The Cell-Cycle Revisited

Cell Cycle

The **cell cycle** is divided into four stages, **G1 -> S -> G2 -> M** (mitosis and cytokinesis).

At G1, the cell can chose between:

- becoming quiescent
- differentiate
- grow and divide

During S-phase, DNA is faithfully duplicated, during G2, mitosis is prepared and cellular material is finally segregated during mitosis and cytokinesis -> leading to two daughter cells.

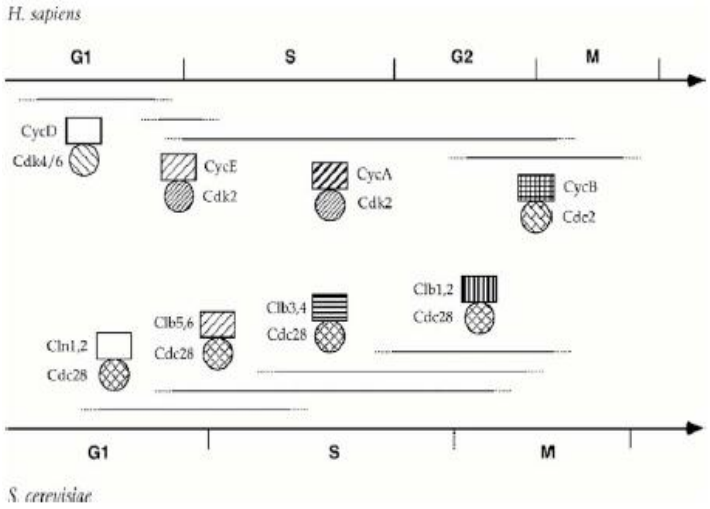


Figure 1: Major CDK-cyclin complexes in animals and budding yeast. Lines indicate approximate timing of activation and function of the indicated complexes. CycD/Cdk4/6; CycE/Cdk2; CycA/Cdk2; CycB/Cdc2; Cln1,2/Cdc28; Clb5,6/Cdc28; Clb2,4/Cdc28; Clb1,2/Cdc28.

Regulation is achieved by an endogenous oscillator, driven by **cyclin-dependent kinases (CDKs)**. Several **checkpoint systems** guarantee the correct order of events. CDKs are regulated directly or their associated cyclin is (required for kinase activity).

- Phosphorylation of Thr160: activating, improving substrate binding
- Phosphorylation of Tyr15 (and Thr14 in multicellular organisms): inhibiting, blocks kinase activity, regulated by Wee1 (kinase) and Cdc25 (phosphatase)
- Phosphorylation of Wee1 and Cdc25 by CDKs, Wee1 inhibited and Cdc25 activated -> **positive feedback loop**
- Initial trigger: Accumulation of mitotic cyclins
- **CDK-inhibitors (CKIs)** bind to CDK-cyclin complex, mask active site
    - **Cip/Kip** family (e.g. p21, p27) inhibits Cdk2- and Cdk4/6-cyclinD complexes involved in G1 and G1/S control
    - **Ink4** family (e.g. p15, p16) specific for Cdk4/6-cyclinD complexes
    - Yeast: **Far1** (inhibits Cdc28-Cln, G1 arrest), **Sic1** (inhibits Cdc28-Clb, timing of S phase)
- Cyclin proteins are regulated through protein

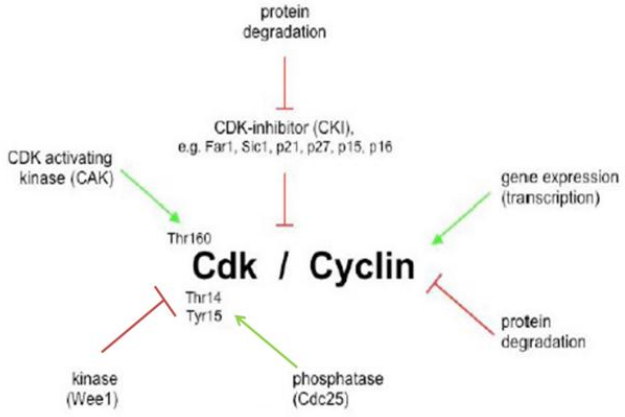


Figure 2: Regulation of CDKs. The numbers of the phosphorylated residues are taken from human Cdk2.

degradation, transcriptional programs and additional programs, such as regulated intracellular localization of CDKs or cyclin subunits

Budding Yeast Life Cycle

Budding yeast can proliferate in both a haploid and a diploid state. Cells of two different mating types (a and α) can mate to form a zygote. The mating reaction is induced by the binding of pheromones of the opposite mating type to receptors at the plasma membrane.

A haploid cell traversing G1 but did not yet pass **Start** has options:

- Initiate mating (if partner is present)
- Exit cell-cycle (absence of nutrient supplies) -> quiescence (G0)
- Enter the cell-cycle (if critical cell size reached -> DNA replication, bud formation, spindle pole duplication)

Cell-cycle entry is default, blocked by presence of pheromones and absence of nutrients. A diploid cell can:

- Enter cell-cycle
- Enter quiescence (in the absence of nutrients)
- Undergo meiosis (sporulation) if starved for nitrogen and carbon

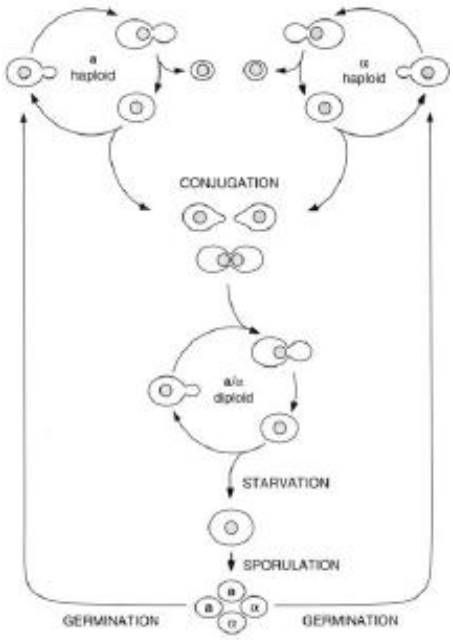


Figure 3: Life cycle of budding yeast

Transcriptional Programs

Introduction

Temporarily restricted transcription:

- In bacteriophages: Lytic cycles, early and late genes, modification of host RNA polymerases
- In eukaryotes: Specific transcription factors in a series of consecutive, interdependent waves

In budding yeast, up to 20% of all genes are suggested to be periodically expressed.

G1 Control in Budding Yeast

Regulation of Start:

- CDC28: Main CDK in *S. cerevisiae*
- CLN3: Cyclin, highly unstable, relatively constant transcription, slight peak late M-early G1 phase
- CLN1, CLN2: Cyclins, transcription highly cell-cycle regulated
- SWI4, SWI6, MBP1

- WHI5
- CLB1-6: B-type cyclins, overlapping functions, Clb5/6 -> onset of DNA replication; Clb3/4 -> spindle formation; Clb1/2 -> trigger anaphase (1/2 can replace 3-6, not vice versa)

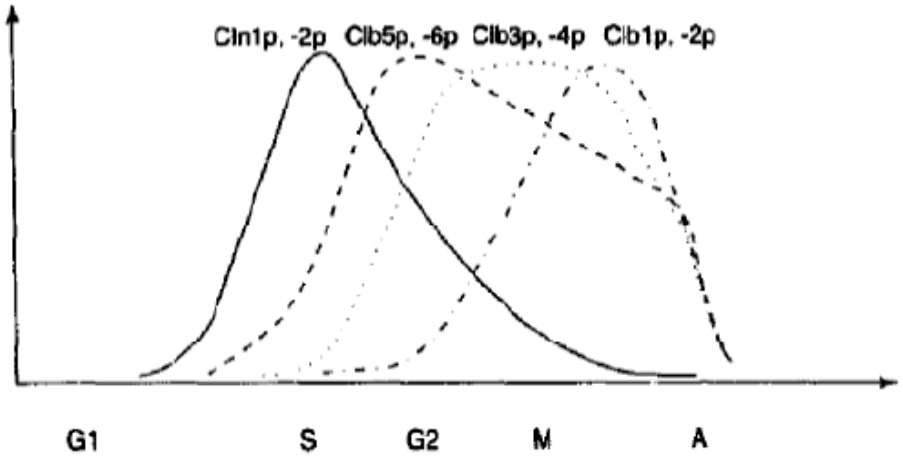


Figure 4: Transcriptional expression profiles of the budding yeast cyclins.

G1 cyclins are partially redundant; expression of any will allow passing Start. Phenotypes below provide some hints. Cells staying in G1 longer will become bigger (delay in progression through Start), strains are listed by increasing size:

- wildtype
- cln1Δ and cln2Δ
- cln3Δ and cln1Δ/cln2Δ
- cln1Δcln2Δcln3Δ is dead (arrested just prior to Start)

Cyclins are positive regulators of Start, with Cln3 being rate-limiting in progression through Start and acting upstream of Cln1 and Cln2. Triple mutant dead, Cln3 must have some additional function(s).

Experiments: Promoter of CLN1 (or CLN2) fused to lacZ to check expression levels (colorimetric assay).

Observations:

- No expression in cdc28 mutants
- Low expression in cln3Δ
- Slightly reduced expression in cln1Δ and cln2Δ mutants
- Low expression in cln1Δcln2Δ

-> Positive feedback loop, Cln1 and Cln2 induce their own expression, requiring the Cdc28-Cln3 and Cdc28-Cln1/2 complexes.

Two transcription factors were identified, where mutations resulted in the formation of bigger cells: swi6Δ (stronger phenotype) and swi4Δ. Swi6 is the transactivating component in two different heterodimeric transcription factors that differ in their DNA-binding component:

- Swi6/Swi4 (SBF)
- Swi6/Mbp1 (MBF)

Swi6 is phosphorylated in a Cdc28-dependent manner (but phosphomutants have normal size). Another factor still missing!

This factor x might be an activator of SBF/MBF needing phosphorylation by Cdc28 to be active, or x could be a repressor, becoming inactive upon Cdc28 phosphorylation. Mutants leading to a smaller size have been identified as possible candidates for a repressor.

Whi5 binds to and represses SBF and MBF and is phosphorylated by Cdc28-Cln:

- whi5Δ are smaller than wildtype cells
- Overexpression of Whi5 causes the formation of larger cells
- Mutation of the phosphorylation sites in Whi5 leads to the formation of larger cells

CDK-activity seems to repress a repressor, rather than to activate an activator -> general feature of the mechanisms that CDKs use to regulate cell-cycle events.

Model: Activation of Cdc28-Cln3 triggers expression of Cln1 and Cln2 after phosphorylation and inactivation of Whi5. Increased levels of Cdc28-Cln1/Cln2 accelerate phosphorylation of Whi5 -> positive feedback loop -> high levels of Cdc28-Cln activity -> progression through Start.

Cln1/2 feedback loop sharpens the onset but does not define the timing of Start progression, as the important trigger is Cln3. Regulation of Cdc28-Cln3 is still under investigation.

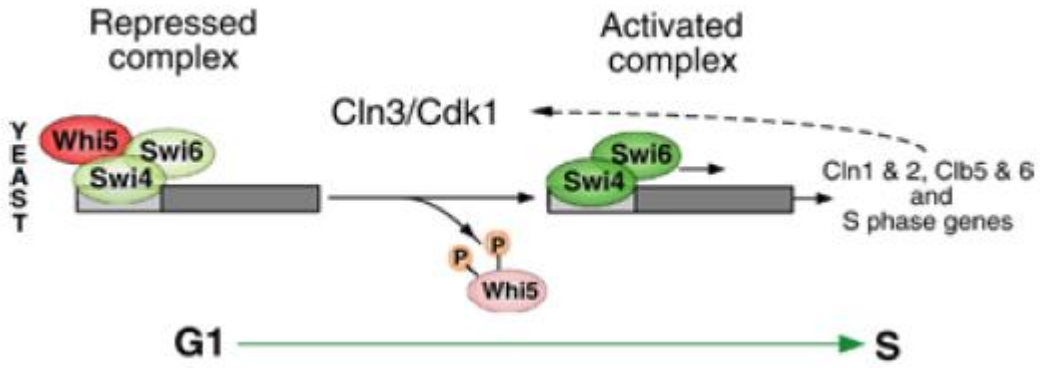


Figure 5: Regulation of G1-specific transcription by Cdc28-Cln in budding yeast.

Downregulation of G1 cyclins happens through Cdc28 activity associated with Clb1-6. Cdc28-Clb2 binds Swi4 and inactivates SBF, and the nuclear import of Swi6 is blocked by Cdc28-Clb-dependent phosphorylation. Cln1/2 mRNA and protein levels persist longer in clb1Δ and clb2Δ mutants.

G1 Control in Animal Cells

Animal counterpart of Whi5 is **Rb** (retinoblastoma protein), no sequence conservation, but the central feature such as the cyclin-based feedback loop triggered by the inactivation of the repressor is present.

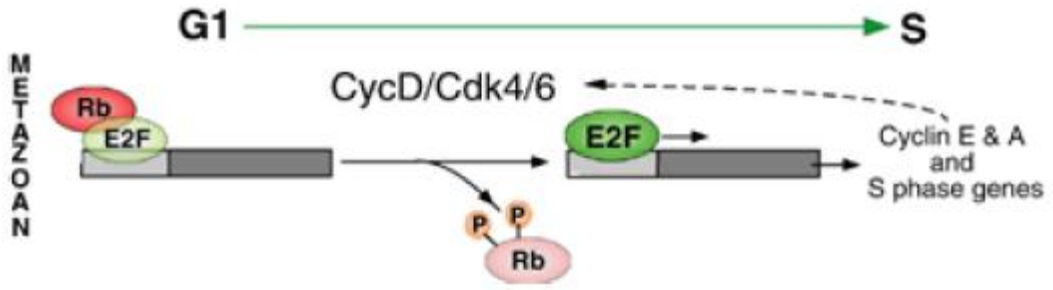
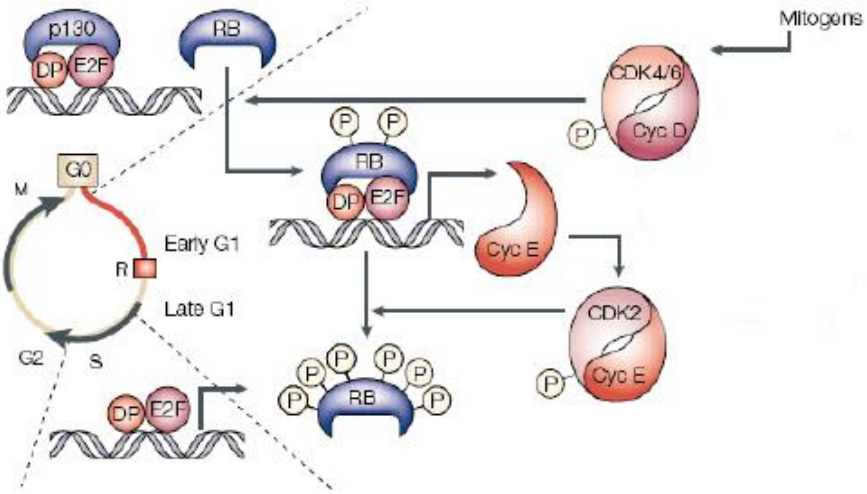


Figure 6: Regulation of G1-specific transcription by CDKs in animal cells.

Rb was originally identified because individuals inheriting a mutant copy are predisposed to develop childhood retinoblastoma (eye cancer). Rb has a tumor suppressor function as negative regulator of G1 progression.

In G0 cells, the heterodimeric transcription factor E2F/DP is bound to p130 (Rb homologue) -> repression of G1/S gene transcription. Entering the cell-cycle, p130 is replaced by Rb, maintaining E2F/DP inactivity. Ras and Map-kinase cascades increase cyclinD synthesis, formation of active Cdk4/6-cyclinD complexes and initial phosphorylation of Rb. Impaired repression leads to induction of some genes, e.g. cyclinE, that itself binds and activates Cdk2. Cdk2-cyclinE hyperphosphorylates and completely inactivates Rb. E2F/DP responsive genes are now fully expressed, e.g. cyclinE -> positive feedback loop.



- Rb = Whi5
- cyclinD = Cln3
- cyclinE = Cln1/2

Figure 7: Regulation of G1 and G1/S transition.

A Global Approach to Transcriptional Regulation

Budding yeast genome -> about 6'000 encoded genes. Using DNA microarrays (mRNA -> cDNA used for hybridization to chip), a catalog of genes whose expression vary periodically within the cell cycle can be generated.

Synchronized vs. asynchronized cells. Synchronization as follows:

- Shift of temperature-sensitive cdc mutants to 37° (restrictive temperature) before releasing them at 25° (permissive temperature); arrest of mutants at a specific time point, e.g. $cdc15^{ts}$ (mitotic exit defect) or $cdc28^{ts}$ (arrested prior to Start)
- Transient exposure of MATa cells to the α-factor pheromone -> arrest before Start
- Elutriation, centrifugation of exponentially growing cells, fractionated by size, only take small unbudded cells

cluster	co-expressed genes	transcription factors
G1	**G1 progression:** CLN1, CLN2 **DNA replication:** CLB5, CLB6, RNR1 (ribonucleotide reductase), POL12 & POL30 (DNA polymerases), CDC45 (initiation of DNA replication), CDC9 (DNA ligase) **Expression of mitotic cyclins:** NDD1 (transcription factor for CLB1,2, requires Cdc28-Clb dependent phosphorylation for activity, degraded during mitosis)	SBF & MBF
S	9 histone genes	SBF & MBF (plus additional factors)
M	**Regulation of mitosis:** CLB1, CLB2, CDC20 (specificity factor of APC/c), SWI5 & ACE2 (transcription factors for SIC1)	Ndd1 together with Mcm1 and Fkh1 or Fkh2
M/G1	**G1 progression:** Sic1 (inhibitor of Cdc28-Clb), 6 MCM genes (establishment of replication-competent origins), CLN3, SWI4 **Mating reaction in response to pheromone:** FAR1	Swi5 & Ace2 (for SIC1)

Figure 8: Summary of some relevant clusters, selected co-expressed genes and the relevant transcription factors.

About 800 out of the 6'000 genes were shown to be periodically expressed. Clustering genes -> find out common transcription factors (same binding sites), mostly more than one.

General Features of Transcriptional Control

- Most clusters contain genes specialized for cell-cycle stage specific functions
- Transcription factors mostly are periodically expressed, often activate genes that are part of the next transcriptional wave and promote the subsequent cell-cycle stage (Swi4 and Ndd1)
- Genes expressed during the later wave can repress genes from the earlier wave (Cdc28-Clb inactivates SBF, MBF terminating Cln1/2 expression)
- Positive feedback loops amplify expression of key regulators -> sharp transitions
- A cluster can prepare several consecutive steps, but delay the later ones as long as required

- Cluster can prepare its own inactivation by promoting the expression of CKIs
- Same cluster can contain genes with opposing functions, keeping options open

A Simplified Model

In this basic transcriptional oscillator, SBF and MBF indirectly activate Clb2. Once activated, Clb2 represses the transcription factors. With SBF and MBF off, Clb2 becomes inactivated, allowing SBF and MBF to recover.

This is an oscillating system with two different states, one with high CDK-activity and one with low CDK-activity. The switch is mainly triggered by the activation of Cdc28-Cln3 after a critical cell size is reached.

Other mechanisms making a contribution are protein degradation and the role of the phosphatase Cdc14.

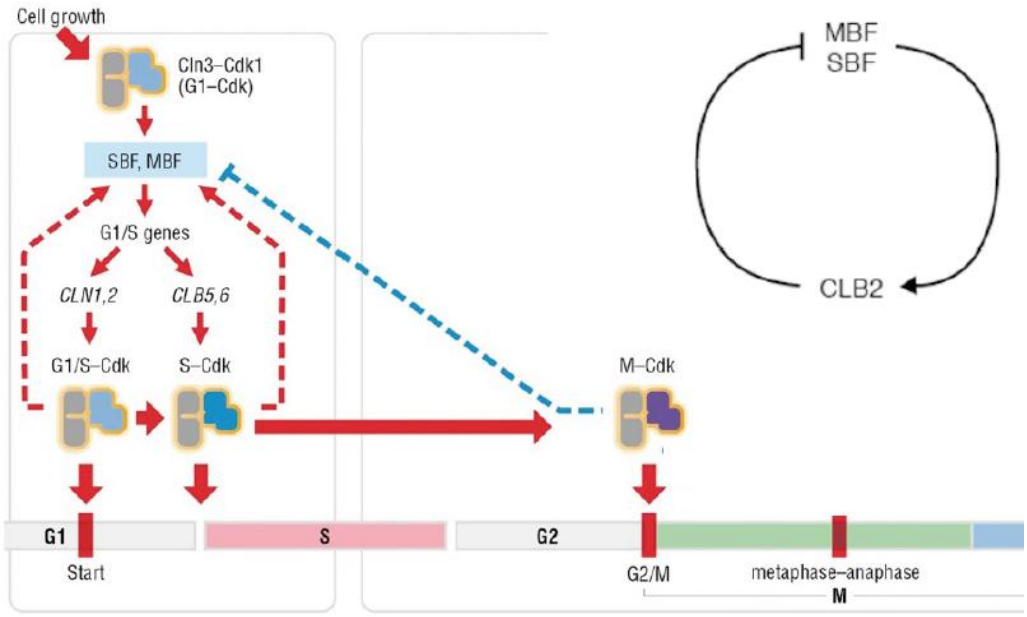


Figure 10: Summary of the cell cycle progression through Start.

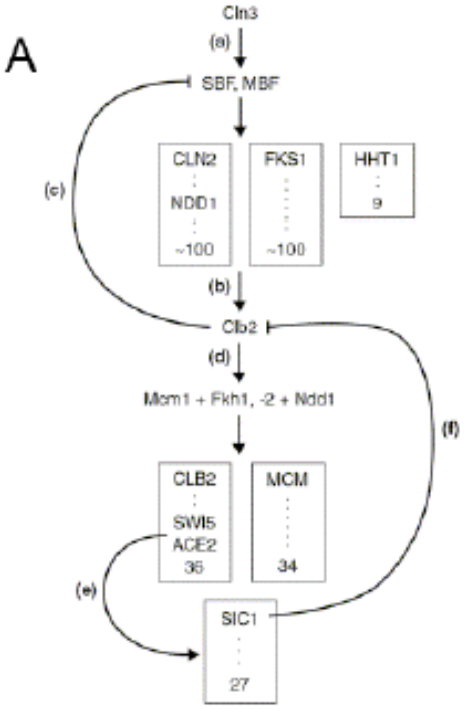


Figure 9: Cell cycle oscillator.

Regulated Protein Degradation

Introduction

Sea urchin embryos: Proteins with a high cell-cycle-correlated periodicity, steadily produced coupled to specific proteolysis -> **regulated protein degradation** important for cell-cycle control.

Targeting of proteins for ubiquitination:

- Activation of the ubiquitin ligase (e.g. APC/c, through signaling pathways intrinsic to cell-cycle, complete and coordinated degradation of target proteins)
- Substrate activation (selectivity dependent on the regulatory context, allows switch-like degradation (Sic1 multiphosphorylation) as well as for selective degradation (constitutively active ligase is SCF)

Regulated degradation and periodic transcription ensure the match of gene expression and gene function. Proteolysis seems to directly control the switches between distinct cell cycle stages whereas transcriptional regulation and the accumulation of key molecules is slower and important for sharpen the transition and help maintaining the stages.

Regulation of CDK-Activity

Central players of the regulation of CDK are Cdc28-Clb complexes and their inhibitor Sic1.

- Low Cdc28-Clb activity (G1) -> spindle disassembly, cytokinesis, prereplicative complex (preRCs) assembly, bud formation initiation
- High Cdc28-Clb activity (S/M) -> DNA replication, spindle pole body (SPBs) duplication, spindle assembly, chromosome segregation

Degradation of Sic1 leads to the activation of Cdc28-Clb whereas degradation of the Clbs results in the inactivation of the CDK complexes.

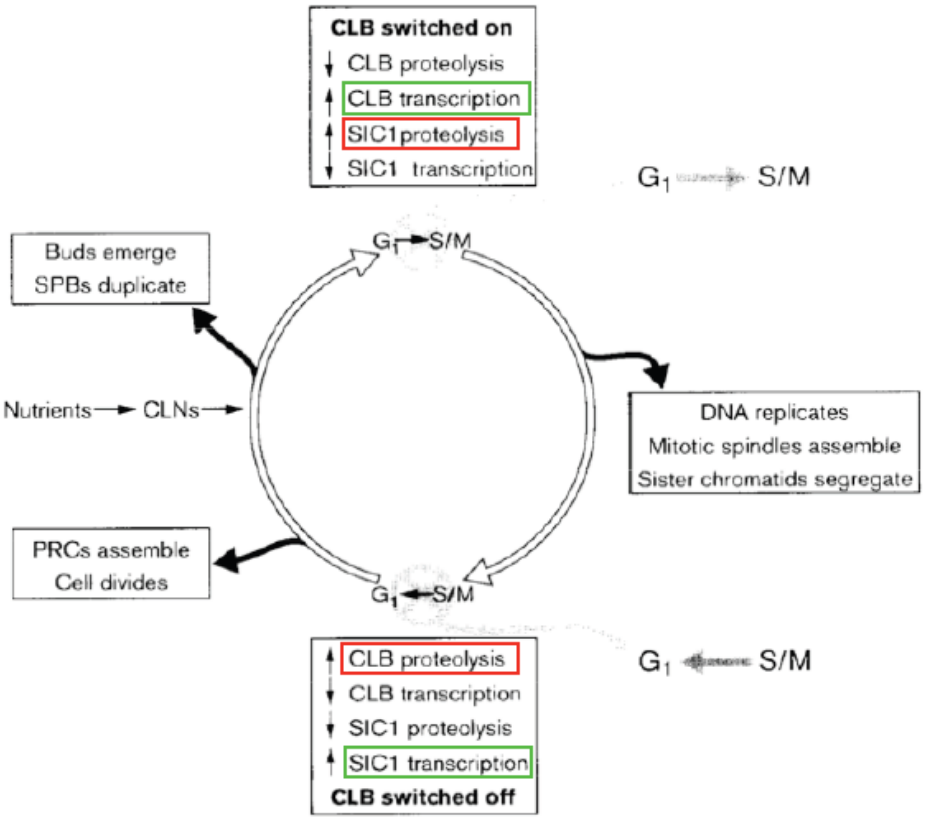


Figure 11: Oscillations between low (G1) and high (S/M) Cdc28-Clb activity are sustained by periodic changes in the activities of proteolytic pathways and transcription factors regulating Clb and Sic1 abundance.

Ubiquitin-Dependent Degradation

Ubiquitin, 76 amino acids, bond between its C-terminus and an amino-group of a lysine residue from the substrate -> requires three enzymes:

- E1: Activating enzyme that forms a thiol ester with the carboxyl group of G76
- E2: Conjugating enzyme that carries the activated ubiquitin molecule as a thiol ester
- E3: Ligase, transfers ubiquitin from E2 to a lysine residue of a substrate
- Polyubiquitin chains (with K48 linkages) -> degradation; monoubiquitin -> trafficking to lysosome

E1 and E2 -> limited number, but huge diversity in E3 ligases (several hundreds). Important for cell cycle:

- E3 ligase SCF: Regulates G1/S progression
- E3 ligase APC/c: Controls mitosis, more precisely the onset of anaphase and mitotic exit

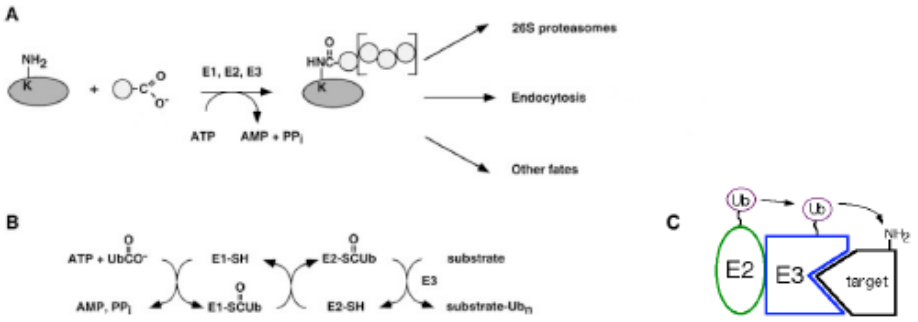


Figure 12: Overview on ubiquitin conjugation, (A) dark grey is substrate, light gray ubiquitin; (B)enzymatic pathway; (C) E3 ligase -> specificity, binds target protein.

SCF and the G1/S Transition

SCF E3 ligase -> several functional subunits, substrate-specific adaptors -> accuracy:

- Cullin1: Structural scaffold, encoded by CDC53
- Skp1: Binds both cullin via its BTB domain and the F-box domain of the substrate specific adaptor
- F-box protein: Adaptor, binding specifically to the substrate
- E2: Brings ubiquitin to ligase, encoded by CDC34
- Ring-finger: Binds and activates E2, encoded by RBX1

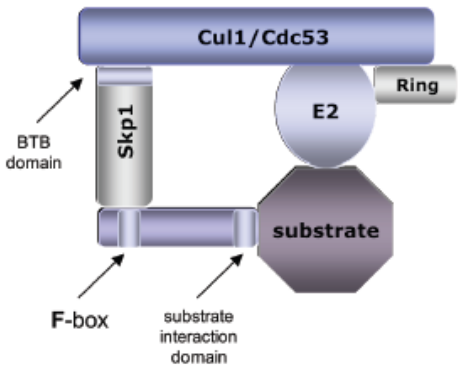


Figure 13: SCF E3 ligase build-up.

SCF ligases are constitutively active. The regulation happens at the level of the substrates (need to be phosphorylated (or modified in some way) to be recognized by the adaptor).

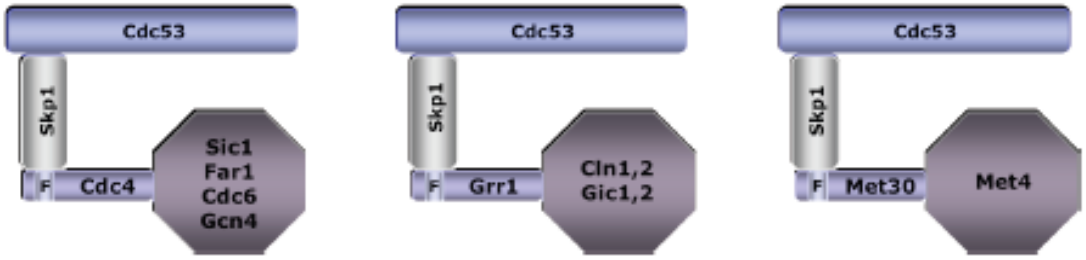


Figure 14: F-box proteins Cdc4, Grr1 and Met30 regulate target proteins, Met4 is a transcriptional activator involved in the regulation of the sulfur amino acid pathway, Gic1/2 are involved in the establishment of cell polarity, Grr1 is required for induction of glucose and amino acid regulated genes, Gcn4 is a transcriptional activator of amino acid biosynthetic genes in response to amino acid starvation.

CDC4 and CDC34 are essential genes. Temperature-sensitive mutants arrest in late G1 at 37° and fail to initiate S-phase. This is caused by the failure to degrade Sic1, and accumulation of Sic1 blocks Cdc28-Clb activity, thereby preventing DNA replication. Multisite phosphorylation of Sic1 normally leads to a very abrupt degradation of the protein. Importantly, the accumulation of the Cdc28-Cln complexes promotes the accumulation of Clbs, but the activation of Cdc28-Clb is only triggered by the degradation of Sic1. G1 cyclins are required for the transition from low to high CDK activity, but not to maintain it. cln1Δcln2Δcln3Δ dead -> plus sic1Δ -> viable! Essential function of cyclins -> promote Sic1 degradation.

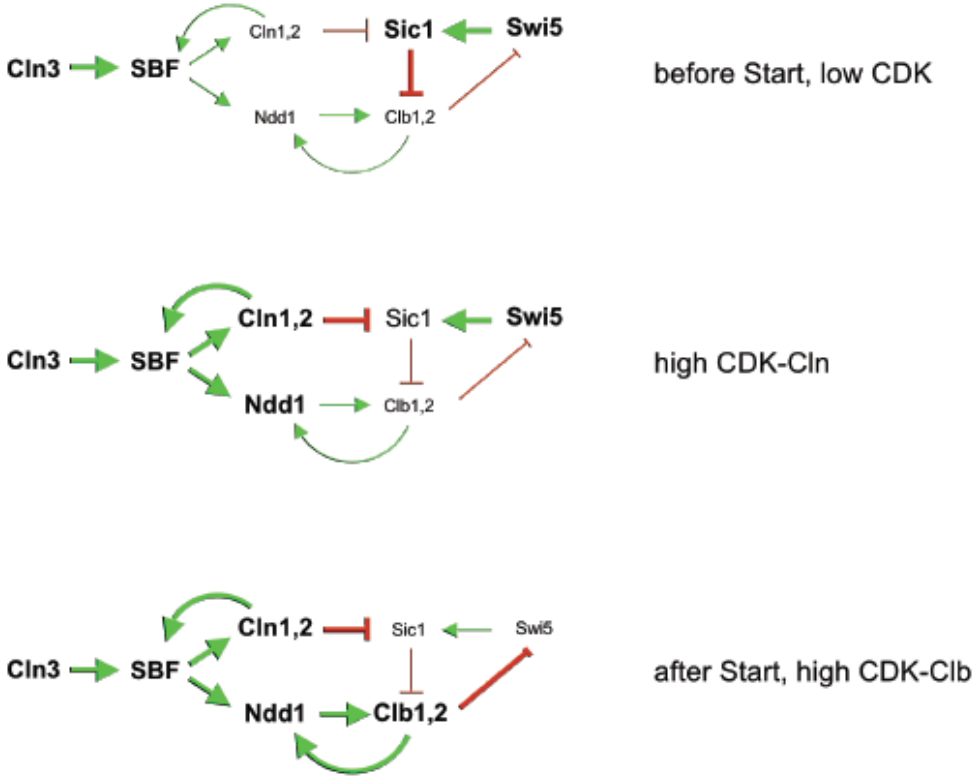


Figure 15: Progression through Start is triggered by the activation of Cdc28-Cln3, followed by increases in Cdc28-Cln1/2 levels and activity and degradation of Sic1. The removal of the CKI leads to the activation of Cdc28-Clb5/6 and therefore to the initiation of DNA synthesis.

The CKI **Far1** is periodically expressed, peaking in early G1 and only inhibiting Cdc28-Cln kinases. Phosphorylation after exposure to pheromones via a G-protein triggered MAP-kinase pathway activates Far1 and leads to the cell cycle arrest during mating. **Ste12**, a transcription factor, is required for regulation of Far1. The Cdc28-Cln-dependent phosphorylation and subsequent degradation of Far1 is required in each G1 phase to allow the accumulation of Cdc28-Cln activity.

The cyclins of the Cdc28-Cln1/2 complexes are phosphorylated and uniquitinated and degraded via SCF^{Grr1}. GRR1 is not essential and the mutant cells form elongated buds, as the accumulation of Cln1/2 leads to cell polarization.

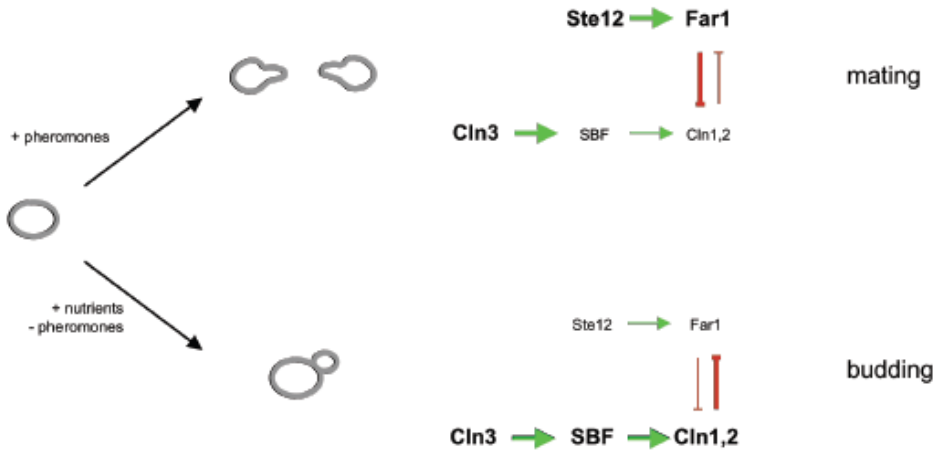


Figure 16: Exposure to pheromones -> induce Far1, block Cdc28-Cln activation. Formation of mating projections, fusion and zygote formation follow. Without pheromones (and enough nutrients), cells pass Start and commit a new cell cycle.

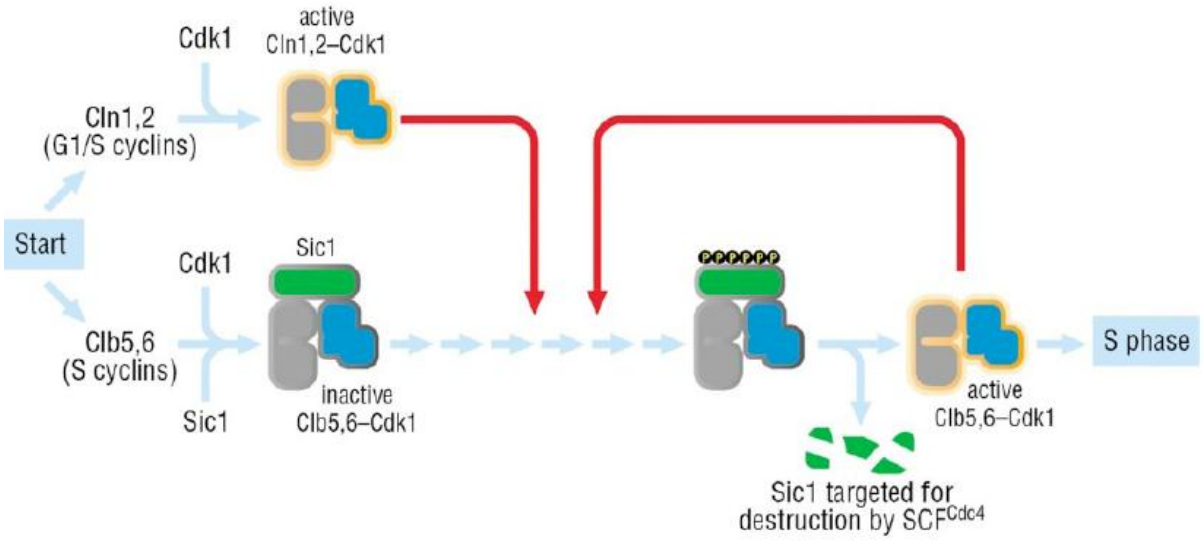


Figure 17: Summary.

APC and the Regulation of Mitosis

APC/c, the anaphase promoting complex, is composed of at least a dozen different subunits, e.g. both a cullin and a ring-finger domain protein (relative to SCF). It can only ubiquitinate substrates with the help of a co-activator protein (directly binding the substrate). Substrates are recognized because they contain a **destruction box** signal (D-box or KEN-box motifs). **Cdc20** and **Cdh1** are the best known co-activators. APC/c major functions are:

- Inactivation of the mitotic kinases at the end of mitosis (degradation of activating subunits or direct degradation of kinase)
- Regulation of anaphase onset

The activity of APC/c is tightly regulated during the cell cycle, mainly through phosphorylation by Cdks. Substrates don't need a modification and APC/c activity is controlled at the level of interaction with the co-activations Cdc20 and Cdh1.

- Cdc20 only interacts with APC/c if several subunits have been phosphorylated by CDKs during early mitosis. APC/c^{Cdc20} is inactivated by dephosphorylation of the relevant subunits at mitotic exit and by degradation of Cdc20 via APC/c^{Cdh1}.
- Cdh1 is prevented from interacting with APC/c until later stages of mitosis, as long as it is itself phosphorylated by the mitotic CDKs. The partial degradation of the mitotic cyclins by APC/c^{Cdc20}, together with the activation of the phosphatase Cdc14 leads to a reduction of Cdh1 phosphorylation. APC/c^{Cdh1} becomes active, and maintains the mitotic cyclins instable throughout G1. At the G1/S transition, phosphorylation of Cdh1 by Cdc28-Cln returns APC/c to its inactive state.

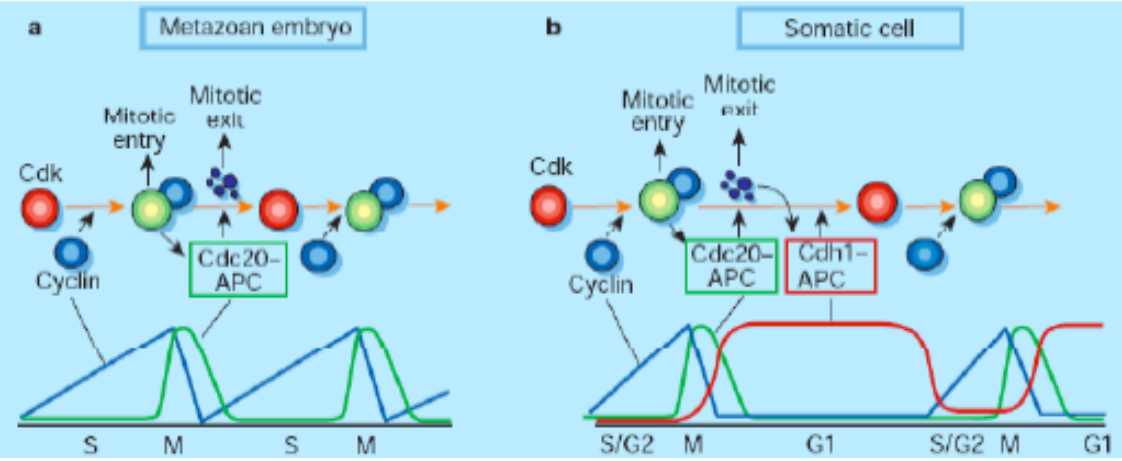


Figure 18: (a) In embryonic cells (alternate between S and M), cyclin-dependent activation of Cdk not only leads to mitotic entry but also promotes activation of APC^{Cdc20}, which then triggers cyclin destruction and mitotic exit. The loss of Cdk activity causes APC^{Cdc20} inactivation, and cyclin can accumulate again. (b) Somatic cells have an additional G1 phase. Cdk inactivation in late mitosis results in the activation of APC^{Cdh1}, which maintains cyclin destruction and Cdk inactivity throughout G1. Inactive CDK is shown in red, while active CDK complexed with cyclin is shown in green.

APC/c^{Cdc20} is important to degrade a first fraction of the mitotic cyclins (Clb1-6) and the anaphase inhibitor Securin (Pds1 in yeast), while APC/c^{Cdh1} takes over to complete the degradation of the mitotic cyclins, and maintains their levels low during G1.

Conclusions:

- CDKs directly phosphorylate Sic1 and promote its ubiquitin-dependent degradation
- Phosphorylation of Cdh1 ensures that APC^{Cdh1} remains inactive and does not degrade Clbs
- The switch regulating high or low Cdk activity, Cdc28-Cln, is not an APC substrate and not inhibited by Sic1

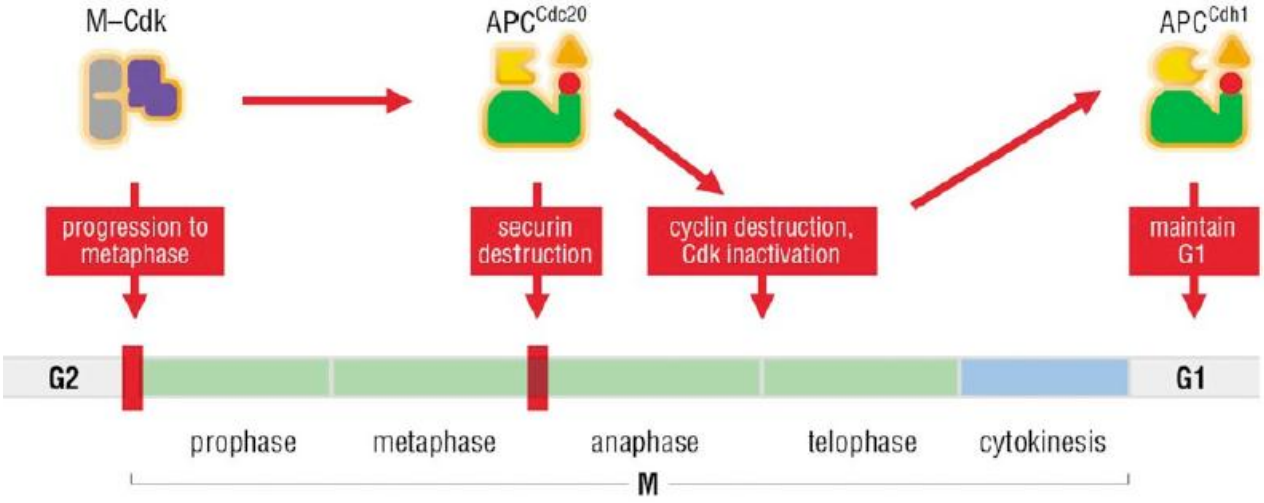


Figure 20: APC summary.

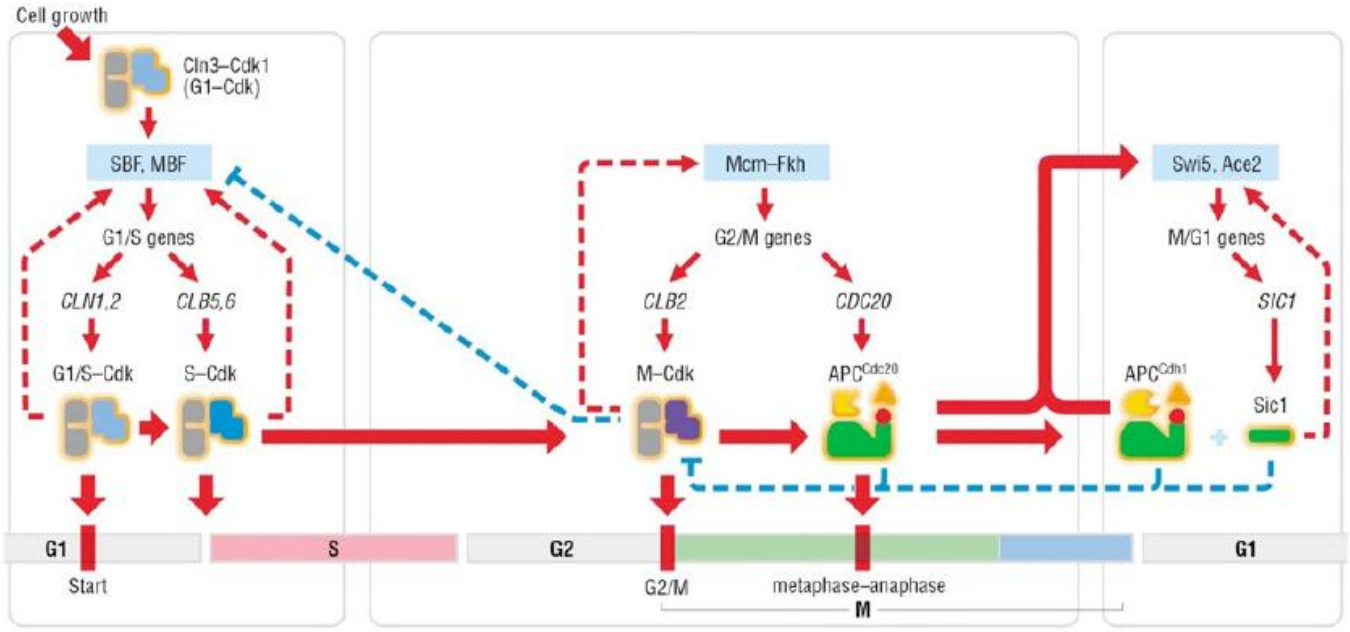


Figure 19: Cell cycle control system in budding yeast, summary.

How to Replicate Once and Only Once

Introduction

Cell fusion experiments:

- Fusing interphase cells (G1, S or G2) to mitotic cells -> initiation of mitosis in interphase cells, so mitosis is dominant over the other cell cycle stages, and the mitosis promoting factor (MPF) is composed of Cdk1-cyclinB (or Cdc28-Clb in budding yeast)
- Fusing G1 or G2 cells to S phase cells -> G1 immediately began replicating DNA, whereas G2 nuclei did not initiate a new round of DNA synthesis, so an activator of DNA replication is present in S-phase cells and G2 cells have a block to replication

Each origin of replication can exist in two different states:

- Pre-replicative complex (preRC) renders an origin competent for firing
- Actual activation of the origin during S-phase leads to the subsequent disassembly of the preRC

Replication Origins

Bacteria have a single, circular chromosome containing one origin of replication (ORI). Premature re-replication is prevented by several systems, including the methylation status of the ORI and regulation of the replication initiator DnaA.

Eukaryotes have linear chromosomes containing many origins of replication (about every 40-150 kb, total of 330 origins in yeast and more than 10'000 in animals). During S-phase, only a subset of the origins actually fires. Two problems arising from the large number of origins:

- Firing must be temporally and spatially coordinated (every chromosome completely duplicated)
- Avoid re-replication (replication forks have to inactivate not fired origins when passing them)

Budding yeast replication origins: **Autonomously replicating sequences (ARS)**, about 300bp long, containing an 11 bp core consensus sequence. Origins of other eukaryotes are much more complicated and do not share a consensus sequence.

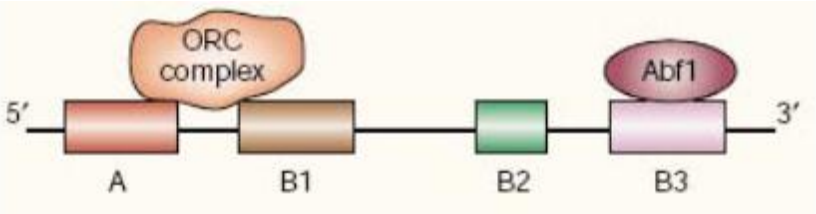


Figure 21: ARS, origin recognition complex binds to domain A containing the consensus sequence (A/TTTTATA/GTTTA/T) and to the B1 domain. Domain B2 contains an easily unwound sequence, whereas domain B3 is a binding site for the transcription factor Abf1, which appears to facilitate DNA replication.

By knowing the core consensus sequence, the **origin recognition complex (ORC)** binding it could be isolated. It is the eukaryotic replication initiator, which is used as a platform to assemble the replication complex.

The Pre-Replicative Complex (preRC)

Eukaryotic origins of replication direct the formation of the **pre-replicative complex (preRC)**, a large protein complex. It is responsible for the assembly of two bidirectional DNA replication forks. Formation of the preRC involves the assembly of a number of replication factors:

- **ORC**: Origin-recognition complex, six subunits, selects the sites for subsequent initiation of DNA replication by binding to the replication origin in an ATP-dependent manner. Yeast: ORC subunits constitutively bound to origins. Animal cells: Association of subunits is cell-cycle regulated.
- **Cdc6**: AAA ATPase, highly related to Orc1, assembles into the preRC after ORC, required to load the Mcm proteins onto DNA. Budding yeast: Abundance of Cdc6 is cell cycle regulated (onset of S phase, phosphorylated by Cdc28-Clb5/6 and degraded by SCF^{Cdc4}). Animal cells: Phosphorylation of Cdc6 promotes its export from the nucleus, dependent on exportin Crm1.
- **Cdt1**: Involved in recruitment of the Mcm complex, precise function unclear. Cdt1 is exported to the cytoplasm in a CDK-dependent manner during S, G2 and M phase. In animal cells, Cdt1 is the target of the replication inhibitor Geminin.
- **Mcm complex** (Mcm2-7p): Six subunits, Mcm2-7, all contain AAA ATPase domain, forming a ring enclosing a single strand of DNA. Open and closed configuration, ATPase activity required for the loading of the ssDNA into the ring. Assembly needs coordinated function of ORC, Cdc6 and Cdt1.

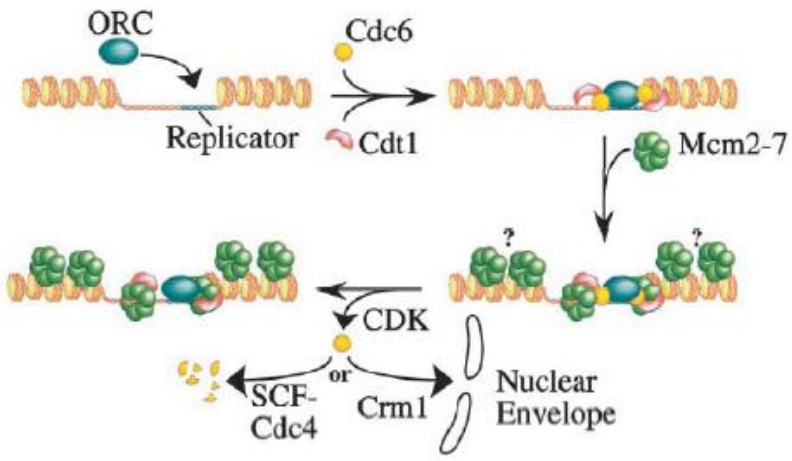


Figure 22: Model for preRC formation.

The Mcm complex seems to play a role also during the elongation (not only initiation) step of DNA replication (independent of ORC, Cdc6 and Cdt1), probably functioning as DNA helicase. Budding yeast: The intracellular localization of Mcm is tightly regulated during the cell cycle, nuclear during G1 and S, and exported during G2 and M phase.

Animal cells: Mcm constantly in the nucleus, but association with the chromatin progressively weakens after cells have proceeded through S-phase.

Regulation of preRC Formation

- High levels of CDK-activity are required to activate the origins of replication as cells enter S phase
- High levels of CDK-activity are required to prevent re-initiation from origins during S, G2 and M

PreRC formation is prevented by **CDK-activity** in at least four different, redundant ways (which makes it clear how important prevention of re-replication is):

1. Phosphorylates and inactivates Cdc6 (either by degradation in yeast or by promoting export to the cytoplasm in animals); Cdc6 phosphorylation can be catalyzed by Cdc28-Cln; since actual activation of origins depends on Cdc28-Clb activity, this mechanism ensures that the ability to make new preRCs is lost before the origins can fire
2. CDKs phosphorylate members of the Mcm complex, disfavoring the incorporation of it into new preRCs (nuclear localization or chromatin association changed, not known yet)
3. CDKs downregulate ORC-activity
4. CDKs promote nuclear export of Cdt1 in budding yeast

In animals, **Geminin** has also been shown to inhibit preRC formation. It appears to function independent of CDK, ORC or Cdc6. It is thought to block Cdt1 activity and thereby inhibiting the recruitment of Mcm onto DNA. Geminin accumulates during S-phase, remains abundant during G2 and is degraded in mitosis.

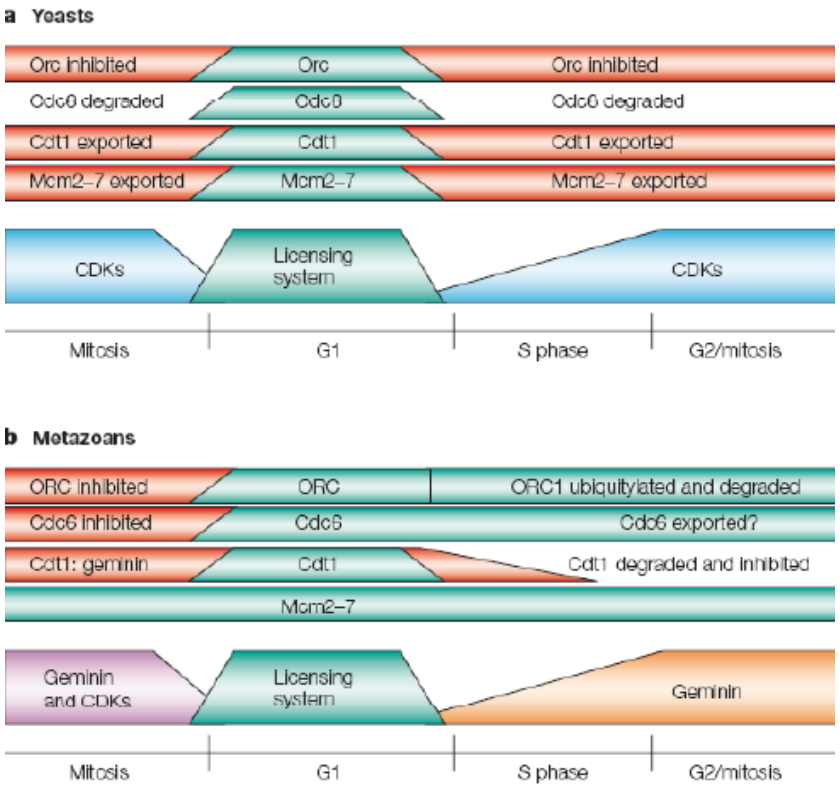


Figure 23: Cell cycle regulation of the licensing system.

Initiation of DNA Replication

The preRCs mark potential sites for DNA replication. Initiation of DNA synthesis depends on the recruitment of factors, unwinding of the DNA and loading of replication fork components. Important kinases:

- CDK (Cdc28-Clb5/6 in budding yeast)
- DDK (Cdc7 and its regulatory subunit Dbf4)

Cdc28-Clb5/6 can directly bind to Cdc6 and thereby be recruited to the replication origins. The direct CDK targets are not clear (neither how they contribute to the initiation of DNA replication). Importantly, Mcm proteins are displaced from origins as DNA replication is initiated (probably travelling ahead of replication fork). Combined degradation/inactivation of Cdc6 and Cdt1 and displacement of Mcm proteins ensure that used origins have to be reactivated by reformation of the preRC.

Closing the Circle

Alternation mechanism ensures that replication only takes place once per cycle by the oscillations of CDK-activity. At one state, the origin of replication is competent for the recruitment of initiation factors but not for initiation and at the other state, the origin is competent for initiation but not for recruitment of initiation factors.

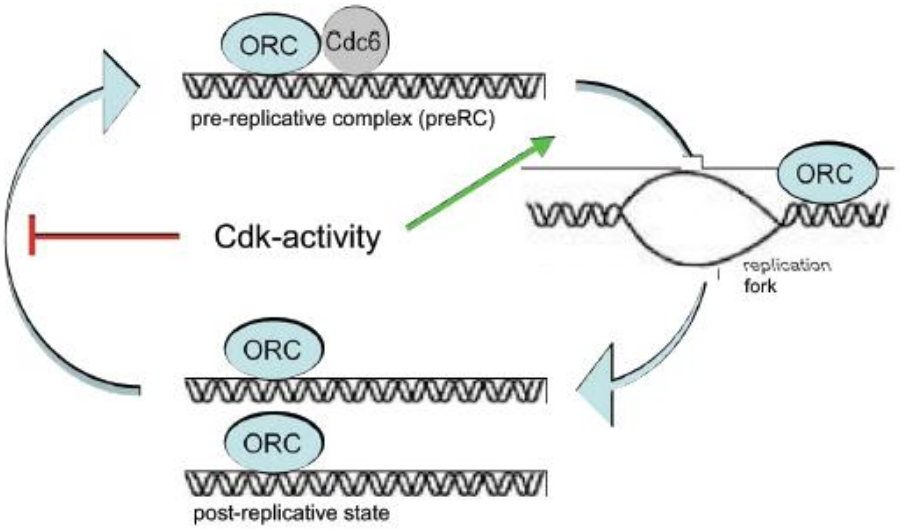


Figure 24: Simplified diagram showing the dual role of CDK-activity during the DNA-cycle.

Low CDK-activity permits the assembly of preRC complexes by allowing Cdc6 to accumulate in the nucleus. At the same time, mitosis cannot occur in the absence of high CDK-activity. In reverse, high CDK-activity is required for the initiation of DNA replication at the beginning of S phase, but at the same time blocks the assembly of preRCs and thereby inhibits origin refiring. The presence of high CDK-activity then triggers mitosis.

For mitosis to be completed, the mitotic CDKs have to be inactivated. By consequence, preRC formation and S phase can only occur after mitosis has been completed. Due to the presence of **DNA replication**

and **DNA damage checkpoints**, mitosis does not start before S phase. These surveillance mechanisms block the onset of mitosis as long as replication is not completed or DNA damage has been detected.

Mitosis - Bipolar Attachment and the Metaphase to Anaphase Transition

Introduction

How ensure, that the next event is only initiated once the previous is successfully completed? In embryonic cells, the relative timing of the cell cycle engine solves the problem, as reactivation of Cdk1-CyclinB and mitotic entry take longer than completion of DNA replication. In these cells, checkpoints are absent (fast and synchronous divisions in embryonic cells). In somatic cells, this is not enough and further checkpoints and mechanisms are needed.

When fusing G1 to S phase cells, they did not enter mitosis until the G1 nuclei has finished DNA replication. This suggests the presence of a feedback control arresting the cell cycle at the G2/M transition. Order can be established by two ways:

1. Coupling of events so that a later step is only carried out when the previous has occurred
2. Surveillance mechanisms, checkpoints

Checkpoints sense whether an event has been completed correctly, and if not, cell cycle progression is halted until the stage is properly completed. They are typically not essential for progression per se and are generally only revealed after a perturbation of specific events. Good evidence for the presence of a checkpoint is provided in cases where a chemical, a mutant or other conditions releave the dependency of the later event on the completion of the earlier one.

Completion and correct order of the following are needed during mitosis:

- Bipolar attachment (spindle assembly checkpoint)
- Anaphase onset (spindle assembly checkpoint)
- Exit from Mitosis
- Physical separation of the two daughter cells during cytokinesis

Other mechanisms include:

- DNA replication checkpoint in G2/M
- DNA damage checkpoint in G1/S, S and G2/M
- Spindle orientation checkpoint in M
- Chromosome segregation checkpoint in late M
- Morphogenesis checkpoint G2/M

Bipolar Attachment

Sister DNA molecules must be disentangled and then segregated to opposite poles of the cell to ensure successful proliferation (and avoiding aneuploidy and pathologies such as cancer). Mitosis can be subdivided into several events:

- Entry into mitosis
- Prophase (chromosome condensation, nuclear envelope (NE) breakdown, spindle assembly)
- Metaphase (bipolar chromosome attachment, chromosomes to metaphase plate)
- Anaphase (chromosome migration to poles (anaphase A), spindle elongation (anaphase B))
- Exit from mitosis
- Cytokinesis (cell separation, chromosome decondensation, NE reassembly, interphase microtubule array reformation)

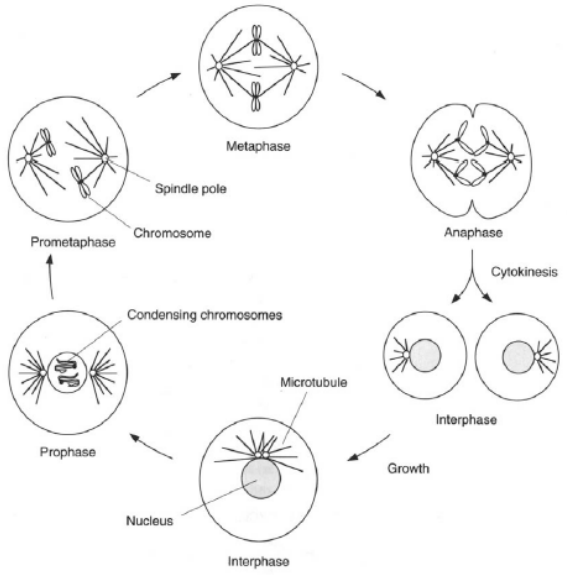


Figure 25: Stages of mitosis.

Key steps: **Bipolar attachment** of the sister chromatids and the **onset of anaphase**. Each sister kinetochore must attach to microtubules which extend to opposite poles of the spindle, bipolar chromosome attachment, chromosome biorientation or amphitelic attachment. **Kinetochores** are proteins that assemble on the centromeric DNA. They are composed of two regions, the inner kinetochore is closely associated with the DNA and the outer kinetochore

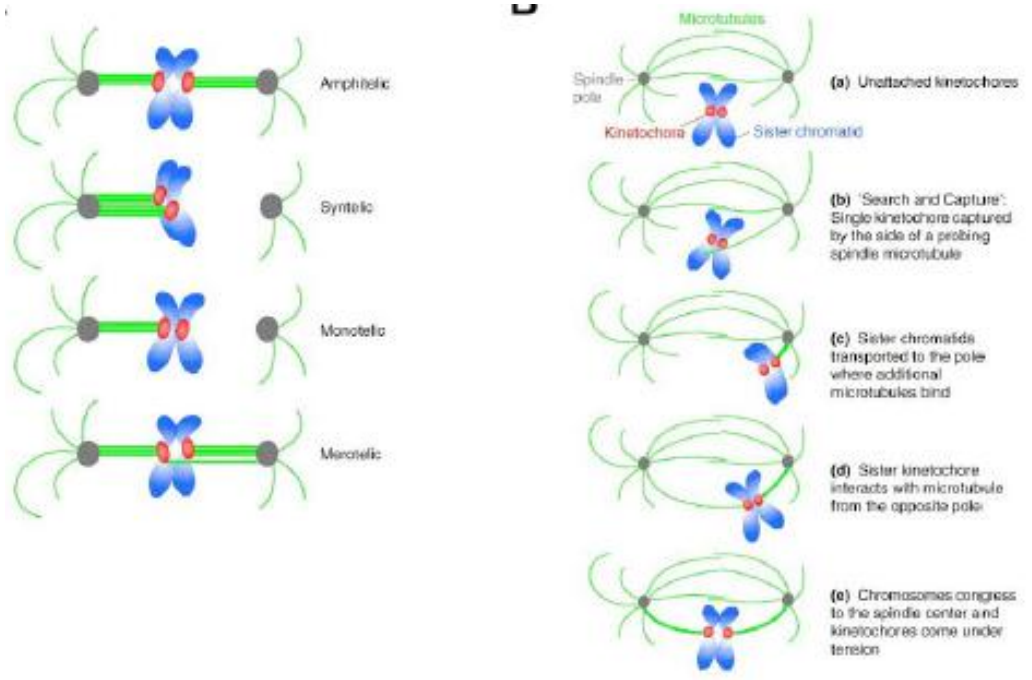


Figure 26: Mechanisms of chromosome attachment. (A) Kinetochore-microtubule arrangements, syntelic and monotelic attachments will lead to missegregation. (B) Dynamic instability allows microtubules to search for kinetochore attachments (captured kinetochore microtubules are stabilized).

interacts with the plus ends of microtubules. Animal cells: Several kinetochore microtubules per chromosome. Yeast: Only one microtubule binding site. The process of correct bipolar orientation can be explained by the **search and capture model**.

In yeast, no NE breakdown takes place. Microtubules are nucleated by the **spindle pole bodies (SPB)** that are embedded in the nuclear envelope. Nucleoplasmic face generates nuclear microtubules (spindle) and cytoplasmic face nucleates cytoplasmic microtubules (function during nuclear and spindle positioning).

During late G1 and early S, the SPBs duplicate (semi-conservative) and separate to form a bipolar spindle. Bud inherits the old SPB (in 90% of the cases) shown by an experiment using dsRed bound to Spc42. dsRed needs several hours to be fluorescent. Yeast cells were forced into stationary phase for four hours, so one single SPB acquired red fluorescence. After re-entry of G1, SPB duplication was completed and the new SPB will not be red (not enough time) before budding takes place.

During G1, chromosomes are attached to SPBs (both kinetochores), which means that for bipolar attachment, these interactions have to be broken. **AuroraB** (Ipl1 in budding yeast), a protein kinase, has been shown to be essential for establishment of chromosome biorientation. AuroraB forms together with **INCENP** (inner centromere protein, Sli15 in budding yeast) and **Survivin** (Bir1 in budding yeast) the **chromosome passenger complex**. The proteins uniformly localize to the chromatin in prophase, to the kinetochore in metaphase and relocalize to the spindle midzone during anaphase. Temperature-sensitive ipl1 mutants:

- Permissive temperature -> Mother but not daughter cell lose chromosomes during mitosis
- Restrictive temperature -> Ipl1 fully inactive, mother cells contain less DNA than daughter cells (both sister chromatids segregate to the daughter cell)

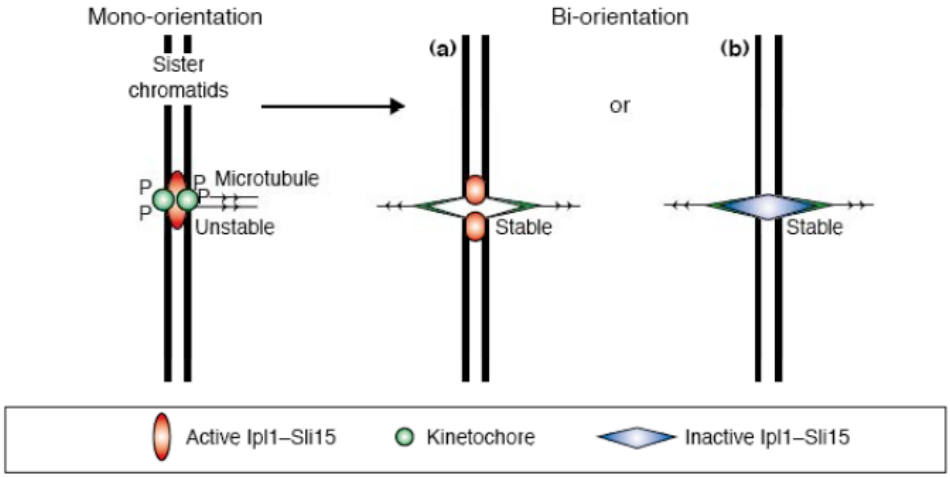


Figure 27: Model for re-orientation of kinetochore-spindle pole connections. Ipl1-dependent phosphorylation of kinetochore components renders kinetochore-microtubule attachments prone to re-orient. Only when a chromosome is bioriented are the kinetochore components dephosphorylated, owing to (a) kinetochore delocalization from Ipl1-Sli15 or (b) Ipl1-Sli15 inactivation by chromatin stretching around centromeres. The dephorphorylation of the kinetochore components makes biorientation stable. P=phosphorylation.

Model: AuroraB is required to detach incorrect kinetochore-microtubule interactions. Incorrect attachments have to be destabilized by AuroraB until correct biorientation is achieved. Overexpression of AuroraB prevents chromosome attachment by constantly destabilizing kinetochore-microtubule interactions. Upon bipolar attachment, the kinetochore-microtubule interaction is stabilized (probably through inactivation of the destabilizing activity of AuroraB).

Two current models, where AuroraB becomes inactive as soon as tension is exerted on sister kinetochores:

- Inhibition once kinetochores are under tension
- Kinetochore proteins (phosphorylated by AuroraB) pulled away from the chromatin-associated kinase as a result of the tension, involving CENP-A (also required for biorientation)

The Metaphase-Anaphase Transition

Metaphase spindle: Equilibrium of forces generated by motors pushing apart the antiparallel microtubules found in the spindle midzone and counteracting forces exerted by the bioriented, tightly bound sister chromatids. Cohesion between sister chromatids is generated during the replication process. Cohesion is also fundamental for regulation of anaphase onset. **Destruction of cohesion** at the metaphase/anaphase transition is sufficient to **trigger segregation** of sister chromatids to opposite poles.

Cohesion extends along the complete chromosomes and is mediated by the multisubunit complex **cohesin**. Cohesin is composed of a heterodimer of the SMC **Smc1** and **Smc3**, and **Scc1**. The proteins form a closed ring, and a fourth protein, **Scc3**, is bound to Scc1 but does not make direct contact with the Smc1/3 heterodimer. SMC proteins bind ATP, and the binding and/or hydrolysis are thought to regulate opening and closing of the ring -> cohesion establishment.

Scc1 contains several cleavage sites for the protease **Separase** (Esp1 in budding yeast). Most of the cell cycle, separase is found in a complex with **Securin** (Pds1 in budding yeast). Securin appears to be important for the correct folding of Separase, but also keeps the folded

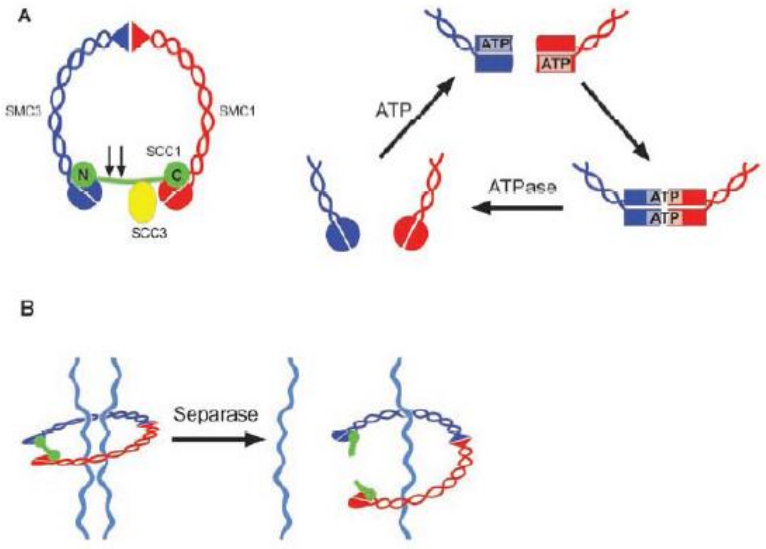


Figure 28: Structure of cohesion, and possible mechanism by which it might hold sister chromatids together, arrows indicate Separase cleavage sites on Scc1.

protein inactive. The **regulated degradation of Securin** at the metaphase/anaphase transition by APC^{Cdc20} leads to **activation of Separase**, **proteolytic cleavage of Scc1** and opening of the cohesion ring. Mitotic spindle is under tension and therefore, the release of chromatid cohesion is sufficient to trigger **chromosome segregation**.

Experiment budding yeast: Cleavage site for viral TEV protease introduced to Scc1, with ts-mutations of separase esp1-1 or APC/c subunit Cdc20. Cells only initiated anaphase if the TEV protease was expressed -> Scc1 cleavage is sufficient to trigger segregation (degradation of Clbs not required). More complex in animal cells!

The Spindle Assembly Checkpoint

Checkpoints are surveillance mechanisms consisting of 3 components:

- Sensors detecting the defect
- Signaling cascades transmitting and amplifying the signal
- Effectors arresting cell cycle progression as long as appropriate

The **spindle assembly checkpoint (SAC)** inhibits anaphase onset until bipolar chromosome attachment has been completed. One single unattached kinetochore is enough to block anaphase onset. The mechanisms are highly conserved. **Mad2** protein shows a dynamic association pattern with kinetochores, gets recruited to unattached kinetochores and gets activated -> **Mad2***. Mad2* dissociates from the kinetochore, binds Cdc20 co-activator of the APC/c and thereby blocks APC^{Cdc20} activity. Securin/Pds1 cannot be degraded which leads to a mitotic arrest prior to anaphase onset. Somehow, Mad2*-Cdc20 can catalyze its own production -> positive feedback loop. This is why one unattached kinetochore is enough to arrest the cell cycle. Once all kinetochores are attached, Mad2* is removed and APC^{Cdc20} becomes active -> initiation of anaphase (degradation of Securin/Pds1 and cyclins).

- Occupancy model: Checkpoint recognizes the lack of microtubule attachment to the kinetochore
- Tension model: Checkpoint senses the absence of tension

-> Not clear yet!

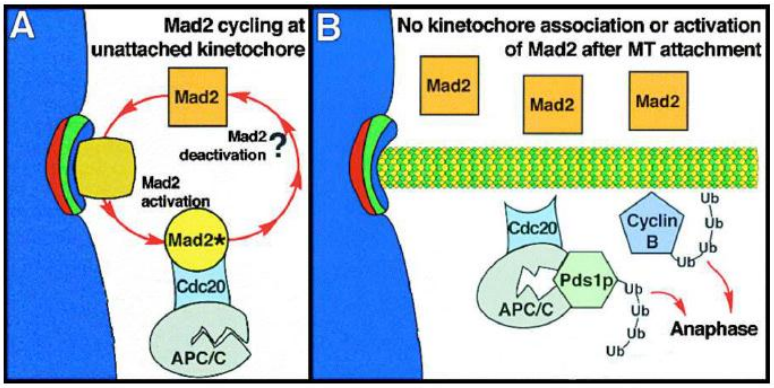


Figure 29: Activation of Mad2 at kinetochores.

Mitotic Exit

Introduction

Chromosome segregation -> exit mitosis:

- Mitotic spindle disassembly
- Chromosome decondensation
- Removal of mitotic determinants
- Reversion of CDK-dependent phosphorylations

-> Cytokinesis and assembly of preRCs. Inactivation of mitotic CDKs required, mainly based on ubiquitin-dependent degradation of mitotic cyclins. In most eukaryotes, bulk of degradation at metaphase/anaphase transition, in budding yeast, two waves of degradation.

Cdc14 (protein phosphatase) is essential for inactivate mitotic CDKs in *S. cerevisiae*. It reverses CDK phosphorylation events, and activates APC/c and Sic1 (plus many other targets that are dephosphorylated) -> rapid resetting of cell cycle G1.

FEAR system sets the stage for finishing mitosis (only transient Cdc14 release) and MEN system triggers mitotic exit plus a spatial sensor, monitoring the position of the elongated anaphase spindle relative to plane of cytokinesis.

Cdc14 Function and Regulation

Exit from mitosis takes place after chromosome segregation, involves cytokinesis, decondensation of chromosomes, the disassembly of the spindle and reassembly of the nuclear envelope. Inactivation of mitotic CDKs is required and achieved by the degradation of cyclins. **Cdc14** (phosphatase) contributes to this process:

- Reversion of CDK-dependent phosphorylations on key regulators (Cdh1, CKI Sic1, Swi5…)
- Probably dephosphorylates most CDK-Clb substrates

Cells lacking CDC14 arrest in late anaphase with high CDK activity. In budding yeast, two regulatory networks for exiting mitosis are required, both regulating the activity of Cdc14 by sequestration in the nucleolus:

- Cdc14 early anaphase release network (FEAR)
- Mitotic exit network (MEN)

Activity of Cdc14 is controlled by cell cycle dependent changes in its association with the inhibitor **Net1**. Net1 localizes to the nucleolus, more precisely to the replication fork barriers in the rDNA repeats and sequesters Cdc14 during most of the cell cycle. Upon phosphorylation of Net1 by Cdc28-Clb and Cdc5

(polo-like kinase), Cdc14 is released. The activation of FEAR only promotes a transient activation of Cdc14, correlating with a transient relocalization of the phosphatase into the nucleus before it becomes sequestered in the nucleolus. The activity of MEN leads to a complete and stable release of Cdc14 from the nucleolus (even into the cytoplasm).

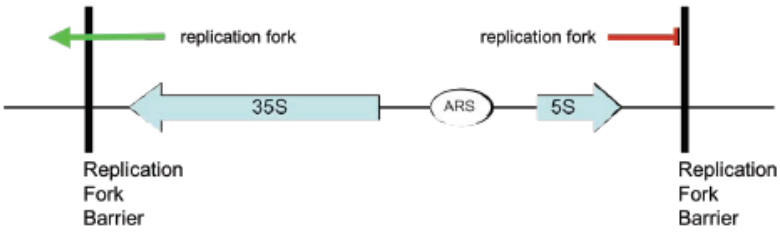


Figure 30: Replication fork barriers (RFBs) in budding yeast rDNA repeats. Inhibit replication forks in the direction opposite to rDNA transcription.

FEAR seems to have two main functions:

- Regulating anaphase events (stabilization of spindle midzone and segregation of rDNA repeats)
- Priming of MEN to permit its activation

MEN mutants arrest in late anaphase with high CDK activity and do not show any anaphase-specific phenotypes.

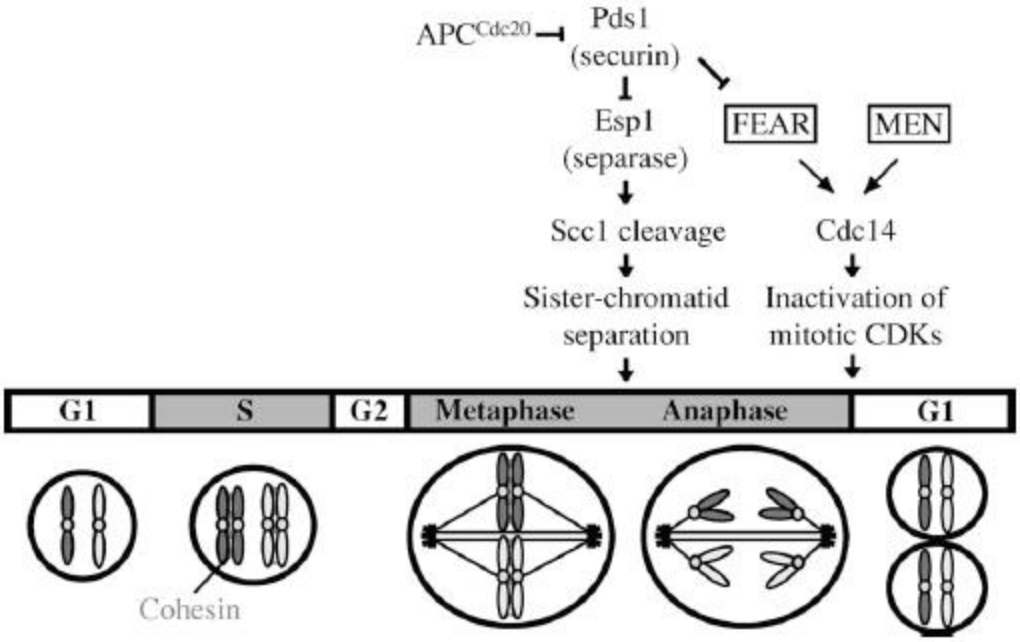


Figure 31: Regulation of mitosis in budding yeast.

FEAR

Components:

- Cdc28-Clb
- Cdc5
- Net1

- Separase (Esp1)
- $PP2A^{Cdc55}$ (type 2A phosphatases, abundant protein phosphatases, scaffold subunit, catalytic subunit, one of 3 regulatory subunits (e.g. Cdc55) -> substrate specificity)

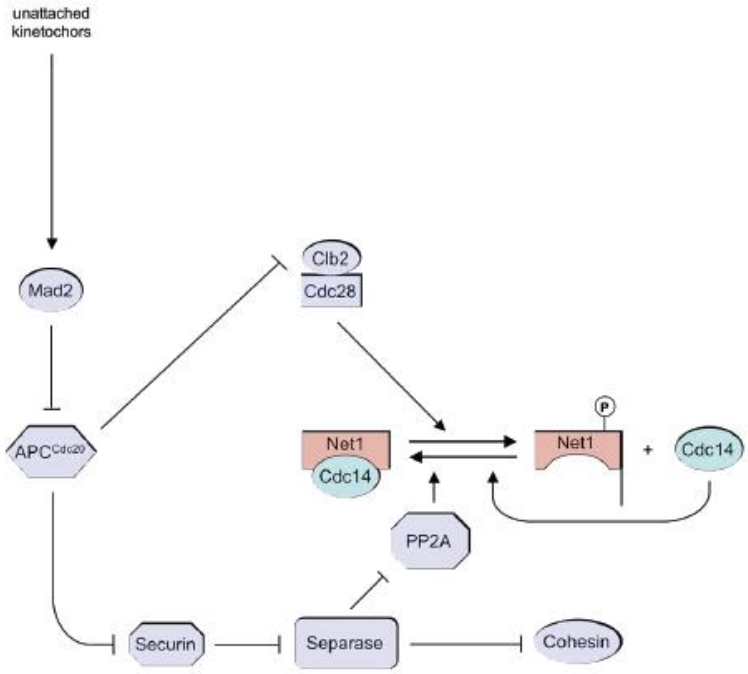


Figure 32: FEAR network, including spindle assembly checkpoint (SAC).

Until metaphase, Net1 is hypophosphorylated because $PP2A^{Cdc55}$ is very active. Cdc14 is inactivated. If the SAC is overcome, anaphase initiates, Securin (Pds1) is degraded (via APC^{Cdc20}) and Separase (Esp1) becomes active. Separase binds Cdc55 and inhibits $PP2A^{Cdc55}$ activity. This then leads to hyperphosphorylation of Net1 and the release of Cdc14 into the nucleus.

CDK-activity is reduced to about 50% and Cdc14 dephosphorylates Net1. Together, these processes lead to the accumulation of hypophosphorylated Net1 and re-binding of Cdc14 to its inhibitor. The Cdc14 activation by FEAR is therefore only transient.

MEN

MEN shares similarity to a Ras-like signaling cascade. The **GTPase Tem1** is controlled by **GEF Lte1** and the bipartite **GAP Bfa1/Bub2**. It activates a protein kinase cascade composed of Cdc15 (upstream) and Dbf2.

Regulation of Tem1 depends on the intrinsic asymmetry of budding yeast cells. Lte1 localizes to the cortex of the daughter cell. The kinase Kin4 (keeps Bfa1/Bub2 active) localizes exclusively to the mother cell. The anaphase spindle is asymmetric as well, Tem1 and GAP localize to the SPB going to the daughter cell, while Cdc15 and Dbf2 are detected on both SPBs. Model: Once

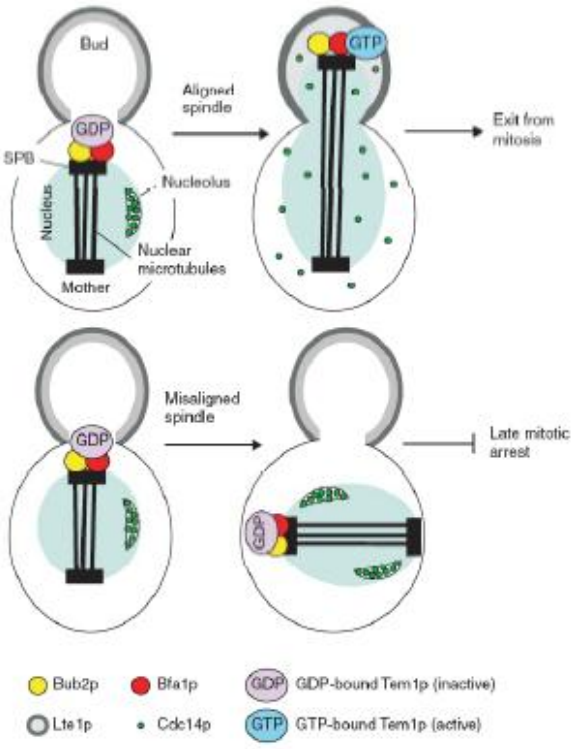


Figure 33: Spindle position checkpoint.

spindle has fully elongated and positioned, Tem1 gets activated by Lte1 (bud-localized). This triggers activation of Cdc15 and Dbf2, the complete phosphorylation of Net1 and the stable release of Cdc14 (cytoplasm). It is unclear if Net1 is a direct target of Dbf2. Dbf2 is active in both nuclei and Cdc14 gets released in mother and daughter cell. In cells with a misaligned spindle, Cdc14 is not activated, and mitosis cannot be exited.

As Tem1 has a high intrinsic GEF rate, even Ite1 mutants are able to activate MEN: Bfa1/Bub2 loses its function because Kin4 (activator) localizes exclusively to the mother, and enough Tem1-GTP is formed in the daughter. Cells lacking BUB2 function are able to exit mitosis even in the presence of an incorrectly aligned mitotic spindle, and in those cases, both nuclei end up in the mother cell.

Coordination of FEAR and MEN

Not only Tem1 but also Cdc15 is regulated. In early anaphase, high CDK-Clb activity phosphorylates and blocks Cdc15. Transient activation of Cdc14 removes phosphorylations (FEAR) making sure MEN is activated after FEAR.

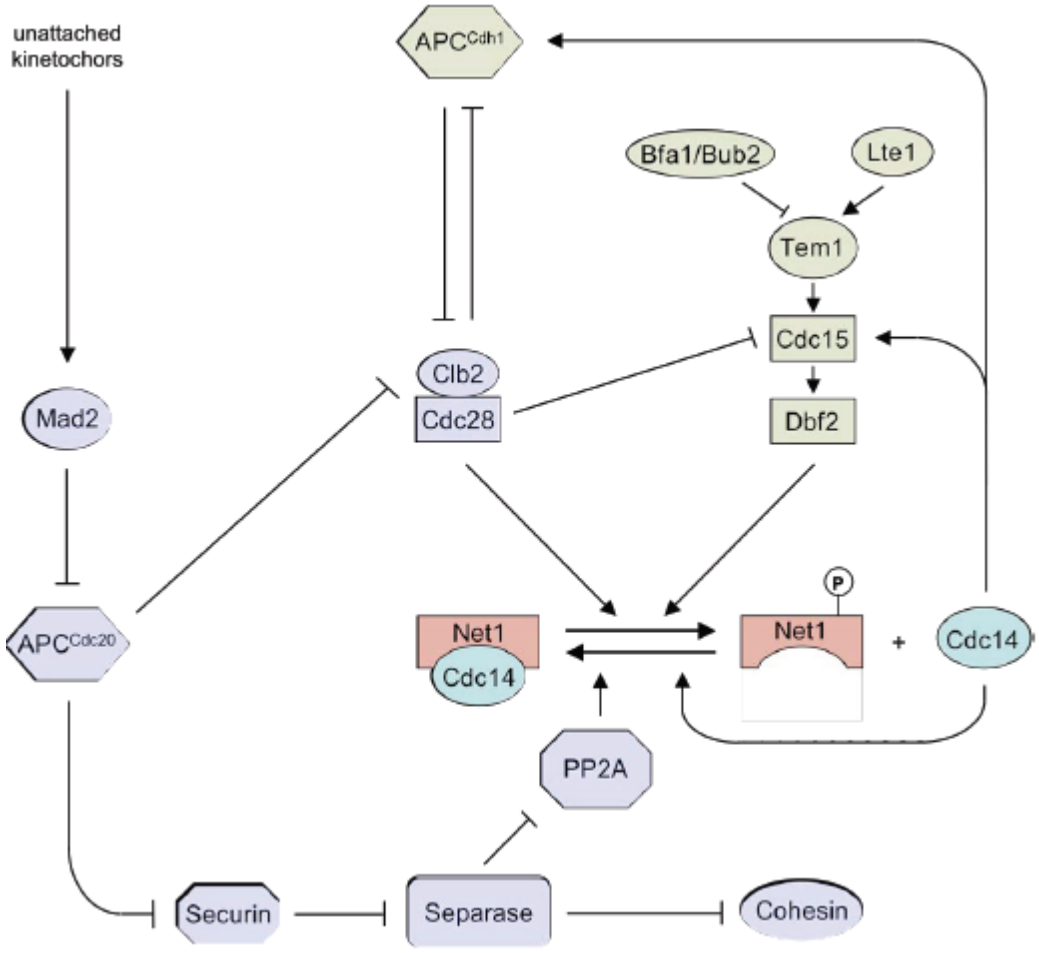


Figure 34: Mitotic regulatory network in budding yeast, including SAC, FEAR and MEN.

Spindle Positioning

Introduction

Mitotis spindle:

- Correct positioning -> proper segregation
- Spatial and temporal coordination of cell division and cytokinesis
- Mitotic spindle microtubules (MTs) -> furrow formation, positioning of cleavage site

Asymmetric positioning of spindle -> asymmetric cell division (cellular diversity, stem cells, yeast -> mother cell (big) and daughter cell (bud, small). Asymmetric spindle positioning is driven by regulated forces that can pull or push the spindle.

Asymmetric Cell Division

Different sister cells can be generated in two ways:

- Polar cells can divide asymmetrically to generate daughter cells different from the mother cell
- Two initially identical sister cells could become different as a consequence of a later event

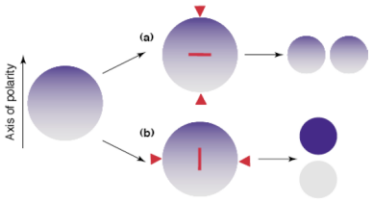


Figure 35: Symmetric (a) and asymmetric cell division (b).

Mutlistep process for **asymmetric cell division:**

1. Establishment of cortical polarity in response to internal and/or external cues
2. Rearrangement of actin cytoskeleton and enforcement of polarity axis, asymmetric distribution of fate determinants
3. Alignment of mitotic spindle, division plane parallel to spindle axis
4. Cytokinesis -> daughter cells containing different subsets of cell fate determinants

Defects in asymmetric cell division can deregulate the number of stem cells and lead to cancer.

Neuroblasts: Stem cells essential to form the central nervous system of *Drosophila*. Daughter cells arising from one neuroblast stem cell:

- One bigger, apical cell that remains a stem cell
- Smaller basal cell that will become a differentiating ganglion mother cell (GMC)

Cell fate determinant here is **Prospero**, which segregates exclusively to the GMC. Prospero seems to repress genes important for self-renewal and at the same time promotes genes required for the differentiation of GMCs into neurons. Misaligned spindles can lead to stem cell loss because both cells inherit Prospero -> both cells GMCs. Loss of Prospero leads to an excess of neuroblasts -> tumors in larval brain.

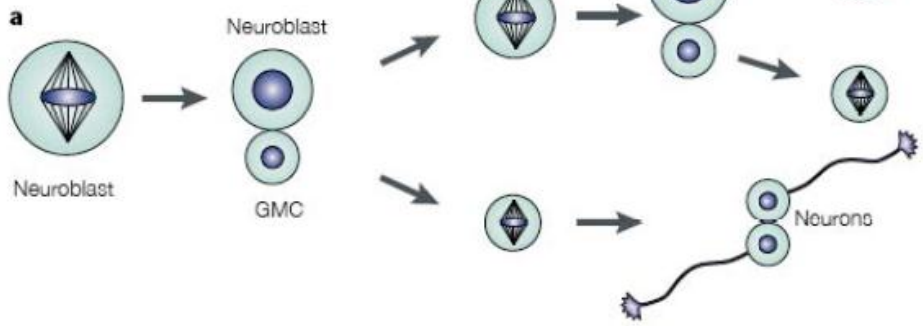


Figure 36: Asymmetric cell division in *Drosophila* neuroblasts. Larger daughter cell stays stem cell, smaller cell (GMC) divides into two neurons.

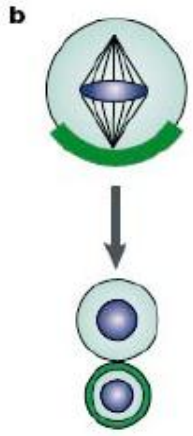


Figure 37: Proteins localizing into a basal cortical crescent and segregate into GMC.

Spindle Positioning in Budding Yeast

In budding yeast, every cell division is asymmetric (daughter growing as bud). Mutation in tub1 (one of the two genes encoding α-tubulin) was shown to strongly affect spindle positioning in budding yeast. The mutation affects the astral microtubules but not the formation of the intranuclear spindle. Astral microtubules interact with the cell cortex and were shown to be involved in the positioning of the mitotic spindle.

There are probably several redundant pathways controlling spindle positioning. This is why a number of mutants with partial defects were identified (20% of cells showed a phenotype at 18° -> shows microtubule-related phenotypes):

- DHC1: Dynein heavy chain
- KAR9: Related to APC (Adenomatous Polyposis Coli), tumor suppressor mutated in colon cancer

DHC1 and KAR9 function in independent but functionally redundant pathways.

- dhc1Δkar9Δ: Cells not viable, accumulation of binucleated cells with one bud or tetranucleated cells with two buds, proteins function in redundant pathways
- bik1Δdhc1Δ: Viable, proteins function in the same pathway
- bik1Δkar9Δ: Not viable
- bim1Δdhc1Δ: Not viable
- bim1Δkar9Δ: Viable

The two pathways were named **KAR9 pathway** (**Kar9, Bim1, Myo2**) and **DHC1 pathway** (**Dhc1, Bik1, dynein/dynactin**). Cells lacking Kar9 function were found to have random spindle alignment during early

cell cycle stages (corrected during anaphase). In contrast, Dhc1 mutants properly pull their spindles towards the bud neck before mitosis, but about 20% of these spindles lose their alignment during anaphase -> Spindle positioning regulated by early Kar9 pathway (prior to anaphase) and the late Dhc1 pathway (during anaphase). The dynein pathway is thought to bring the spindle towards the bud neck with help of pulling forces generated by the minus-end directed movement of cortically anchored dynein.

The Kar9 Pathway

In normal cells, 90% of old SPBs end up in the daughter cell. In kar9Δ, SPB segregation is almost random. This means that Kar9 is required for proper SPB inheritance. Kar9 interacts with Myo2, a type-V myosin, and Bim1, an EB1 homologue and microtubule-plus-end binding protein. Microtubules originating from the old spindle pole body are actively oriented towards the bud, while those from the new SPB are shorter and oscillate randomly.

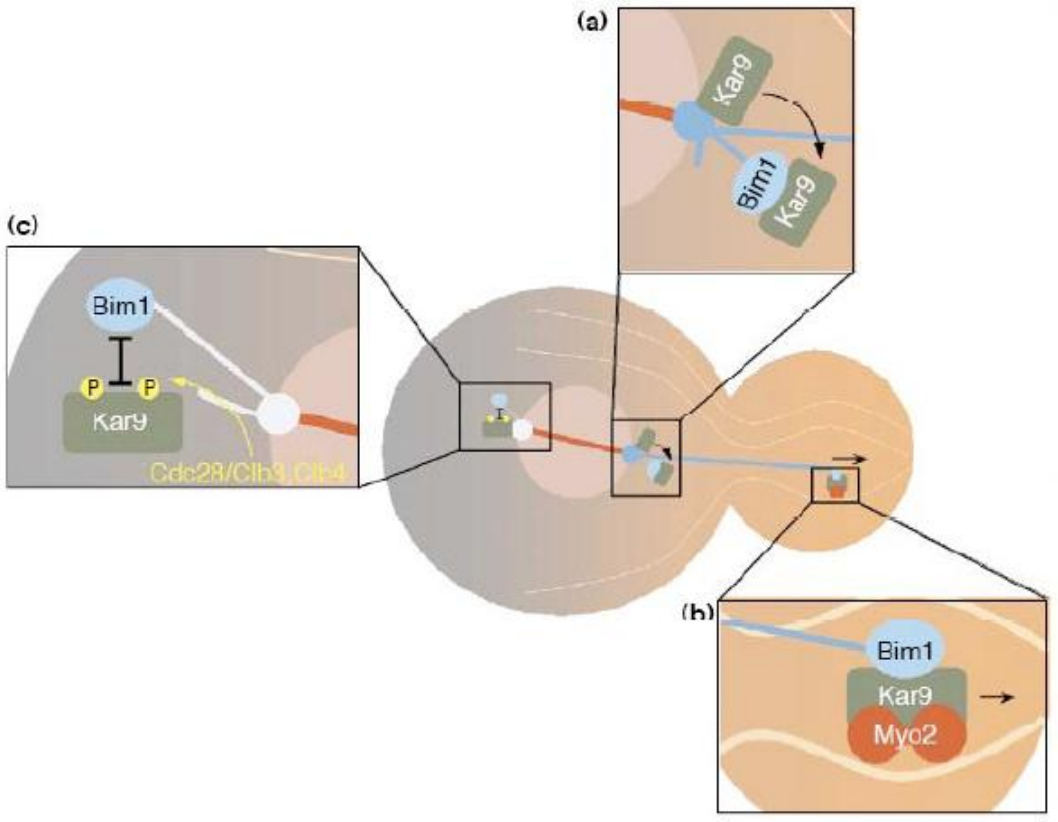


Figure 38: (a) Loading of Kar9 onto MT is an active and spatially regulated process. It occurs at the old spindle pole. Kar9 loaded from the spindle pole onto MTs through its interaction with Bim1. (b) MT-bound Kar9 physically interacts with the type V myosin Myo2, which guides MTs along the polarized actin cables. Actin cables are nucleated at the bud cortex. (c) At the spindle pole destined to the mother cell, Cdc28-Clb4 phosphorylates Kar9 and represses its interaction with Bim1, thereby preventing Kar9 loading onto MTs at the distal spindle pole, which does not orientate towards the bud.

The plus-ends of microtubules originating from the old SPB (not the new ones) are bound by a complex of Bim1, Kar9 and Myo2, where Kar9 functions as a linker (between Bim1 and Myo2, fusion protein Bim1-Myo2 compensates absence of Kar9). Bim1, Kar9 and Clb4 are loaded onto microtubules via the SPB and subsequently transported to the microtubule tips, while Myo2 associates directly with the complex at the tip. Myo2 then walks along actin cables, towards their cable plus-ends localized in the bud. This turns the old SPB towards the bud, and thereby orients the spindle. The new SPB also contains Bim1, but for reasons discussed further down Kar9 and by consequence Myo2 are not able to associate with Bim1 in this context. Therefore, the new SPB will not be pulled towards the bud.

Kar9 Localization and Activity

Kar9 can be phosphorylated by Cdc28-Clb4. The phosphorylation state affects its asymmetric localization. Non-phosphorylatable Kar9 mutants exhibit no asymmetric localization. Inactivation of Cdc28 where Kar9 already localized asymmetrically leads to Kar9 redistribution to both SPBs. Cdc28-Clb4 seems to be active on the new, distal SPB, locally phosphorylating Kar9 and thereby inhibiting Bim1-dependent Kar9 recruitment. Surprisingly, the major fraction of Clb4 localizes to the old SPB.

Mechanism:

- Kar9Δ: Clb4 is lost from SPB and microtubule tip, suggesting that Kar9 interacts with and recruits Clb4 to microtubules, consistent with model in which Kar9 acts as a CKI for Cdc28-Clb4
- On the old SPB, Kar9 would stably associate with the abundant Cdc28-Clb4 and inhibit Cdc28 activity towards Kar9
- On the new SPB, Kar9 would get constantly phosphorylated by Cdc28-Clb4 -> factor dissociating Kar9 from Cdc28-Clb4?
- Maybe Tem1, modification of Kar9 by ubiquitin-like peptide SUMO
- Tem1 regulated by GAP Bfa1/Bub2 that is active on the old, daughter-bound SPB -> activation of Tem1 on the new SPB, asymmetric sumoylation of Kar9, dissociation of Kar9 from Cdc28-Clb4 and phosphorylation of Kar9
- tem1 mutants have Kar9 localized to both SPBs
- Tem1 two functions:
    - On new SPB prior to mitosis to generate spindle asymmetry
    - On old SPB to trigger exit from mitosis

Stem Cells and Cancer

Self-renewal:

- Important for development of an organism
- Inherent risk of cancer

Cell fate determinants such as Prospero segregate asymmetrically into differentiating daughter cells. These factors suppress self-renewal, and are required to keep the number of stem cells constant. Mutants divide symmetrically and lead to uncontrolled accumulation of stem cells and tumor formation.

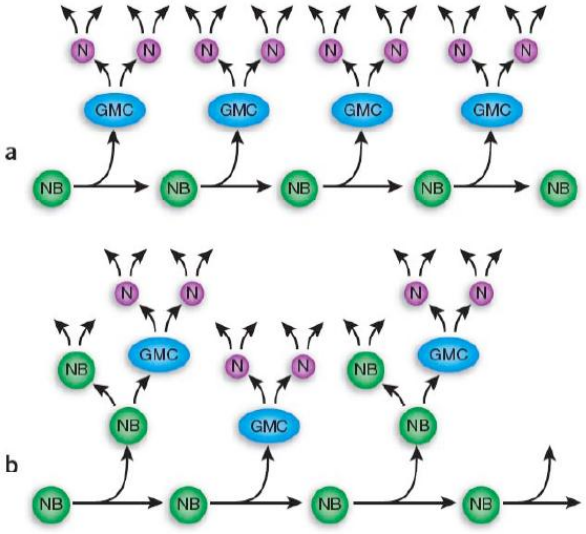


Figure 39: (a) Neuroblast (NB) divides in a stem cell like fashion. (b) Loss of asymmetry -> increasing numbers of NB.

The adenomatous polyposis coli (APC) gene is required for the asymmetric division of spermatogonial cells in *Drosophila*, and apc mutant animals accumulate increasing numbers of stem cells. APC is also an important tumor suppressor in the mammalian intestinal epithelium. Although it is not known if APC regulates asymmetric division of stem cells in this tissue, it is intriguing that colorectal cancer cells have properties very similar to epithelial stem cells.

Taken together, loss of polarity in stem cells results in symmetric modes of division and compromised inheritance of cell fate determinants, and may promote tumorigenesis by leading to the uncontrolled expansion of stem cell numbers.