenbaum, J. (2007) Intraflagellar transport Protein 27 is a small G protein involved in cell-cycle control. *Curr. Biol.* **17**, 193–202.

Quarmby, L.M. and Parker, J.D.K. (2005) Cilia and the cell cycle? *J. Cell Biol.* **169**, 707–710.

Rasala, B.A., Chao, S.-S., Pier, M., Barrera, D.J. and Mayfield, S.P. (2014) Enhanced genetic tools for engineering multigene traits into green algae. *PLoS ONE*, **9**, e94028.

Rasi, M.Q., Parker, J.D.K., Feldman, J.L., Marshall, W.F. and Quarmby, L.M. (2009) Katanin knockdown supports a role for microtubule severing in release of basal bodies before mitosis in Chlamydomonas. *Mol. Biol. Cell* **20**, 379–388.

Robbens, S., Khadaroo, B., Camasses, A., Derelle, E., Ferraz, C., Inzé, D., Van De Peer, Y. and Moreau, H. (2005) Genome-wide analysis of core cell cycle genes in the unicellular green alga *Ostreococcus tauri*. *Mol. Biol. Evol.* **22**, 589–597.

Robreau, G. and Le Gal, Y. (1975) Isolation and partial characterization of *Chlamydomonas reinhardtii* chromatin. *Biochimie*, **57**, 703–710.

Rogozin, I.B., Basu, M.K., Csürös, M. and Koonin, E.V. (2009) Analysis of rare genomic changes does not support the unikont-bikont phylogeny and suggests cyanobacterial symbiosis as the point of primary radiation of eukaryotes. *Genome Biol. Evol.* **1**, 99–113.

Russell, P. and Nurse, P. (1986) cdc25+ functions as an inducer in the mitotic control of fission yeast. *Cell*, **45**, 145–153.

Russell, P. and Nurse, P. (1987) Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. *Cell*, **49**, 559–567.

Sabelli, P.A. and Larkins, B.A. (2009) The contribution of cell cycle regulation to endosperm development. *Sex. Plant Reprod.* **22**, 207–219.

Salisbury, J.L. (2007) A mechanistic view on the evolutionary origin for centrin-based control of centriole duplication. *J. Cell. Physiol.* **213**, 420–428.

Salisbury, J.L., Baron, A.T. and Sanders, M.A. (1988) The centrin-based cytoskeleton of *Chlamydomonas reinhardtii*: distribution in interphase and mitotic cells. *J. Cell Biol.* **107**, 635–641.

Schoppmeier, J., Mages, W. and Lechtreck, K.-F. (2005) GFP as a tool for the analysis of proteins in the flagellar basal apparatus of Chlamydomonas. *Cell Motil. Cytoskeleton* **61**, 189–200.

Schulze, T., Prager, K., Dathe, H., Kelm, J., Kießling, P. and Mittag, M. (2010) How the green alga *Chlamydomonas reinhardtii* keeps time. *Protoplasma*, **244**, 3–14.

Silflow, C.D., Liu, B., LaVoie, M., Richardson, E.A. and Palevitz, B.A. (1999) Gamma-tubulin in Chlamydomonas: characterization of the gene and localization of the gene product in cells. *Cell Motil. Cytoskeleton* **42**, 285–297.

Silflow, C.D., LaVoie, M., Tam, L.-W., Tousey, S., Sanders, M., Wu, W., Borodovsky, M. and Lefebvre, P.A. (2001) The Vfl1 Protein in Chlamydomonas localizes in a rotationally asymmetric pattern at the distal ends of the basal bodies. *J. Cell Biol.* **153**, 63–74.

Spudich, J.L. and Sager, R. (1980) Regulation of the Chlamydomonas cell cycle by light and dark. *J. Cell Biol.* **85**, 136–145.

Straley, S.C. and Bruce, V.G. (1979) Stickiness to glass circadian changes in the cell surface of *Chlamydomonas reinhardi*. *Plant Physiol.* **63**, 1175–1181.

Taillon, B.E., Adler, S.A., Suhan, J.P. and Jarvik, J.W. (1992) Mutational analysis of centrin: an EF-hand protein associated with three distinct contractile fibers in the basal body apparatus of Chlamydomonas. *J. Cell Biol.* **119**, 1613–1624.

Tamanoi, F. (2011) Ras signaling in yeast. *Gene Cancer* **2**, 210–215.

Tenenboim, H., Smirnova, J., Willmitzer, L., Steup, M. and Brotman, Y. (2014) VMP1-deficient Chlamydomonas exhibits severely aberrant cell morphology and disrupted cytokinesis. *BMC Plant Biol.* **14**, 1–13.

Tulin, F. and Cross, F.R. (2014) A microbial avenue to cell cycle control in the plant superkingdom. *Plant Cell* **26**, 4019–4038.

Turmel, M., Lemieux, C. and Lee, R.W. (1980) Net synthesis of chloroplast DNA throughout the synchronized vegetative cell-cycle of Chlamydomonas. *Curr. Genet.* **2**, 229–232.

Umen, J.G. (2005) The elusive sizer. *Curr. Opin. Cell Biol.* **17**, 435–441.

Umen, J.G. and Goodenough, U.W. (2001) Control of cell division by a retinoblastoma protein homolog in Chlamydomonas. *Genes Dev.* **15**, 1652–1661.

Vítová, M., Bisová, K., Hlavová, M., Kawano, S., Zachleder, V. and Cízková, M. (2011a) *Chlamydomonas reinhardtii*: duration of its cell cycle and phases at growth rates affected by temperature. *Planta*, **234**, 599–608.

Vítová, M., Bisová, K., Umysová, D., Hlavová, M., Kawano, S., Zachleder, V. and Cízková, M. (2011b) *Chlamydomonas reinhardtii*: duration of its cell cycle and phases at growth rates affected by light intensity. *Planta*, **233**, 75–86.

Voigt, J. and Münzner, P. (1987) The Chlamydomonas cell cycle is regulated by a light/dark-responsive cell-cycle switch. *Planta*, **172**, 463–472.

Vos, J.W., Safadi, F., Reddy, A.S. and Hepler, P.K. (2000) The kinesin-like calmodulin binding protein is differentially involved in cell division. *Plant Cell* **12**, 979–990.

Wang, D., Kong, D., Wang, Y., Hu, Y., He, Y. and Sun, J. (2003) Isolation of two plastid division ftsZ genes from *Chlamydomonas reinhardtii* and its evolutionary implication for the role of FtsZ in plastid division. *J. Exp. Bot.* **54**, 1115–1116.

Wang, P., Malumbres, M. and Archambault, V. (2011) The greatwall-PP2A axis in cell cycle control. In *Methods in Molecular Biology* (Noguchi, E. and Gadaleta, M.C., eds). New York: Springer, pp. 99–111.

Warr, J.R. (1968) A mutant of *Chlamydomonas reinhardii* with abnormal cell division. *J. General Microbiol.* **52**, 243–251.

Wood, C.R., Wang, Z., Diener, D., Zones, J.M., Rosenbaum, J. and Umen, J.G. (2012) IFT proteins accumulate during cell division and localize to the cleavage furrow in Chlamydomonas. *PLoS ONE*, **7**, e30729.

Woodcock, C.L., Safer, J.P. and Stanchfield, J.E. (1976) Structural repeating units in chromatin. I. Evidence for their general occurrence. *Exp. Cell Res.* **97**, 101–110.

Wright, R.L., Salisbury, J. and Jarvik, J.W. (1985) A nucleus-basal body connector in *Chlamydomonas reinhardtii* that may function in basal body localization or segregation. *J. Cell Biol.* **101**, 1903–1912.

Wright, R.L., Adler, S.A., Spanier, J.G. and Jarvik, J.W. (1989) Nucleus-basal body connector in *Chlamydomonas*: evidence for a role in basal body segregation and against essential roles in mitosis or in determining cell polarity. *Cell Motil. Cytoskeleton* **14**, 516–526.

Yang, Y., Glynn, J.M., Olson, B.J., Schmitz, A.J. and Osteryoung, K.W. (2008) Plastid division: across time and space. *Curr. Opin. Plant Biol.* **11**, 577–584.

Zachariae, W. and Nasmyth, K. (1999) Whose end is destruction: cell division and the anaphase-promoting complex. *Genes Dev.* **13**, 2039–2058.

Zamora, I. and Marshall, W.F. (2005) A mutation in the centriole-associated protein centrin causes genomic instability via increased chromosome loss in *Chlamydomonas reinhardtii*. *BMC Biol.* **3**, 15.

Zhang, R., Patena, W., Armbruster, U., Gang, S.S., Blum, S.R. and Jonikas, M.C. (2014) High-throughput genotyping of green algal mutants reveals random distribution of mutagenic insertion sites and endonucleolytic cleavage of transforming DNA. *Plant Cell* **26**, 1398–1409.