10 Yeast Growth and the Cell Cycle

10.1 Vegetative Reproduction in Yeast

Mitotic division is initiated when cells attain a critical cell size and are stimulated by exogenous or endogenous signals during interphase. A prerequisite is that DNA synthesis guarantees proper duplication of the genetic material and that all substrates critical to anabolic pathways are available. In mammalian cells, the mitotic phase can be subdivided into a number of steps that are characterized by particular states of orienting the spindle pole bodies, sorting and movement of the duplicated chromosomes, and finally, cell separation after cytokinesis (Figure 10-1). While all processes pertinent to the cell cycle have been highly conserved among all eukaryotes, ascomycetous fungi, and in particular *S.cerevisiae*, exhibit several peculiarities with respect to cell division and cytokinesis [Bouquin et al., 2000; Bidlingmaier et al., 2001].

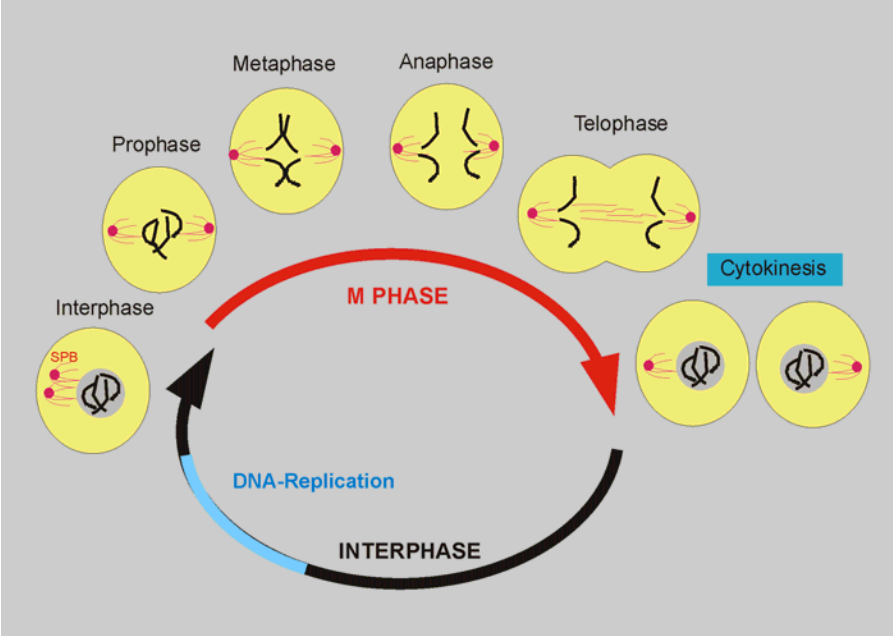

Figure 10-1: Phases in the mitotic cycle.

10.1.1 Yeast Budding

Budding is the most common mode of **vegetative growth** in yeasts and multilateral budding is a typical reproductive characteristic of ascomycetous yeasts, including *S. cerevisiae*. Yeast buds are initiated when mother cells attain a critical cell size at a time coinciding with the onset of DNA synthesis. This is followed by localized weakening of the cell wall and this, together with tension exerted by turgor pressure, allows extrusion of cytoplasm into an area bounded by new cell wall material. The regulation of particular cell wall synthetic enzymes and transport of specific bud plasma membrane receptors are key steps in the emergence of a bud. Chitin forms a ring at the junction between the mother cell and the newly emerging bud to finally result in the generation of a daughter cell. After cell separation, this ring will be retained at the surface of the mother cell and form the so-called bud scar (Figures 10-2 and 10-3), and a birth scar at the surface of the daughter. The number of bud scars left on the surface of a yeast cell is a useful determinant of cellular age.

Several recent papers have been devoted to budding in yeast [Roemer et al., 1996; Barral et al., 1999; Barrett et al., 2000; Manning et al., 1999; Ni & Snyder, 2001; Sheu et al., 2000; Swaroop et al., 2000; Vogel et al., 2000].

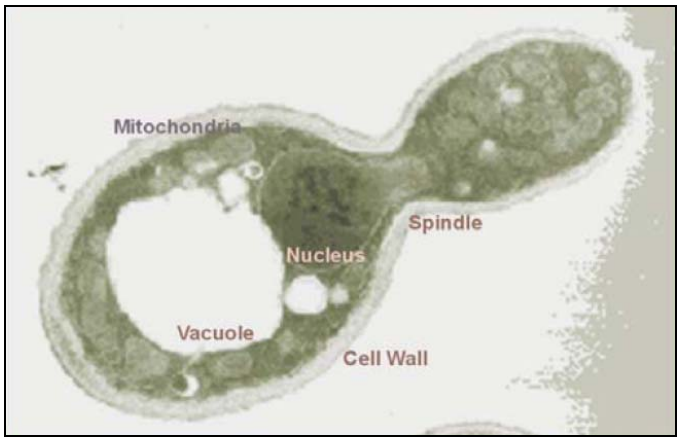

Figure 10-2: Budding yeast cell.

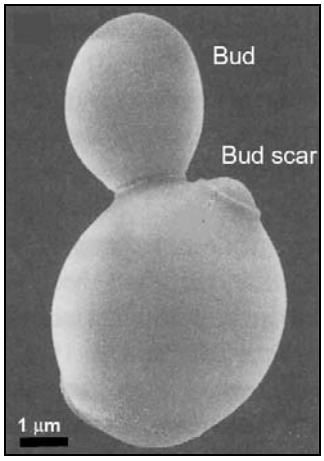

Figure 10-3: Yeast bud and bud scar.

Table 10-1: Examples of components important for budding

Genes	Gene product characteristics	Mutant phenotypes
General bud-site selection		Random budding in haploids and diploids
BUD1/RSR1	Ras-related protein	
BUD2	GTPase-activating protein (GAP)	
BUD5	GDP-GTP exchange factor (GEF)	
Axial bud-site selection		Bipolar budding in haploids
BUD3	Novel	
BUD4	GTP_binding domain	
AKL1	a-factor protease	
BUD10/AXL2	Type-I plasma membrane glycoprotein	
Polarity establishment		Round, multinucleate cells unable to bud
CDC24	GEF for Cdc42p	
CDC42	Rho/Rac GTPase	
BEM1	SH3 domains	
Diploid bud-site selection		Random budding in diploids/mother cells
ACT1	Actin	
SPA2	Coiled-coil domain	
RSV161, RSV167	SH3 domain	
BNI1, BUD6, BUD7	?	
BUD8	?	
BUD9	?	
Septin-ring		Bipolar budding in haploids
CDC3, CDC10, CDC11, CDC12	10-nm filament ring components GTP-binding domain	

During bud formation, only the bud but not the mother cell will grow. Once mitosis is complete and the bud nucleus and other organelles have migrated into the bud, cytokinesis commences and a septum is formed in the isthmus between mother and daughter. A ring of proteins, called septins (Figure 10-4), are involved in positioning cell division in that they define the cleavage plain which bisects the spindle axis at cytokinesis. These septins encircle the neck between mother and daughter for the duration of the cell cycle.

In *S. cerevisiae*, cell size at division is asymmetrical with buds being smaller than mother cells when they separate. Also cell division cycle times are different, because daughter cells need time (in G1 phase) to attain the critical cell size before they are prepared to bud.

Budding is not a randomized, uncontrolled process; cellular geometry is explicitly important in localizing budding sites. Numerous studies have endeavoured to explain at the cellular and molecular level how polarized cell growth is regulated and how the site of emerging buds is chosen. Bud site selection depends on several physiological and genetic factors. For example, cell mating type is important, and **a** and α haploid cells are kwon to exhibit an axial budding pattern, where as **a**/α diploids exhibit a bipolar budding pattern. Axial budding means that mother and daughter cells form a new bud near the preceding bud scar and birth scar, respectively. Bipolar budding is when daughter cells bud firstly away from their mother, while mother cells either bud away or toward daughter cells.

The hierarchy of cell polarity is governed by the interplay of various genes that dictate the orientation of cytoskeletal elements. For example, the bud-site selection genes (*BUD* genes) are required for determining the orientation of actin fibres, and genes for bud formation (such as *CDC24, CDC42, BEM1*) direct cell surface growth to the developing bud. Budding is strictly connected to events in the cell cycle (see below) in that cyclins and cyclin-dependent kinases play a decisive role in actin assembly and in localizing and timing of bud emergence.

It may be mentioned briefly that fission yeasts, like *Schizosaccharomyces pombe*, divide exclusively by forming a cell septum analogous to the mammalian cell cleavage furrow, which constricts the cell into two equal-sized daughters.

10.1.2 Yeast Septins

Septins are highly conserved cytoskeletal elements found in fungi, mammals, and all eukaryotes examined thus far, with the exception of plants [Barral et al., 2000; Casamayor & Snyder, 2004]. The septin proteins assemble into filaments that lie underneath the plasma membrane. In *Saccharomyces cerevisiae*, where they were first identified, septins are visible as electron-dense cortical rings at the mother bud neck. In multicellular organisms, they are found at the cleavage furrow and other cortical locations. Consistent with their localization, septins have been shown to be required for cytokinesis in yeast, *Drosophila melanogaster*, and mammalian cells.

Recent evidence in yeast has demonstrated that septins participate in a variety of other cellular processes, including cell morphogenesis, bud site selection, chitin deposition, cell cycle regulation, cell compartmentalization, and spore wall formation. Since septins participate in many cellular processes, it is not surprising that a diverse set of proteins have been found associated with the yeast septin cytoskeleton.

The septins have a highly conserved structure. They contain a central GTP-binding domain flanked by a basic region at the amino terminus, and most septins contain a coiled-coil domain at the carboxy terminus. In yeast, five septins, Cdc3, Cdc10, Cdc11, Cdc12, and Shs1, localize to the mother bud neck in vegetatively growing cells. Cdc3 and Cdc12 are essential for growth at all temperatures, whereas Cdc10 and Cdc11 are required only at elevated temperatures. Shs1 is a nonessential septin. Cells containing temperature- sensitive mutations in either *CDC3*, *CDC10*, *CDC11*, or *CDC12* delay at a G2 checkpoint and arrest at the restrictive temperature, forming extensive chains of highly elongated cells.

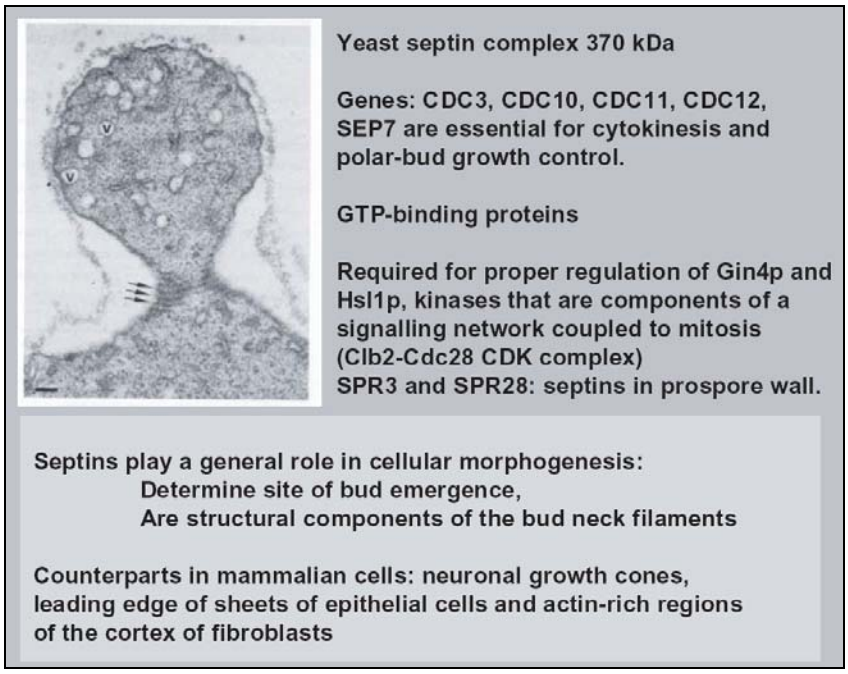

Figure 10-4: Functions of septins.

Table 10-2: Diversity of septin expression and function.

Gene	Function	Localization	Biochemistry
CDC3 *CDC10* *CDC11* *CDC12*	Essential for cytokinesis and polar-bud growth control. Cdc3p and Cdc12p required for viability, but not Cdc10p and Cdc11pin some backgrounds. Reqired for proper regulation of the Gin4p and Hsl1p kinases	Bud neck, site of bud emergence, base of schmoo	Found in 370 kDa complex that can form filaments in vitro
SEP7	Required in vivo for proper regulation of Gin4p kinase	Bud neck, site of bud emergence	Can complex with Cdc3p, Cdc10, Cdc11p, Cdc12p
SPR3	Sporulation efficiency	Prospore wall	No data
SPR28	No obvious phentype	Prospore wall	No data

Table 10-3: Budding yeast septin-protein interations.

Protein	Function	Septin-dependent localization	Genetic/physical interactions	Localization
Gin4p Hsl1 Kcc4	Protein kinases that function in septin localization and cell cycle progression	Yes	Gin4p interacts physically and genetically with septins	Bud neck, site of bud emergence
Bni4p	Reqired for normal chitin depositionand morphology	Yes	Two-hybrid interaction with Cdc10p and Chs4p	Bud neck

Chs3p Chs4p	Required for normal chitin synthesis	Yes	Synthetic lethal interaction between Chs4p and Cdc12p	Bud neck, site of bud emergence
Yck1p Yck2p	Casein kinase I homologs, required for septin localization, cytokinesis, morphogenesis and endocytosis	No	Unknown	Bud neck, sites of polarized growth, plasma membrane
Bud3p Bud4p Spa2p	Bud site selection	Yes Yes ??	Unknown Unknown Synthetic lethal interaction with Cdc10p	Bud neck, site of bud emergence
Bni1p	Cytokinesis/morphogenesis	??	Synthetic lethal interaction with Cdc12p	Bud tip
Myo1p	Type II myosin. Plays a role in cytogenesis	Yes	Unknown	Bud neck
Arf1p	Morphogenesis during mating	??	Two-hybrid interaction with Cdc12p	Base of mating projections

10.1.3 Yeast Spindle Pole Body (SPB)

Nucleation of microtubules by eukaryotic microtubule organizing centers (MTOCs) is required for a variety of functions, including chromosome segregation during mitosis and meiosis, cytokinesis, fertilization, cellular morphogenesis, cell motility, and intracellular trafficking. Analysis of MTOCs from different organisms shows that the structure of these organelles is widely varied even though they all share the function of microtubule nucleation. Despite their morphological diversity, many components and regulators of MTOCs, as well as principles in their assembly, seem to be conserved [Segal et al., 2001; Jasperson et al., 2004; .Cheeseman & Desay, 2004].

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The **spindle pole body** (SPB) is the sole site of microtubule organization in the budding yeast *Saccharomyces cerevisiae*. SPBs are embedded in the nuclear envelope throughout the yeast life cycle and are therefore able to nucleate both nuclear and cytoplasmic microtubules. The small size of the yeast SPB, its location in a membrane, and the fact that nearly all genes involved in SPB function are essential have presented significant challenges in its analysis. Nevertheless, the SPB is perhaps the best-characterized microtubule organizing center (MTOC).

The SPB is a cylindrical organelle that appears to consist of three disks or plaques of darkly staining material (Figure 10-5): an outer plaque that faces the cytoplasm and is associated with cytoplasmic microtubules, an inner plaque that faces the nucleoplasm and is associated with nuclear microtubules and a central plaque that spans the nuclear membrane. One side of the central plaque is associated with an electron-dense region of the nuclear envelope termed the half-bridge. This is the site of new SPB assembly because darkly staining material similar in structure to the SPB accumulates on its distal, cytoplasmic tip during G1 phase of the cell cycle.

Careful analysis of SPB size and structure indicates that the SPB is a dynamic organelle. In haploid cells, the SPB grows in diameter from 80 nm in G1 to 110 nm in mitosis. The molecular mass of a diploid SPB, including microtubules and microtubule associated proteins, is estimated to be 1–1.5 GDa. However, only 17 components of the mitotic SPB have been identified to date (Table 10-4).

Table 10-4: Yeast spindle pole body components.

Protein	SPB location	Role in SPB function
Tub4	γ-tubulin complex	MT nucleation
Spc98	γ-tubulin complex	MT nucleation
Spc97	γ-tubulin complex	MT nucleation
Spc72	OP, HB	γ-tubulin binding protein
Nud1	OP, satellite	MEN signalling
Cnm67	IL1, OP, satellite	Spacer, anchors OP to CP
Spc42	IL2, OP, satellite	Structural SPB core
Spc29	CP, satellite	Structural SPB core
Cmd1	CP	Structural Spc110 binding protein
Spc110	CP to IP	Spacer, γ-tubulin binding protein
Ndc1	SPB periphery	Membrane protein, SPB insertion
Mps2	SPB periphery	Membrane protein, SPB insertion
Bbp1	SPB periphery	SPB core, HB linker to membrane
Kar1	HB	Membrane protein, SPB duplication
Mps3	HB	Membrane protein, SPB duplication
Cdc31	HB	SPB duplication
Sfi1	HB	SPB duplication
Mpc54	MP	Replace Spc72 in meosis I
Spo21	MP	Replace Spc72 in meosis II

CP= Central plaque; HB=half-bridge; IL1=Inner layer 1; IL2= Inner layer 2; IP=Inner plaque; MEN= ; cMT= Cytoplasmic MT; nMT= Nuclear MT; OP=Outer plaque.

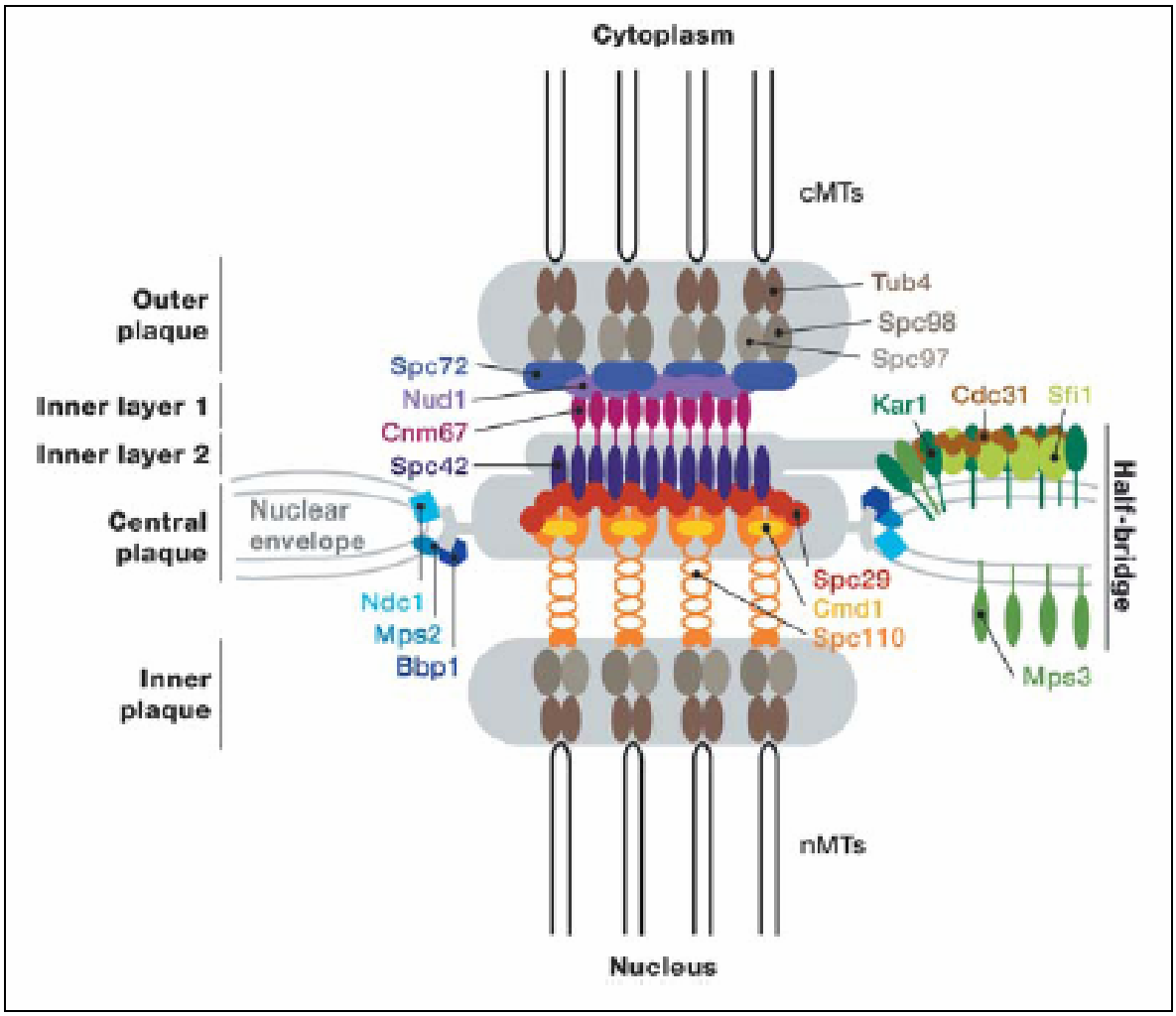

Figure 10-5: Location of protein components of the spindle pole body.

Regulators of **SPB duplication** and function associate with the SPB during all or part of the cell cycle. Mps1, a conserved protein kinase required for multiple steps in SPB duplication and also for the spindle checkpoint, localizes to SPBs and to kinetochores

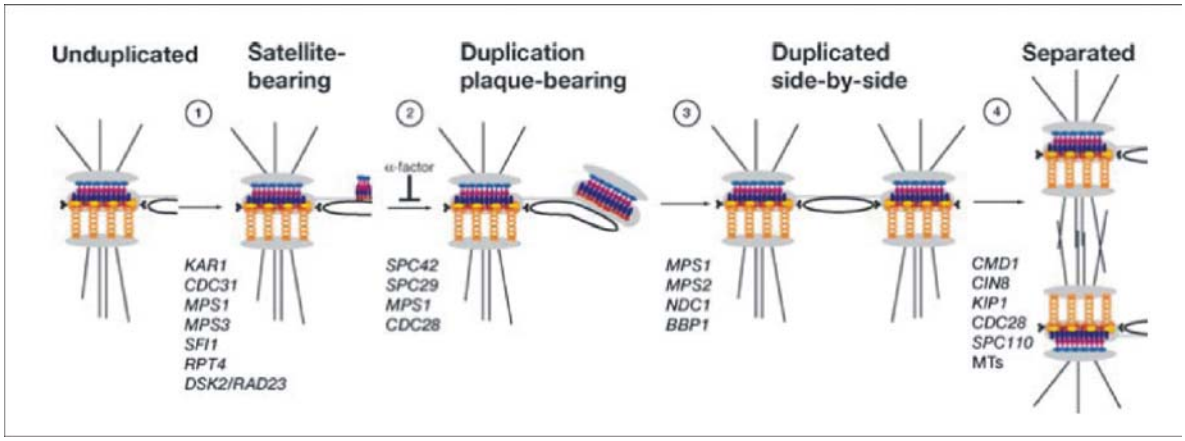

Figure 10-6: SPB duplication pathway.

SPB duplication (Figure 10-6) can be divided into three steps: (1) half-bridge elongation and deposition of satellite material, (2) expansion of the satellite into a duplication plaque and retraction of the half-bridge, and (3) insertion of the duplication plaque into the nuclear envelope and assembly of the inner plaque. Following completion of SPB duplication, the bridge connecting the side-by-side SPBs is severed, and SPBs move to opposite sides of the nuclear envelope (4). The requirements for various gene products in each step are shown in the figure. *SPC72*, *NUD1*, and *CNM67* are probably required for step 2. SPBs are not synthesized de novo. Consequently, every time a cell divides it must duplicate its SPB, as well as its genome, to ensure that both the mother and daughter cell contain one copy of all 16 chromosomes and one SPB. SPB duplication occurs in G1 phase of the cell cycle; however, defects in SPB duplication are not detected until mitosis when cells fail to form a functional bipolar spindle. Generally, SPB defects cannot be reversed at this point, so cells will eventually attempt chromosome segregation with a monopolar spindle, which results in progeny with aberrant DNA content and/or SPB number. Therefore, accurate SPB duplication during G1 is essential to maintain genomic stability.

10.2 The Yeast Cell Cycle

10.2.1 General

The **cell cycle** can be defined as the period between division of a mother cell and subsequent division of its daughter progeny. The regulatory mechanisms that order and coordinate the progress of the cell cycle have been intensely studied [Mal & Nurse, 1998; Futcher, 2000;.Lauren et al., 2001]. Numerous proteins that have been characterized through mutations are collectively designated as cell division cycle (Cdc) proteins.

The eukaryotic cell cycle involves both continuous events (cell growth) and periodic events (DNA synthesis and mitosis). Commencement and progression of these events can formally been distinguished into pathways for DNA synthesis and nuclear division, spindle formation, bud emergence and nuclear migration, and cytokinesis. However, from a molecular viewpoint these processes are intimately coupled (Figure 10-6).

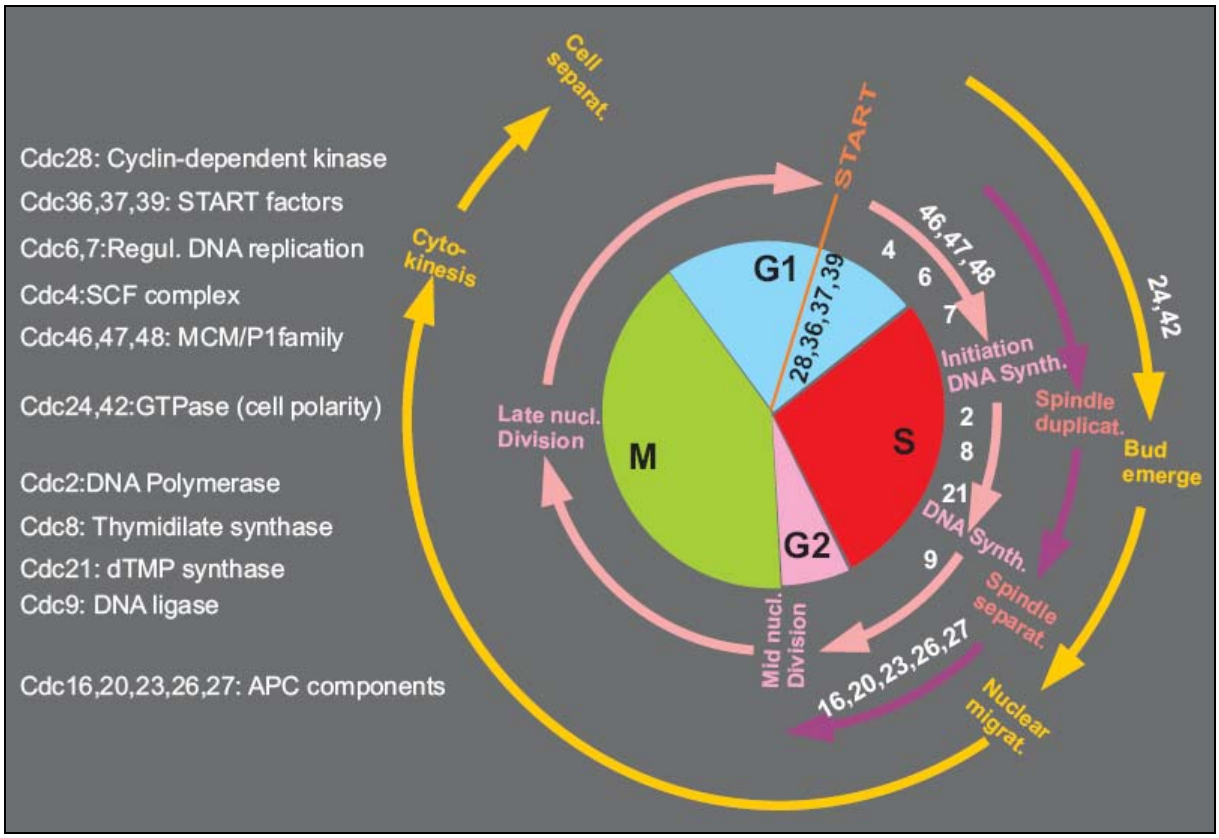

Figure 10-7: Cell cycle phases and physiological processes.

The **periodic events** can be divided into four phases (Figure 10-7): DNA synthesis (S phase); a post-synthetic gap (G2 phase); mitosis (M phase); and a pre-synthetic gap (G1 phase). For division, yeast cells must reach a critical size. The key point in control of the cell cycle is START, the transition that initiates processes like DNA synthesis in S phase, budding and spindle pole body duplication. Once cells have passed START, they are irreversibly committed to replicating their DNA and progressing through the cell cycle. START thus coordinates the cell cycle with cell growth. Nutrient starvation as well as induction of mating blocks passage through START. There are additional **checkpoints** that

arrest cells during the cell cycle to avoid DNA damage or cell death due to events occurring out of order. These control points are situated at the G1-S and G2-M boundaries and can be considered as internal regulatory systems that arrest the cell cycle if prerequisites for progression are not met.

After having passed the cell-size dependent START checkpoint, the level of cyclins (Cln,Clb) dramatically increase. **Cyclins** are periodically expressed and different cyclins (at least 11 in yeast) are known to be involved in the control of G1 (G1 cyclins), G2 (B-type cyclins) and DNA synthesis (S phase cyclins). G1 cyclins are transcriptionally regulated. Cln3p, a particular G1 cyclin, is a putative sensor of cell size, which acts by modulating the levels of other cyclins. In addition to cyclin accumulation, the activity of a cylin-dependent kinase (CDK) which is an effector of START, is induced; this is the gene product of *CDC28*. Cdc28p (also termed Cdk1p) couples with G1 cyclins that activate its kinase potential. Homologues of this 34 kDa protein have been characterized in other eukaryotes. Cdk1 as well as being essential for S phase, is also important in controlling entry into mitosis. Complex formation of Cdk1 has been established with Cln1-3 (at G1), with Clb5,6 (at S), Clb 3,4 (at S/G2) and Clb1,2 (at M). Alternation of cell cycle phases appears to be due to mechanisms that one cyclin family succeeds another. The level of cyclins are controlled by synthesis and programmed proteolysis. In this regard it has been shown that G2 cyclins are necessary for degradation of G1 cyclins, and that G2 cyclin synthesis is coupled to removal of G1 cyclins (Figure 10-8).

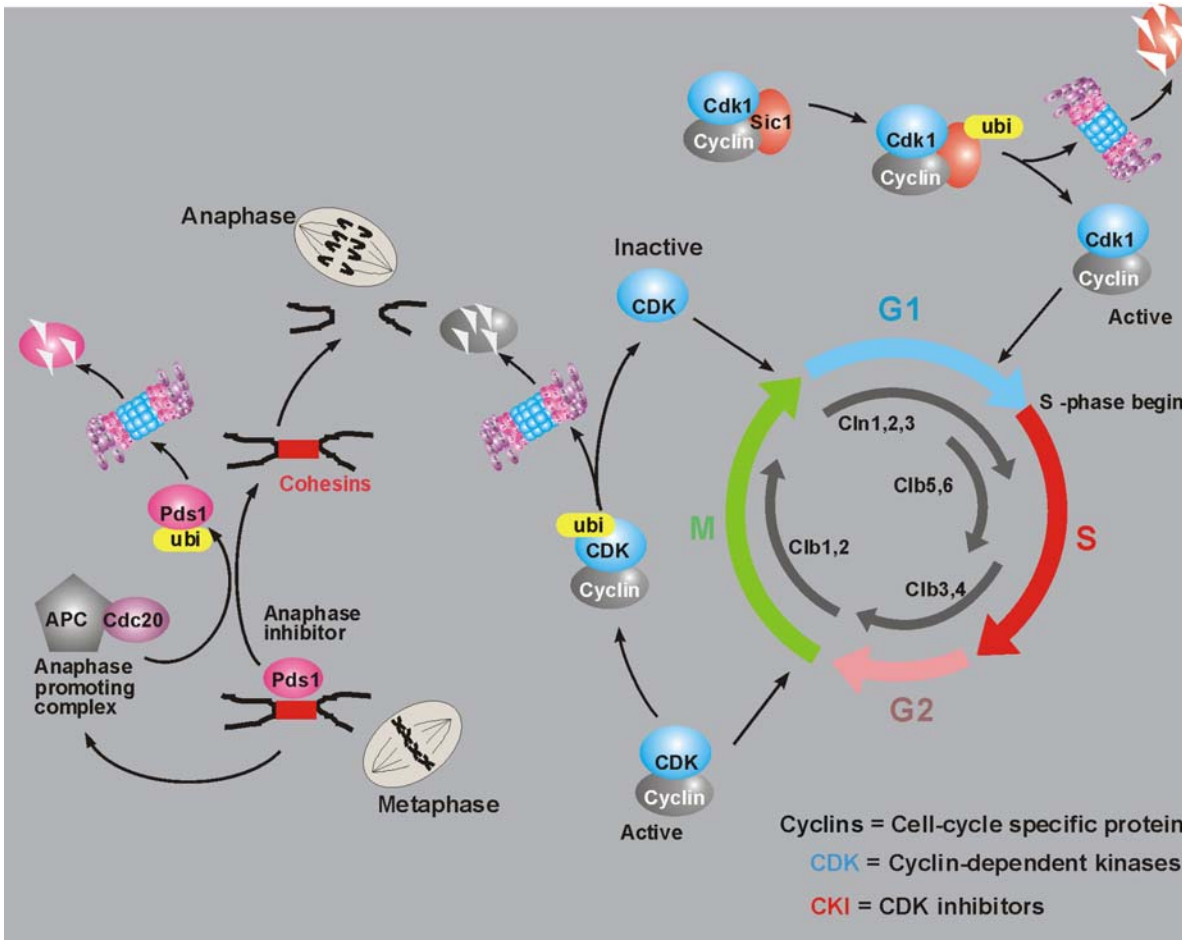

Figure 10-8: Regulation of the yeast cell cycle.

Important players in this game are **inhibitor proteins**, known as CKIs, which block CDK activity in G1. They represent a key mechanism by which the onset of DNA replication is regulated. One such

inhibitor is Sic1p: upon its destruction by programmed proteolysis, cyclin-Cdk1 activity is induced. This degradation then triggers the G1-S transition [Lauren et al., 2001].

In addition to this pathway, cell cycle progression is controlled by the availability of nutrients (Figure 10-9). Nutrient levels (for example, glucose or nitrogenous compounds) regulate the intracellular concentration of cAMP via a small G protein, Ras. The so-called Ras/cAMP pathway is well documented (see chapter 13). Decreasing levels lead to G1 arrest, while increasing levels induce the cAMP-dependent protein kinase (PKA), which then phosphorylates and thereby activates specific transcription factors involved in START.

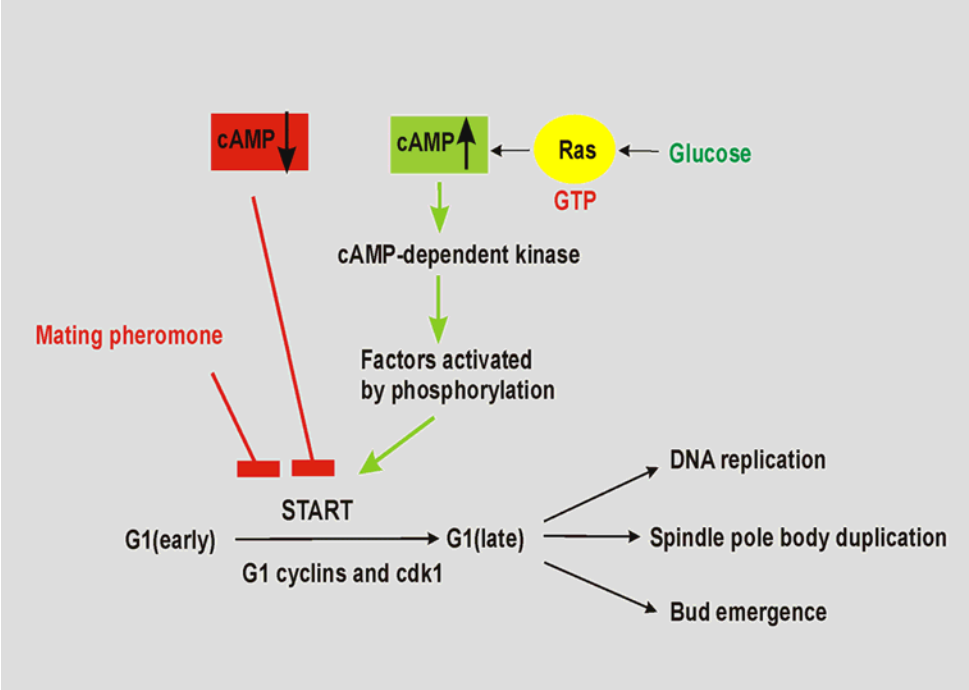

Figure 10-9: G1 regulation in yeast.

G2-M control is characterized by the association of Cdk1 with B-type cyclins. Complexing of the kinase with the cyclins activates the kinase, leading to induction of M phase. At the end of M phase, the mitotic cyclins are removed by programmed proteolysis.

10.2.2 DNA Replication

Chromosome duplication is central to cell division and is tightly controlled during the cell cycle. In eukaryotic cells, chromosome duplication is accomplished by initiating replication forks at many origins of replication on each chromosome; activation of the different **replication origins** is coordinated during S phase [Donaldson et al., 1999; Stillman, 2001; Raghuraman et al, 2001 Iyer et al., 2001; Heun et al., 2001; Wyrick et al., 2001]. ARS elements as substantial elements in yeast replication origins have been discussed above.

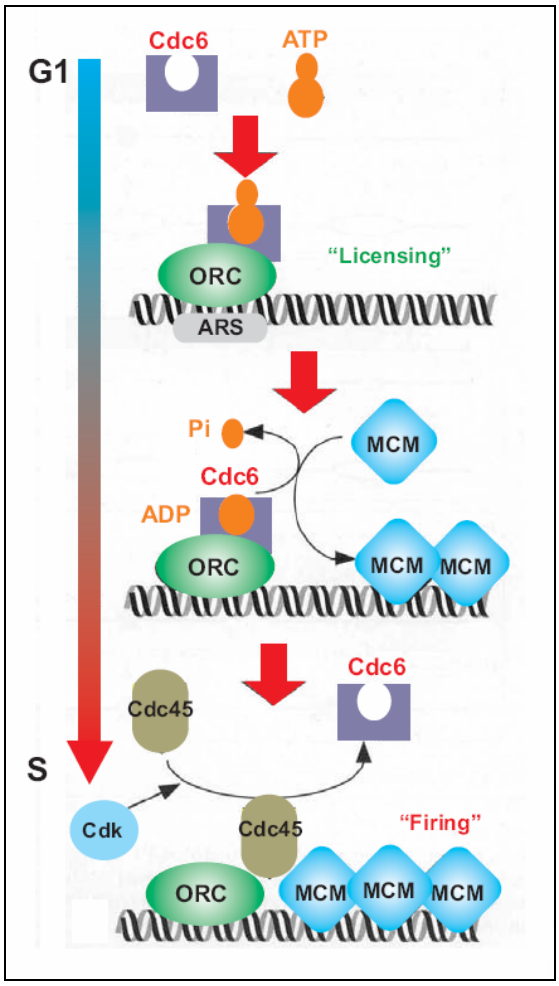

Figure 10-10: Licensing by the origin recognition complex.

A two-step model of replication initiation, in which origins are licensed for firing during G1 but only activated under cellular conditions that preclude their **licensing**, has been proposed. In yeast, the six-protein **origin recognition complex** (ORC) remains bound to DNA throughout the cell cycle and forms the core of the origin complex to which other protein components are recruited. Interestingly, ORC is also involved in silencing of gene expression. The first step in pre-replicative complex formation (Figure 10-10) appears to be the recruitment of Cdc6p by ORC, after which Cdc6p is in turn required for loading of Mcm/P1 protein complexes onto DNA, resulting in the origin being licensed for replication in the subsequent S phase. Cdc6p is an ATPase, and ATP hydrolysis seems essential for the loading of Mcm/P1. The role of the Mcm/P1 gene products is not sufficiently clarified yet. This protein family consists of six members, all of which are involved in the licensing process but their biochemical function is unclear. It may well be that they supply helicase function required in both initiation and elongation phases of replication. Following Cdk activation in late G1, Cdc45p becomes associated with the origin; removal of Cdc6p may stimulate this event.

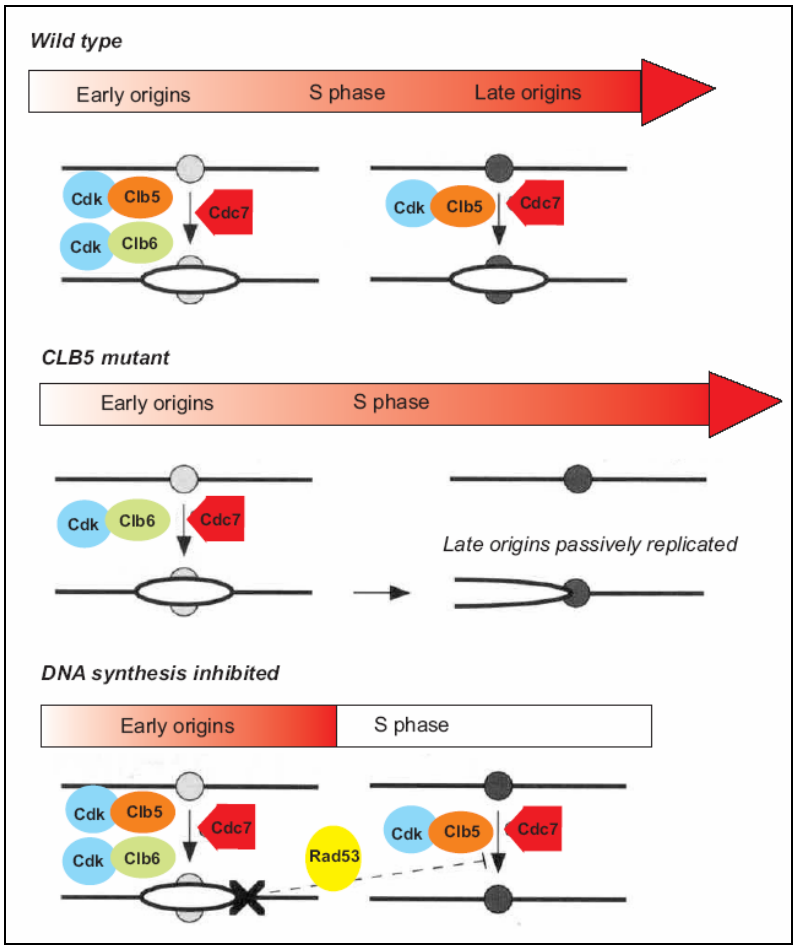

Figure 10-11: Regulation of replication by cyclins.

Interestingly, DNA replication must be restricted to only one round in each cell cycle. Work in yeast has shown that Cdk activity during G2 is required to prevent re-replication of DNA; the mechanism of this inhibition is not known, only the fact that the Cdks are involved in promoting the degradation of Cdc6p.

Progression through the cell cycle is highly coordinated. Replication origin firing during S phase is not random but rather is under strict temporal and spatial control. Replication forks cluster in discrete 'replication factories' within the nucleus and components required for elongation associate with nuclear structural components such as the lamina. Definitely, early and late origins have to be distinguished. Factors that share responsibility for promoting S phase are two B type cyclins, Clb5p and Clb6p, in conjunction with a single cyclin-dependent kinase, Cdc28p. As it apperas, Clb5p is executing the origin firing programme in both early and late origins, while Clb6-Cdc28 can only fire early replication origins. Further, the origin-firing programme is subject to checkpoint controls (Figure 10-11). One of the essential players is Rad53p, which is involved in monitoring successful execution of the programme of DNA replication during S phase, and co-ordinating a controlled arrest if problems are encountered. Rad53p also seems to be required for maintaining the level of nucleotides in the normal S phase.

10.2.3 Spindle Dynamics

In *S. cerevisiae*, the **mitotic spindle** must orient along the cell polarity axis, defined by the site of bud emergence, to ensure correct nuclear division between the mother and daughter cells [Segal and

Bloom, 2001]. Establishment of spindle polarity dictates this process and relies on the concerted control of spindle pole function and a precise programme of cues originating from the cell cortex that directs the cytoplasmic microtubule attachments during spindle morphogenesis. This cues cross talk with the machinery responsible for bud site selection, indicating that orientation of the spindle is mechanistically coupled to the definition of a polarity axis and the division plane.

Spindle morphogenesis in yeast is initiated by the execution of START at the G1-S transition of the cell cycle. Progression through START triggers bud emergence, DNA replication and the duplication of the microtubule-organizing centre (MTOC) - the spindle pole body (SPB) (Figures 10-5 and 10-12).

The single stages of mitosis and intracellular movements can be distinguished by time-lapse phase-contrast microscopy. In addition to the polymerization and depolymerization of tubulin (the major microtubular protein), cytolasmic dynein is a mechanochemical enzyme or motor protein which drives microtubules motility in yeast. Actin filaments, either as cytoskeletal cables or as cortical membrane patches, undergo dynamic changes during the cell cycle. The microtubules emanate from the SPBs toward the new bud and orientate the nucleus and intranuclear spindle at mitosis. The nuclear membrane remains intact throughout mitosis with the mitotic spindle forming intranuclearly between two SPBs embedded in the nuclear envelope. Once the genome replicates, the spindle aligns parallel to the mother bud axis and elongates eventually to provide each cell with one nucleus.

The program for the establishment of **spindle polarity**, primed by cellular factors partioning asymmetrically between the bud and the mother cortex, coupling of this process to bud site selection and polarized growth has been elucidated in some detail [Segal and Bloom, 2001]. Several cortical components implicated in spindle orientation such as Bni1p, a target of the polarizing machinery essential in bud site selection and spindle orientation, and the actin interactor Aip3p/Bud6p are initially localized to the bud tip. Other cortical elements (e.g. Num1p) are restricted initially to the mother cell during spindle assembly.

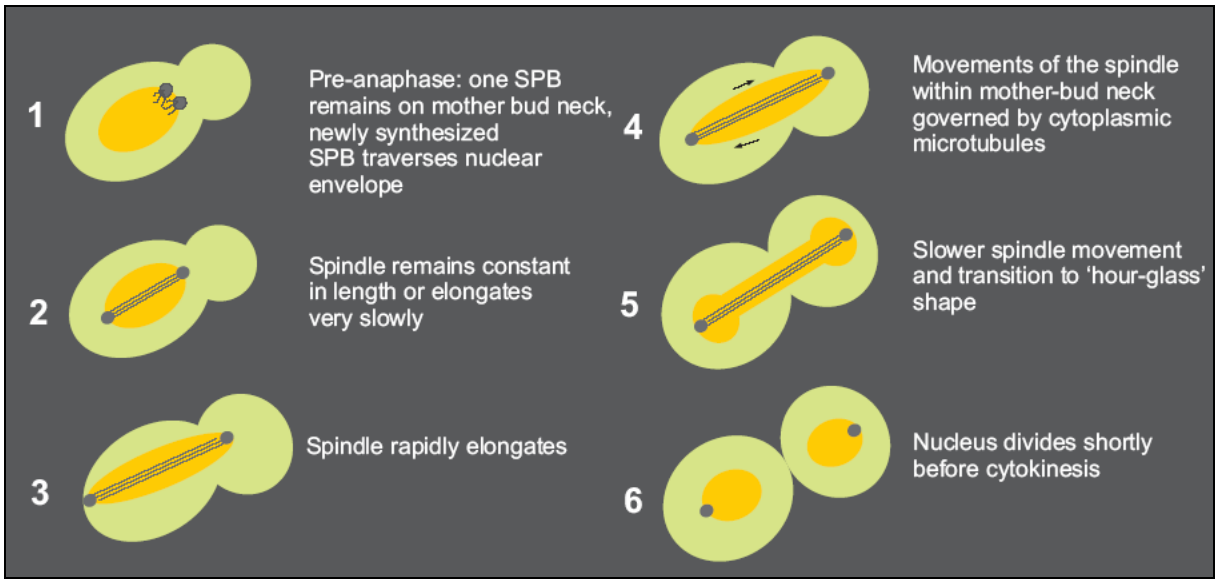

Figure 10-12: Spindle dynamics.

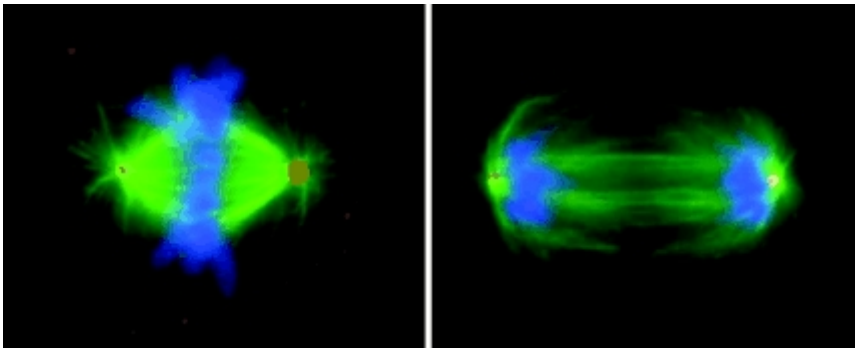

Figure 10-13: Fluorescence imaging of microtubules.

Factors mediating the process of microtubule attachment with the bud cell cortex are Bim1p and Kar9p. Bim1p can directly bind to microtubules and is required for the high dynamic instability of microtubules that is characteristic of cells before spindle assembly. Kar9p has been implicated in the orientation of functional microtubule attachments into the bud during vegetative growth. It is delivered to the bud by a Myo2-dependent mechanism presumably tracking on actin cables. Interaction of the two factors, Bim1p and Kar9p, appears to provide a functional linkage between the actin and microtubule cytoskeletons. In addition, Bud3p, a protein for axial budding of haploid cells, accumulates at the bud neck and is required for the efficient association of Bud6p to the neck region. Further, a variety of motor proteins are necessary in spindle morphogenesis: dynein and the kinesin-like proteins Kip2p and Kip3p, as well as Kar3p are involved in regulating microtubule dynamics, mediating nuclear migration to the bud neck and facilitating spindle translocation (Figure 10-13).

10.2.4 Sister Chromatid Cohesion and Separation

Sister chromatid cohesion is essential for accurate chromosome segregation during the cell cycle [Nasmyth, 1999; Biggins & Murray, 1999; Robert et al.; Nasmyth, 2002; Carnobel & Cohen-Fix; 2002; Uhlmann, 2004]. A number of structural proteins are required for sister chromatid cohesion and there seems be a link in some organisms between the processes of cohesion and condensation. Likewise, a number of proteins that induce and regulate the separation of sister chromatids have been identified.

Chromosome splitting is an irreversible event and must therefore be highly regulated. Once sister chromatids separate from one another, damage to the genome cannot easily be repaired by recombination nor can mistakes in chromosome alignment be corrected. Sister chromatids are pulled to opposite 'halves' of the cell by microtubules that emanate from opposite spindle poles. These microtubules interdigitate and keep the two poles apart. Subsequently, a second set of microtubules attaches to chromosomes through specialized 'kinetochores' and pulls them to the poles. In this way, sister chromatides separate and start to move into opposing poles (Figure 10-14).

However, chromosomes do not remain inactive at this process: cohesion between sister chromatids generates the tension by which cells align them on the metaphase plate. Cohesion also prevents chromosomes falling apart because of double-stranded breaks and facilitates their repair by recombination.

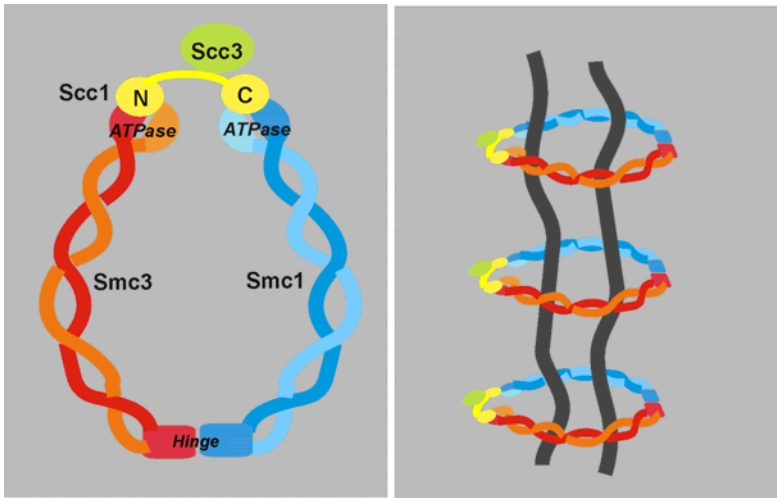

Figure 10-14: Cohesins: the 'glue' between sister chromatids.

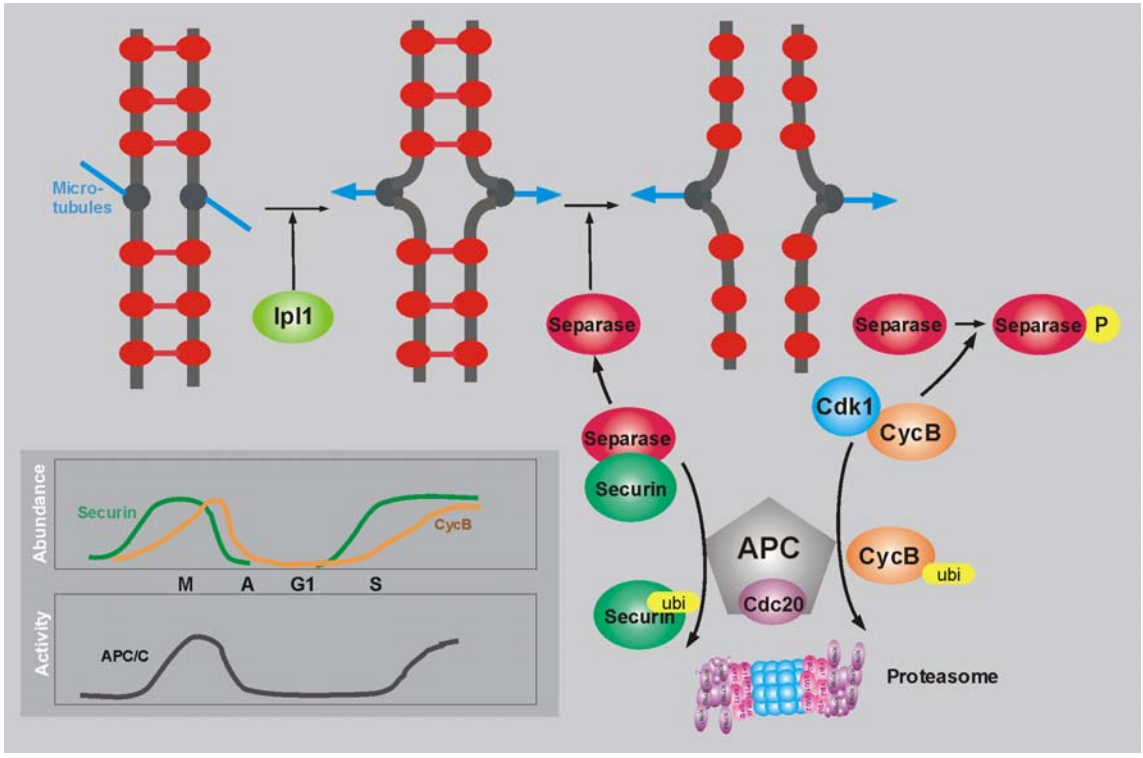

Figure 10-15: Sister chromatid separation.

Cohesin rings. The detection of chromosomal proteins that are essential for sister-chromatid cohesion during G2 and M phases and subsequent analyses have shown that the molecular basis for sister chromatid cohesion is a chromosomal protein complex, called cohesin. This complex consists of at least four subunits which together form a large (>50nm diameter) proteinaceous ring: a pair of structurally similar members of the SMC ('structural maintenance of chromosomes') family, Smc1 and Smt3; and Scc1 and Ssc3; all of which are encoded by essential genes. The establishment of the cohesin complex is mediated by Eco1/Ctf7 (in S phase) and Pds5, and may also depend on Ssc2 and Scc4.

The cohesin complex is shown in Figure 10-14. The circumference of the cohesin ring largely consists of flexible coiled-coil of the Smc1 and Smc3 subunits, binding each other in head to head and tail to tail orientation. The tails are held together by the hinge domains binding each other with high affinity. The Smc1 and Smc3 heads consist of ABC-type ATPase domains that dimerise with each other after binding ATP. Further, Scc1 associates with the Smc heads, whereby its N-terminus binds to Smc3 and its C-terminus to Smc1. This bridge could stabilize the ring structure. ATP hydrolysis weakens the interactions between Smc1 and Smc3 heads, and any remaining link between the heads has to rely on Ssc1. Evidence has been accumulating that the cohesin complex binds tightly to chromatin by encircling and topological trapping. A current model for the trapping mechanism is that ATP hydrolysis of the ABC-like ATPase domains by cohesin induces a change in the interaction of the Smc heads, thus coupling potential conformational changes in the Smc proteins to active transport of chromatin into the ring.

It has to be noted here that it is the Scc1 subunit of cohesin that is cleaved by separase at anaphase onset to relieve sister chromatid cohesion. Separase is tightly regulated: for the rest of the cell cycle, it is inactive by being bound to an inhibitory chaperone, called securin, whose destruction during the spindle checkpoint (see below) takes place only after all chromatid pairs have been aligned correctly on the mitotic spindle.

Condensin. The cohesin binding to chromatin has similarities with chromatin binding to condensin. Condensin, a 13 S complex, consists of two Smc proteins, Smc2p and Smc4p, and contains three other essential subunits, one of which is homologous to Scc1p. Just like cohesin, the topological structure of condensin is a ring. Condensin associates with chromatin independently of ATP, but ATP hydrolysis is needed for the binding reaction. A particular feat of condensin is that chromatin wraps around condensin, generating a torsion in the DNA. Thus it is amenable of contributing to chromosome compaction. In fact, after partial removal of cohesin rings by the separase reaction, condensin replaces cohesin.

Sister chromatid separation. Segregation of chromosomes must be a tightly regulated process (Figure 10-15). The fraction of cohesin that persists on chromosomes until metaphase is responsible for holding sisters together while they bi-orient during prometaphase. It is an absolute requirement that the sister chromatids adopt an amphitelic orientation, i.e. that the spindle apparatus can be activated in a way that allows the traction of sister chromatids to opposite poles of the cell by the microtubules to occur. (Remember that in yeast microtubules connect kinetochores to spindle poles throughout the cell cycle).

Two phases can be distinguished: (i) cohesin's dissociation from and condensin's association with chromosomes occurs in the complete absence of microtubules yet is capable of separating sister chromatids to ~0.5 μm. This first step in the individualisation process is triggered by the participation of PLK ('Polo-like kinase') which will phosphorylate cohesin (and possibly some other proteins). The second step, orienting sister chromatids on the mitotic spindle (Bi-orientation) and attaching microtubules to sister kinetochores, whereby chromosomes come under tension, is promoted by the Aurora-like kinase Ipl1p and controlled by the spindle checkpoint (see below). (ii) The molecular basis for sister chromatid separation in the second phase arose from the discovery of mitotic cyclins whose abundance fluctuates during the cell cycle. As already discussed, mitotic cyclins are regulatory subunits of a cyclin-dependent kinase, Cdk1p, whose activation triggers mitosis into late G2 phase. The sudden degradation of cyclin B as cells enter anaphase has an important role in abolishing Cdk1

activity but is not required for the separation of sister chromatids. The apparatus responsible for targeting cyclin B degradation is a highly conserved multisubunit particle, called the **anaphase promoting complex** (APC/C), which possesses ubiquitin-protein-ligase activty. Ubiquitination mediated by APC/C requires rate-limiting activator proteins that bind to APC; in yeast these are at least two proteins, namely Cdc20p and Cdh1p. These activators specify both substrate specificity and the timing of proteolysis.

In the absence of APC/C function, yeast cells arrest in metaphase, and sister chromatids fail to segregate owing to the persistence of securin, the inhibitor of separase. Securin accumulates within nuclei during late G1 phase, is maintained during G2 and early M phase, but degraded shortly before anaphase, so that separase becomes active. Separase resides in the cytoplasm until cells enter mitosis, whereupon it accumulates at the mitotic spindle until late anaphase. Separase is activated by proteolysis of securin by APC/C.

10.2.5 Spindle Checkpoint

Most eukaryotic cells posses a surveillance mechanism, also called **spindle checkpoint**, that prevents sister chromatid separation when spindles are damaged or chromosomes fail to form spindle attachments [Amon, 1999; Nasmyth, 2002]. The kinetochore of a single lagging chromosome emits a signal capable of blocking separation of all sister pairs. It also blocks any further cell cycle progression. In yeast, this process is triggered by the production of a complex, which contains the proteins Mad1p, Mad2p, Mad3p, and the protein kinases Bub1p and Bub3p (which are essential, integral components of the kinetochore). Bub3p binds to the activator Cdc20p of the APC/C complex and thereby blocks ubiquitination of both securin and cyclin B. In addition, protein kinase Msp1p is also required for spindle pole duplication, and the subunit Ncd10p of the centromere binding complex CBF3 are needed. The Mad complex (Figure 10-16) has been shown to be highly conserved among other eukaryotes.

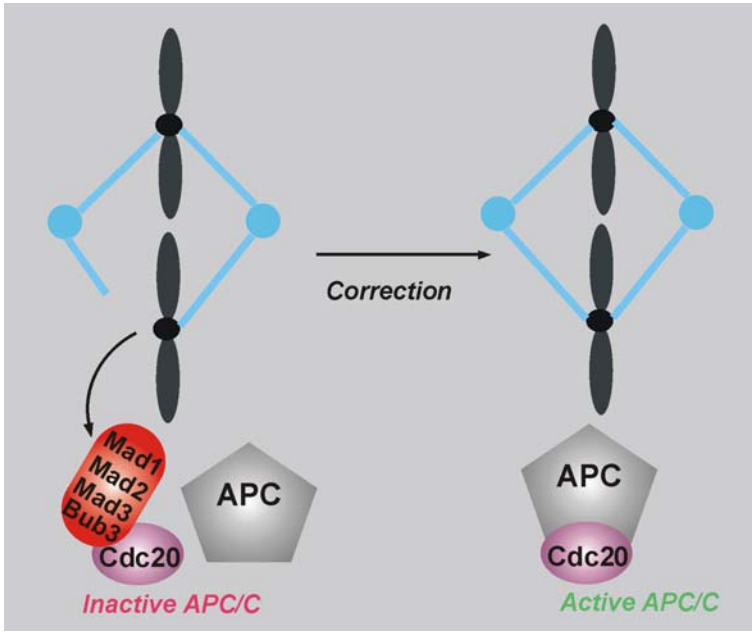

Figure 10-16: The spindle checkpoint.

10.3 Sexual Reproduction

Though vegetative growth is the major way of yeast reproduction, **sexual reproduction** is an alternative when nutrient supplies fall short. The latter process involves the conjugation of cells of opposite mating type [Shimoda, 2004]. A heterokaryon with a diploid set of chromosomes is formed, which is capable of reproduction by budding. Under starvation conditions, meiosis is induced which leads to sporulation and finally to the propagation of four haploid spores that segregate 2:2 (Figure 10-17).

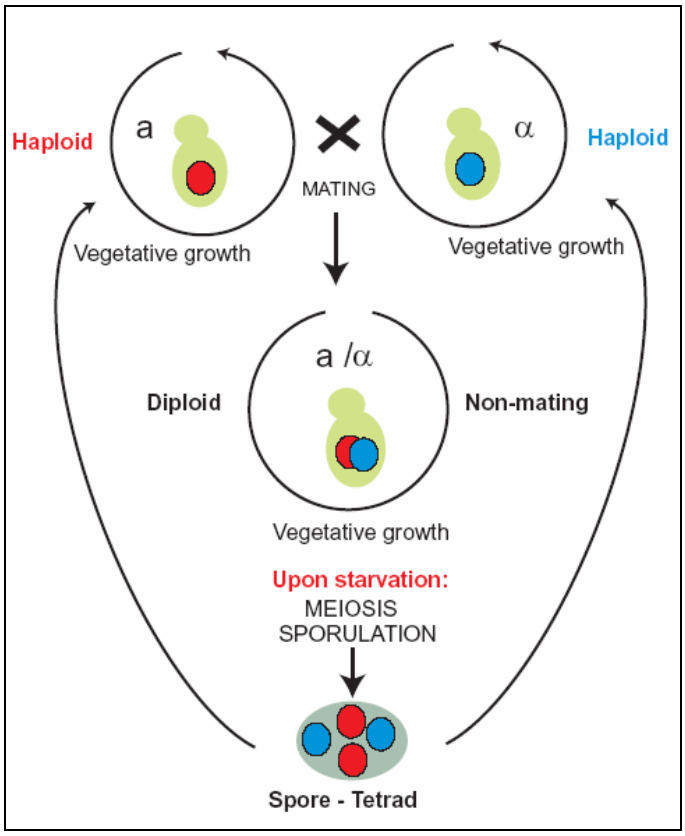

Figure 10-17: Life cycle of S. cerevisiae.

10.3.1 Mating Types and Mating

Cells of opposite mating type (**a** and α) synchronize each others' cell cycles at START in response to their mating factors. Once cells progress through START, they are unable to mate until the next cell cycle. Conjugation occurs by surface contact of specialized projections ('schmoo' formation) on mating cells followed by plasma membrane fusion. The mechanism of the mating response will be discussed in more detail in chapter 13.

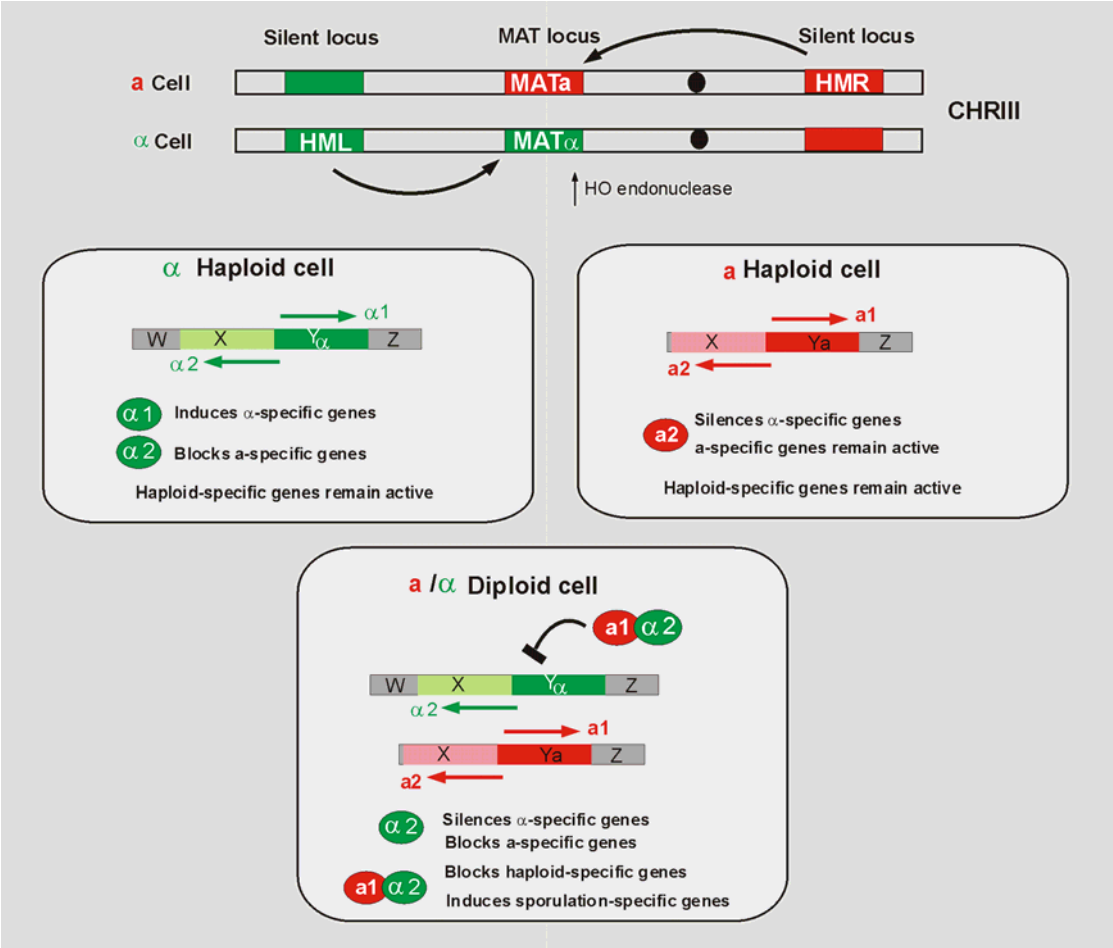

Figure 10-18: Mating type switch.

After **spore germination**, haploid cells have the capability of undergoing mating type switch which maximizes the potential of diploidy. Wild-type strains are homothallic and **a**/α diploids represent the usual vegetative state of this yeast. Industrial (brewing) yeast strains are generally polyploid and do not undergo a sexual life cycle (Figure 10-18).

10.3.2 Meiosis and Sporulation

Meiosis is a variation on the theme of mitosis. It produces haploid gametes that contain recombinant chromosomes, parts of which are derived from one parent and parts of which are derived from the other parent. As in the case of mitosis, meiosis starts with a round of DNA replication in diploid cells, which produces connected sister chromatids (Figure 10-19). **Recombination** between homologous chromatids brings homologues together. During the first meiotic division (meiosis I), sister kinetochores are treated as a single unit, and homologous kinetochore pairs are attached to and are pulled towards opposite spindle poles. However, as long as at least one reciprocal exchange has occurred, cohesion between sister chromatid arms will oppose disjunction of homologues and their segregation to opposite poles of the cell. Thus, loss of sister-chromatid cohesion along chromosome arms is essential for chromosome segregation during meiosis I. Meanwhile, however, cohesion between sister centromeres persists so that it can be later used to align sisters on the meiosis II metaphase plate. The difference in timing of sister chromatid cohesion loss is therefore a crucial aspect. Similar cohesion proteins as required for mitosis seem to work in meiosis.

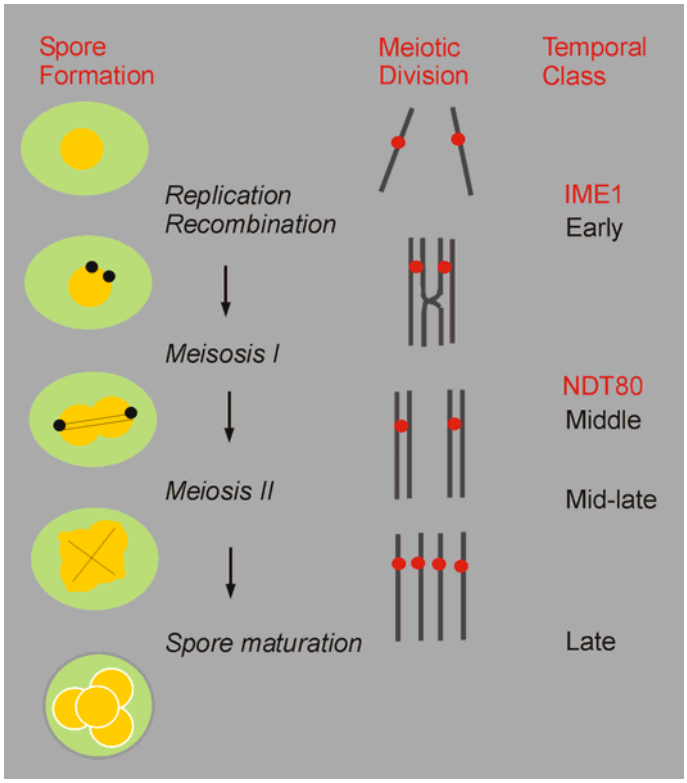

Figure 10-19: Meiosis in yeast.

Sporulation, which in yeast is induced at (nitrogen) starvation, is regulated by a specialized MAP kinase signalling pathway (see chapter 13). In many aspects, starvation is similar to other stress responses in yeast. Spore formation requires the activation of a large number of genes, several of which are involved in the biosynthesis of **spore walls**.

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