BP205 2013
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S. CEREVISIAE AS A PLATFORM FOR QUANTIATIVE BIOLOGY: EVOLUTION, GENOMES, LIFE CYCLE, CELL TYPE-SPECIFICATION, CHROMOSOME BEHAVIOR, and MATING

I. Resources

Guide to Yeast Genetics: Functional Genomics, Proteomics and Other Systems Analysis, Volume 470, Second Edition (Methods in Enzymology) J. Weissman, C. Guthrie, G. Fink (editors). Academic Press 2010

From a to α*: yeast as a model for cellular diferentiation*. H. Madhani Cold Spring Harbor Press 2007

Advanced Genetic Analysis: Genes, Genomes, and Networks in Eukaryotes. P. Meneely. Oxford University Press 2009

Can a Systems Perspective Help Us Appreciate the Biological Meaning of Small Effects? H. El-Samad, HD. Madhani *Developmental Cell*, 21(1): 11-13, 2011.

II. Learning objectives:

•Describe the features of *S. cerevisiae* life cycle

•Describe the molecular basis of cell type specification

•Understand chromosome behavior in mitosis

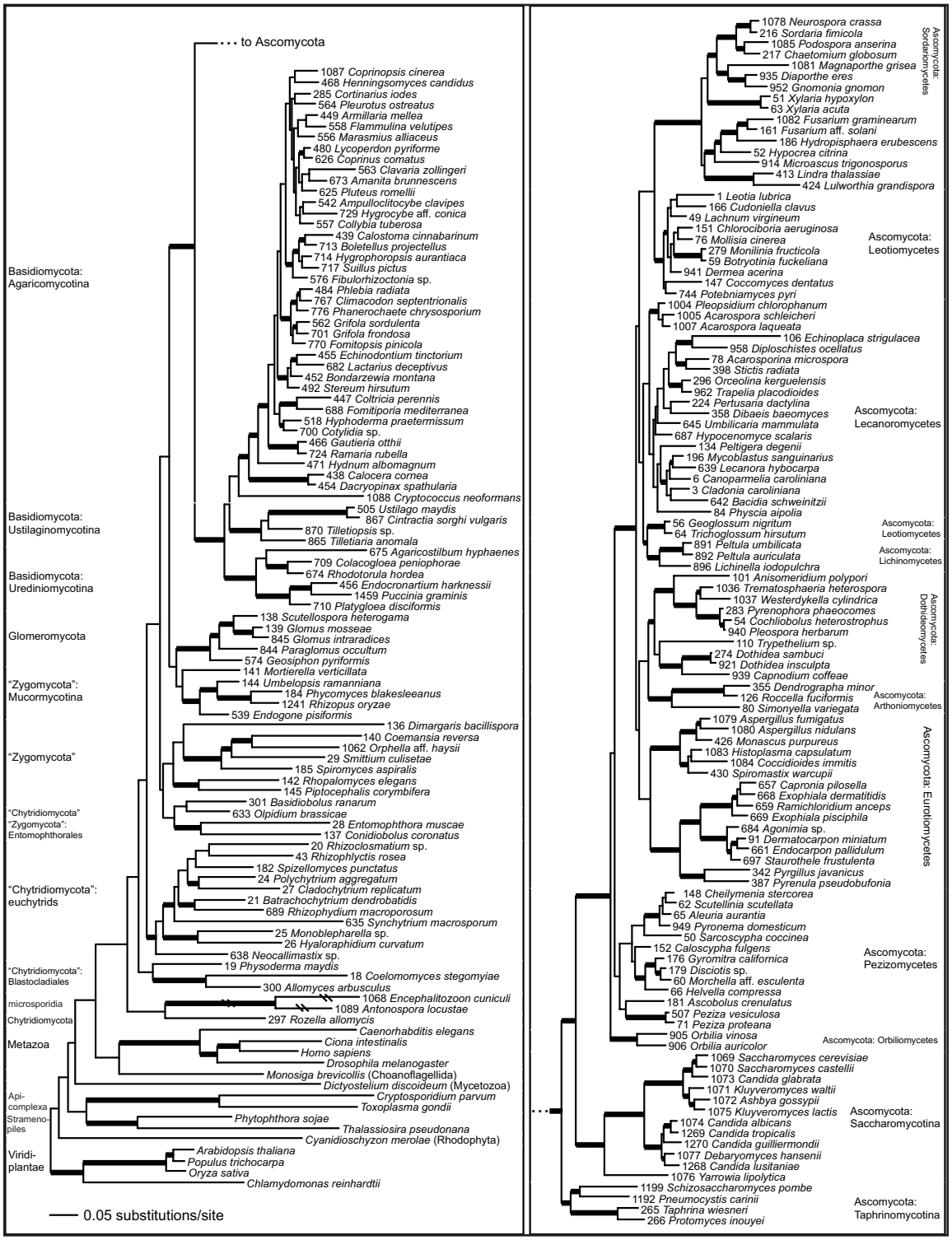
•Understand chromosome behavior in meiosis

•Understand the pheromone response pathway

•List key unsolved questions

Fungi

The fungal kingdom contains at least 70,000 species. Many fungi grow as filaments (e.g. the bread mold Neurospora crassa), but some grow as yeast. Yeasts are defined as fungi which can grow as free-living single cells (as opposed to filaments, the more common growth form of fungi -- e.g. bread mold). The most commonly studied yeasts are *Saccharomyces cerevisiae* and *Schizosacchomyces pombe*. They divide by budding and fission, espectively. A small percentage of fungi are dimorphic -- they can grow as yeast or filaments. *S. cerevisiae* is dimorphic, as are some pathogenic fungi of humans including *Candida albicans* and *Histoplasma capsulatum*. *S. cerevisiae* can grow as single cell yeast or switch to a filamentous from called pseudohyphae, which are invasive filaments of elongated cells.



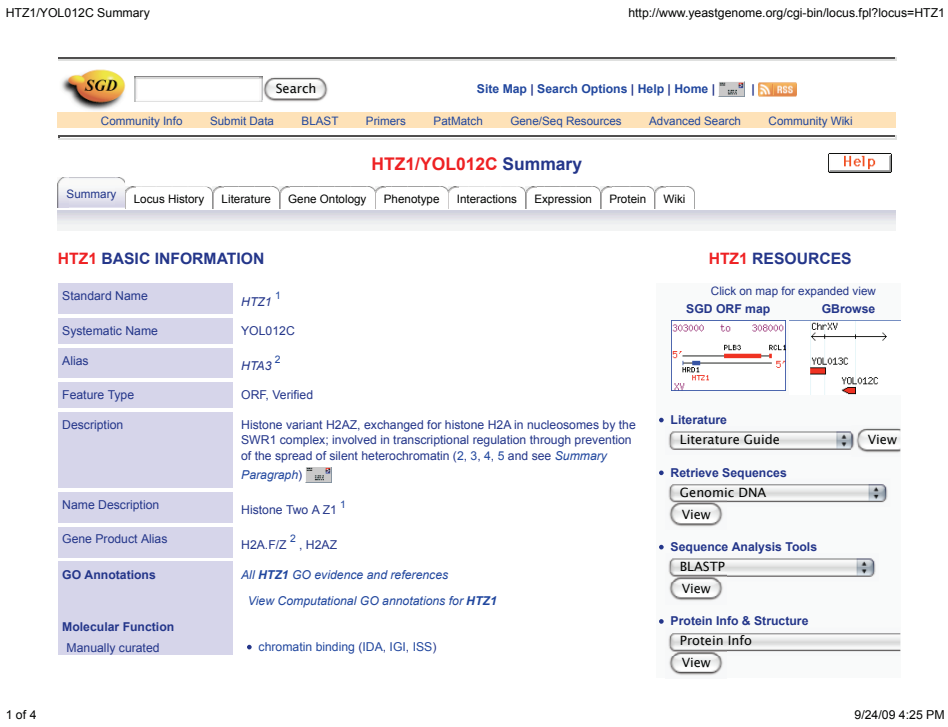
Fungi numerous experimental advantages for experimental studies of eukaryotic biology. For example, *S.cerevisiae* can be cultured in haploid (one set of each chromosome) or diploid (two sets) forms (wild isolates are always diploid). The generation time is rapid, 90 min in rich media.

TABLE IV
SYNTHETIC MINIMAL (SD) MEDIA[a]

Component	Composition
0.67% Bacto-yeast nitrogen base (without amino acids)	6.7 g
2% Dextrose	20 g
2% Bacto-agar	20 g
Distilled water	1000 ml
	Amount per liter
Carbon source	
Dextrose	20 g
Nitrogen source	
Ammonium sulfate	5 g
Vitamins	
Biotin	20 μg
Calcium pantothenate	2 mg
Folic acid	2 μg
Inositol	10 mg
Niacin	400 μg
p-Aminobenzoic acid	200 μg
Pyridoxine hydrochloride	400 μg
Riboflavin	200 μg
Thiamin hydrochloride	400 μg
Compounds supplying trace elements	
Boric acid	500 μg
Copper sulfate	40 μg
Potassium iodide	100 μg
Ferric chloride	200 μg
Manganese sulfate	400 μg
Sodium molybdate	200 μg
Zinc sulfate	400 μg
Salts	
Potassium phosphate monobasic	850 mg
Potassium phosphate dibasic	150 mg
Magnesium sulfate	500 mg
Sodium chloride	100 mg
Calcium chloride	100 mg

[a] This synthetic medium is based on media described by Wickersham[42] and is marketed, without dextrose, by Difco Laboratories (Detroit, MI) as "Yeast nitrogen base without amino acids."

Fungal genomes are generally small (15 Mbp for *S. cerevisiae*, about 6000 genes). For comparison, the *Drosophila* genome encodes about 12,000 genes. Pure cultures of fungi can be grown in chemically defined media (*S. cerevisiae* can grow in ammonium sulfate as the sole nitrogen source and glucose as the sole carbon source), allowing complete control of both genotype and environment. Last, but not least, most fungi have very active homologous recombination system. This allowed the construction of a complete collection of strains containing deletions in virtually every nonessential gene in *S. cerevisiae* and *S. pombe* and partial genome knockout collections in the human pathogens *Cryptococcus neoformans* and *Candida albicans*. Strain collections in which every gene has been tagged by an affinity tag or green fluorescent protein (GFP) have also been constructed.



Genomes of *S. cerevisiae*

S. cerevisiae has 16 linear chromosomes, each with a centromere (the site of microtubule attachment during mitosis) and two telomeres (the ends).

Yeast has several other inherited elements: an endogenous nuclear plasmid (the 2 micron circle), a mitochondrial genome, a double-stranded RNA cytoplasmic virus system that encodes a toxin and resistance (L-A virus), and some strains harbor a cytoplasmic prion: [Psi+] and [URE] were the first two to be described, but many others have been identified.

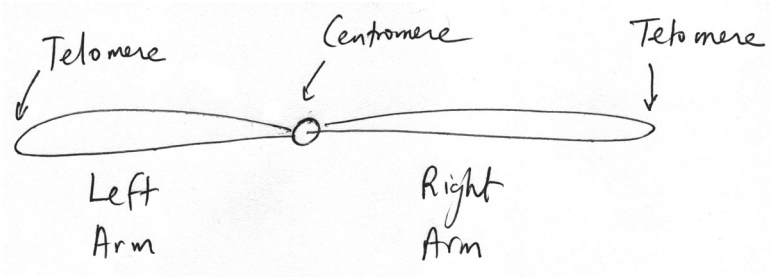
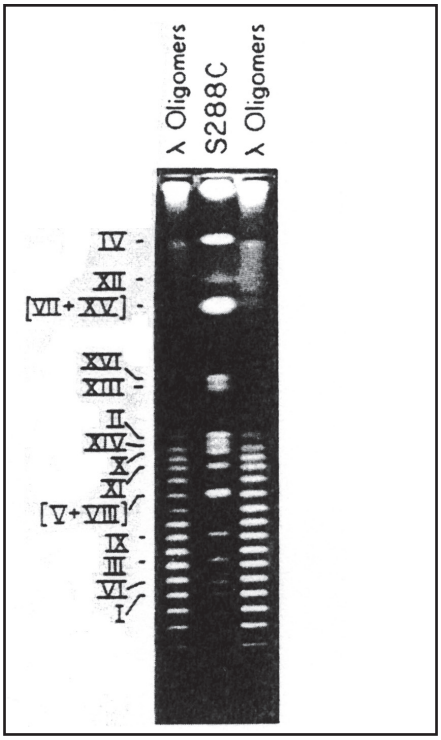
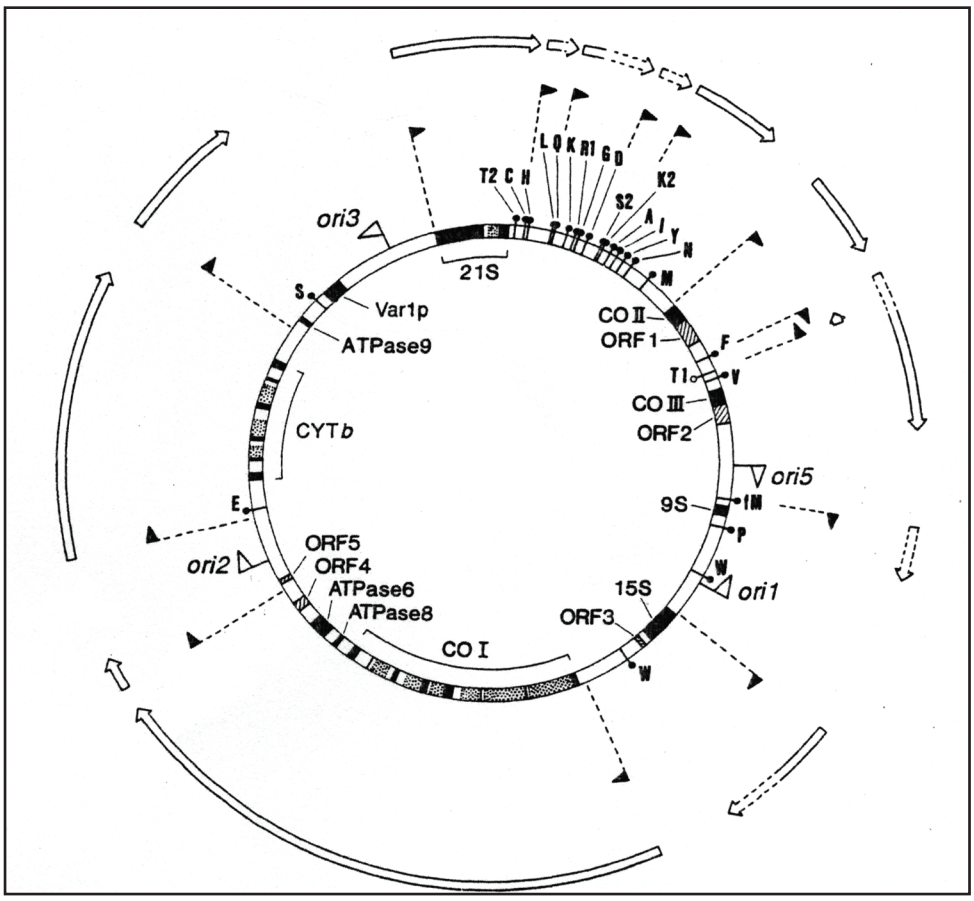


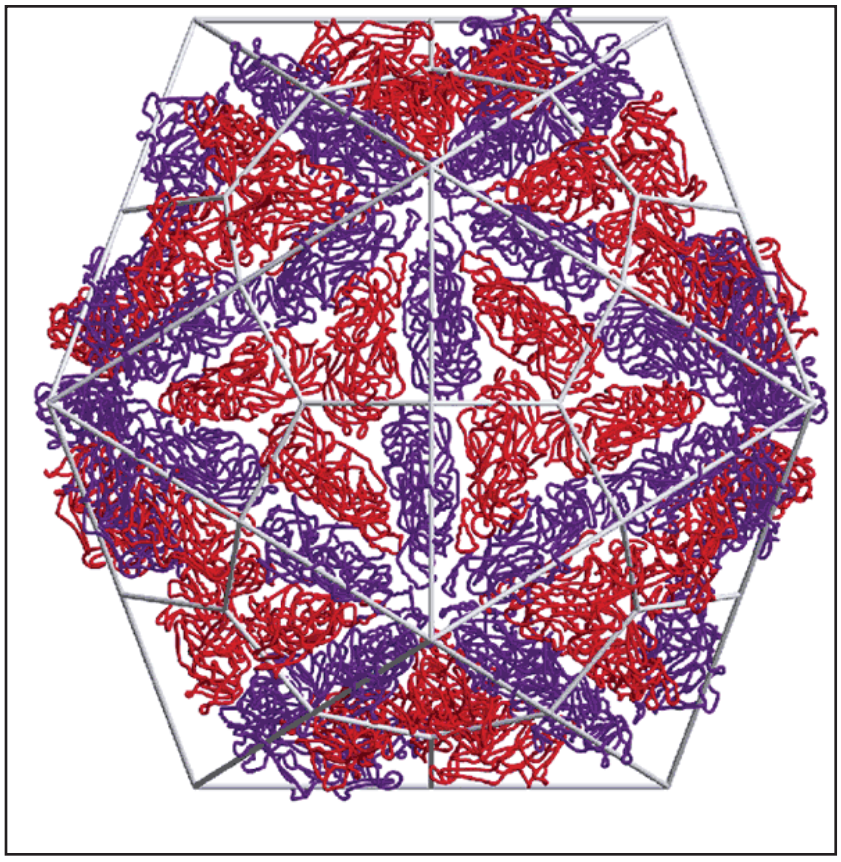
Table 2 Sizes of the yeast chromosomes

Ordered by chromosome number		Ordered by chromosome size	
chromosome	size	chromosome	size
I	240 kb	I	240 kb
II	840	VI	280
III	350	III	350
IV	1,640	IX	440
V	590	V	590
VI	280	VIII	590
VII	1,120	XI	680
VIII	590	X	755
IX	440	XIV	810
X	755	II	840
XI	680	XIII	950
XII	1,095 + rDNA	XVI	980
XIII	950	VII	1,120
XIV	810	XV	1,130
XV	1,130	IV	1,640
XVI	980	XII	1,095 + rDNA
Total	12,490 + rDNA	Total	12,490 + rDNA





Mitochondrial Genome



X-ray structure of L-A virus

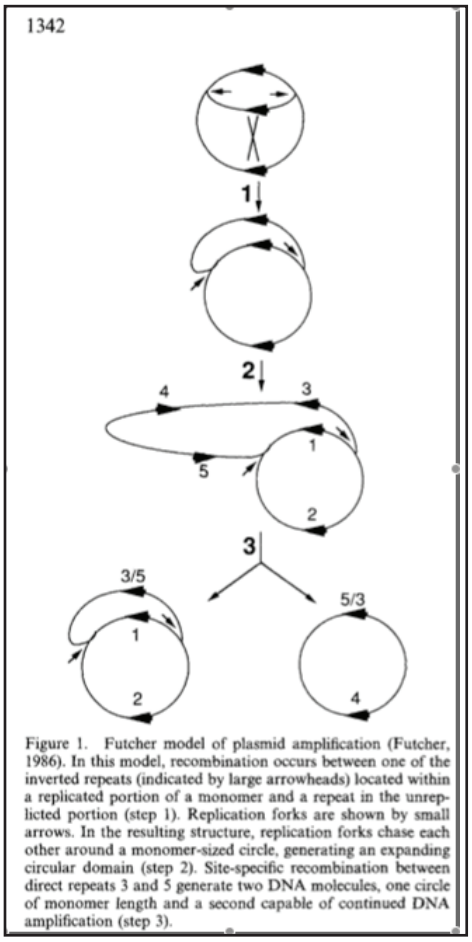


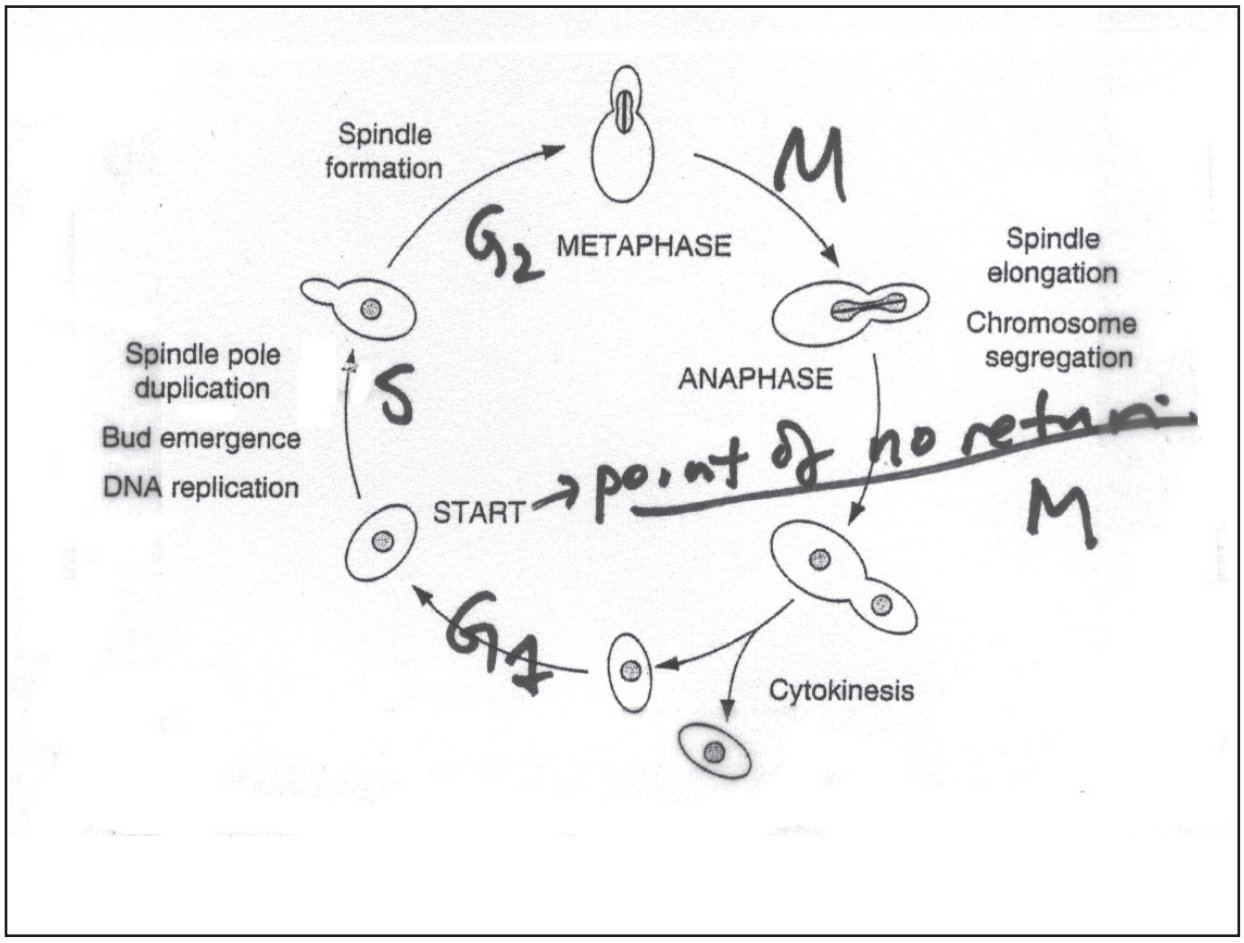
Figure 1. Futcher model of plasmid amplification (Futcher, 1986). In this model, recombination occurs between one of the inverted repeats (indicated by large arrowheads) located within a replicated portion of a monomer and a repeat in the unreplicted portion (step 1). Replication forks are shown by small arrows. In the resulting structure, replication forks chase each other around a monomer-sized circle, generating an expanding circular domain (step 2). Site-specific recombination between direct repeats 3 and 5 generate two DNA molecules, one circle of monomer length and a second capable of continued DNA amplification (step 3).

2 micron circle (plasmid)

Mitotic Cell Cycle

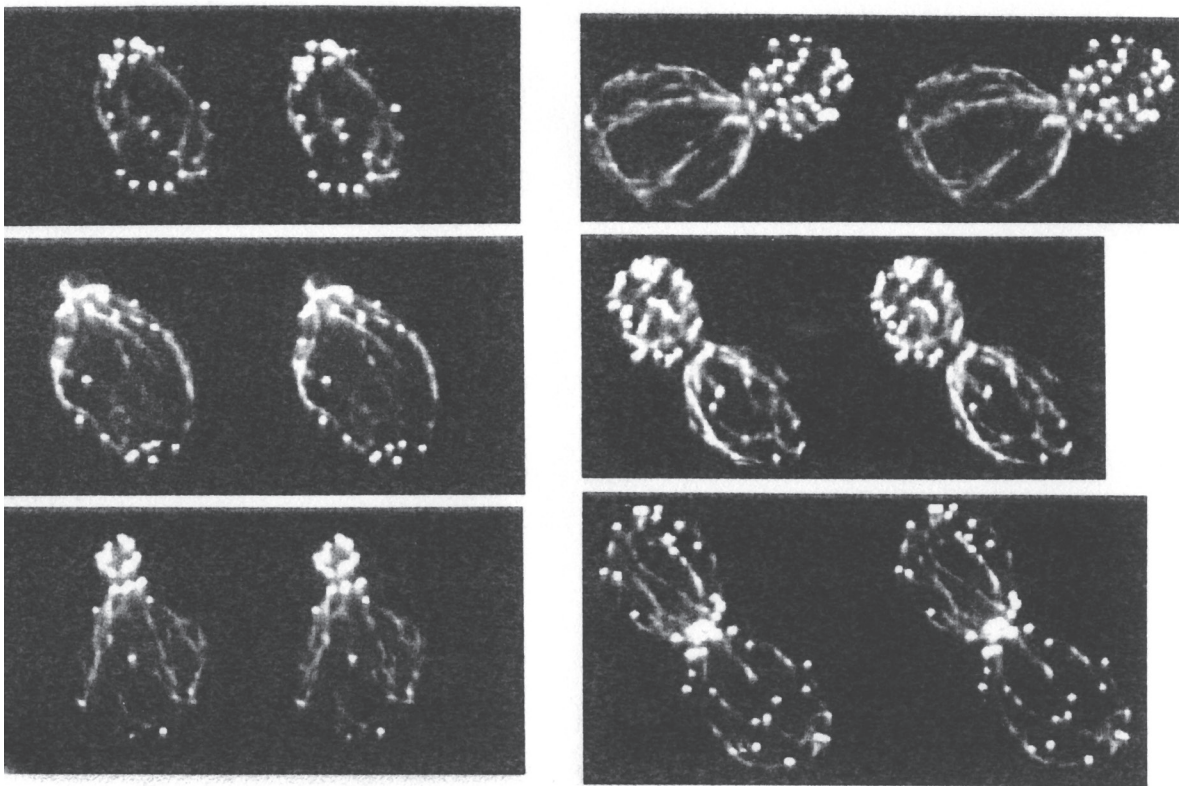
The yeast cell cycle has two purposes with respect to the genome: 1) replicate each chromosome exactly once per division. 2) give each daughter cell one copy of each chromosome at mitosis. Mistakes can mean death (or cancer in humans).

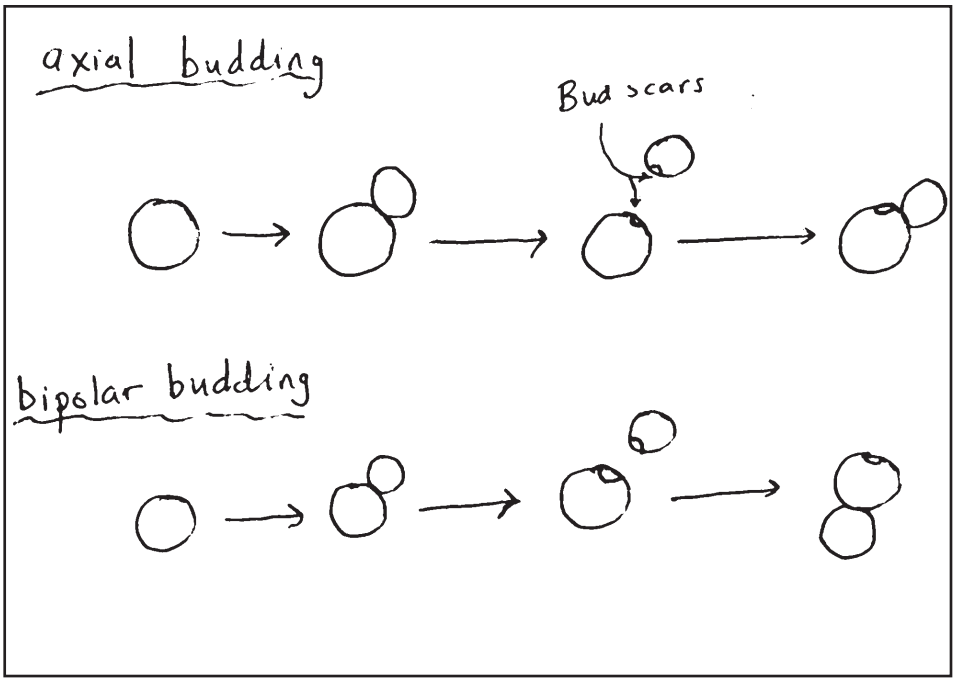
The size of the daughter bud is a visual marker for cell cycle position. G1 cells are unbudded, S phase cells have a small bud, and mitotic cells are "dumbells." G2 is usually very short or nonexistent in yeast. Because yeast divides by budding, every cell division is asymmetric. Daughters are born smaller than mothers. A mother can bud many times, but eventually dies (mother cell senescence or aging).



Budding Patterns

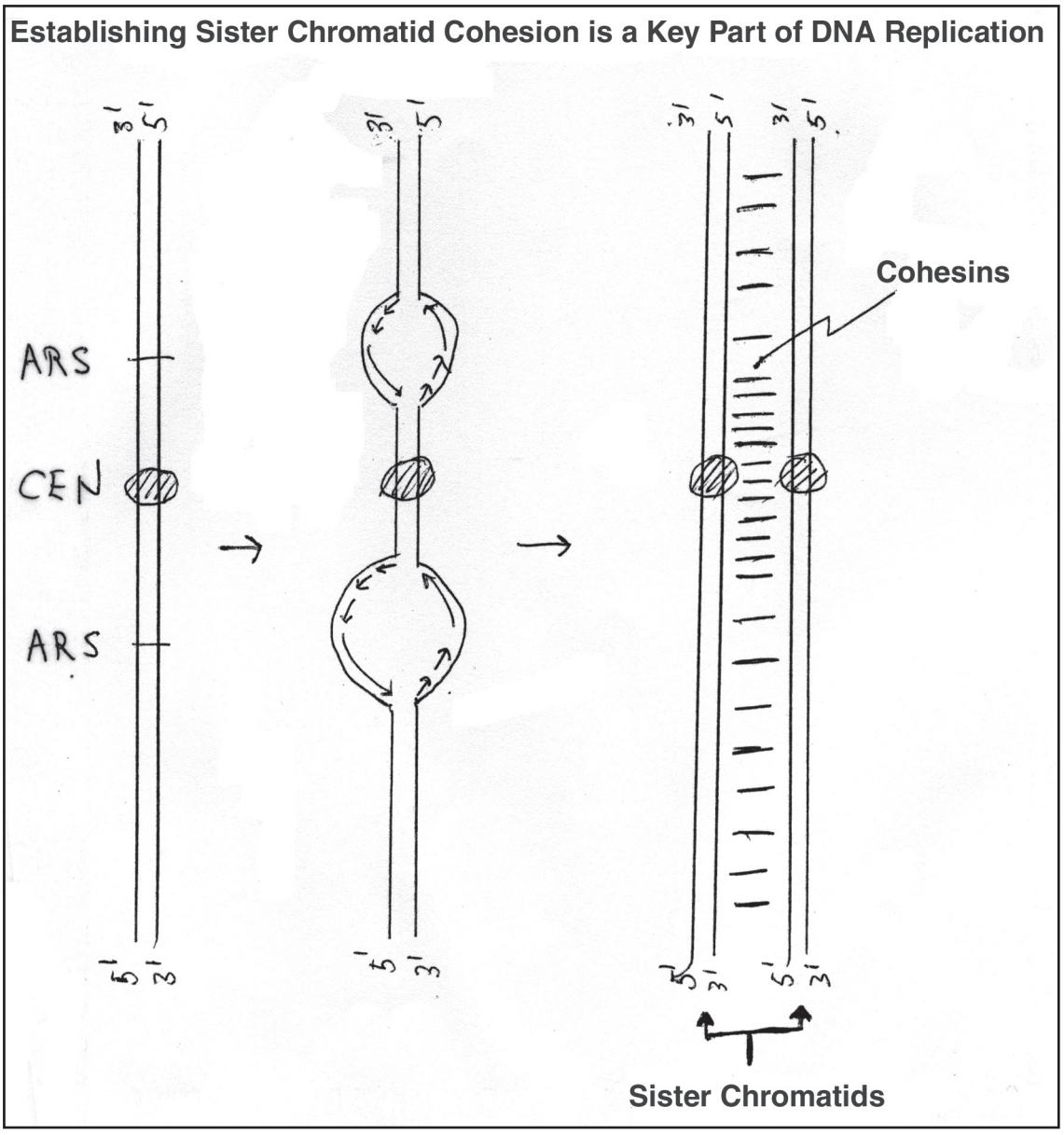
The process of choosing a division site is tightly controlled. Depending on cell-type and environmental conditions, yeast divide on one of two budding patterns: axial or bipolar. In axial budding, new buds are formed proximal to where the previous bud occurred (as evidenced by a bud scar). In bipolar budding, new buds are formed at distal to the previous bud, that is on the other end of the cell. Budding patterns offer a way to study how cells lay down intracellular landmarks to control morphogenesis.



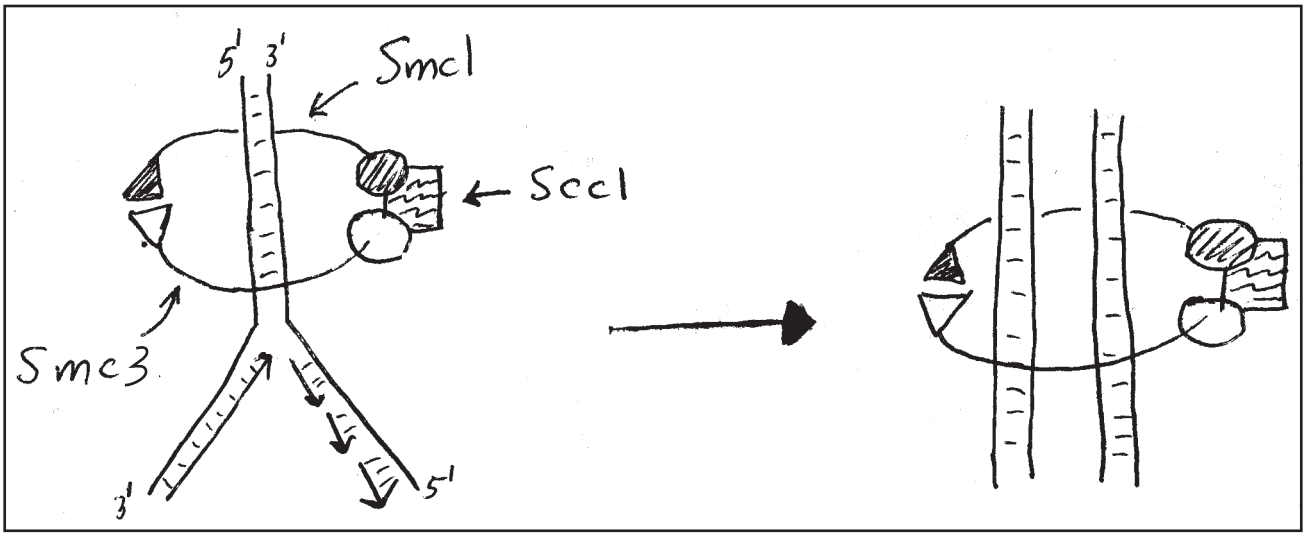


Sister chromatid cohesion

Once cells reach a certain size, S phase and bud growth are simultaneously initiated. How cells know how big they are (size control) is a major unsolved problem. DNA replication (S phase) occurs once and only once. Multiple origins of replication occur on each chromosome and fire bidirectionally. Yeast origins are called ARS elements (autonomously replicating sequences) because they were first isolated as sequences that allow foreign DNA to replicate in yeast. The replicated chromosomes, called sister chromatids, remain associated with each other via a crucial protein-mediated interaction termed sister chromatid cohesion. A complex of proteins responsible for cohesion have been identified, termed the cohesin complex. It includes two members of the Structural Maintenance of Chromosomes (SMC) family and the protein Scc1/Mcd1.

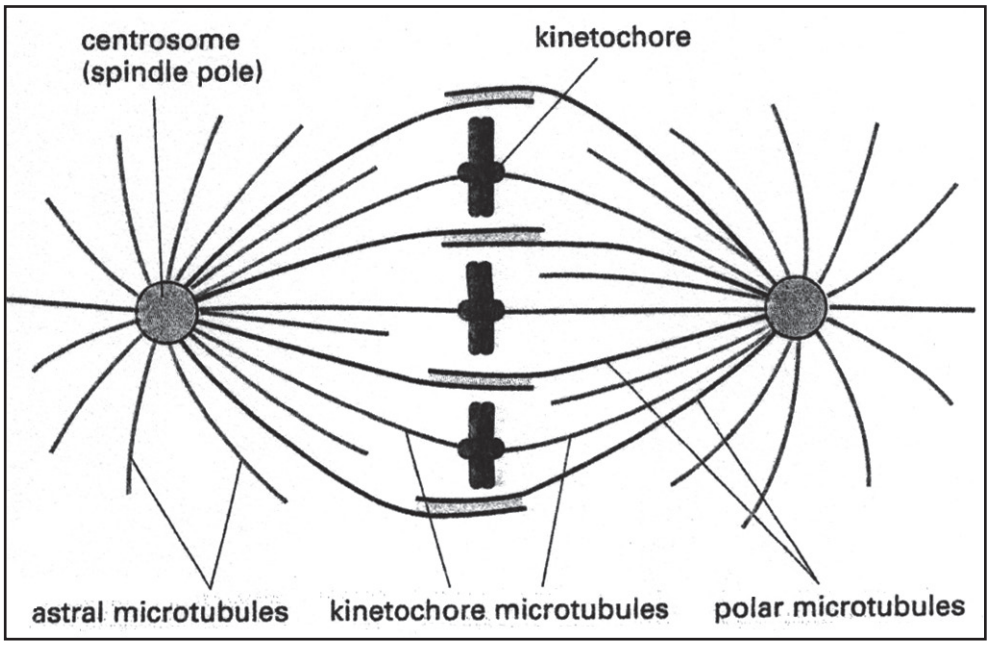


One model for how these proteins mediate cohesion is diagramed below. The idea is that the SMC proteins are loaded onto DNA as a ring which is closed by the Scc1 protein. After DNA replication, the two sister chromatids are held together topologically.

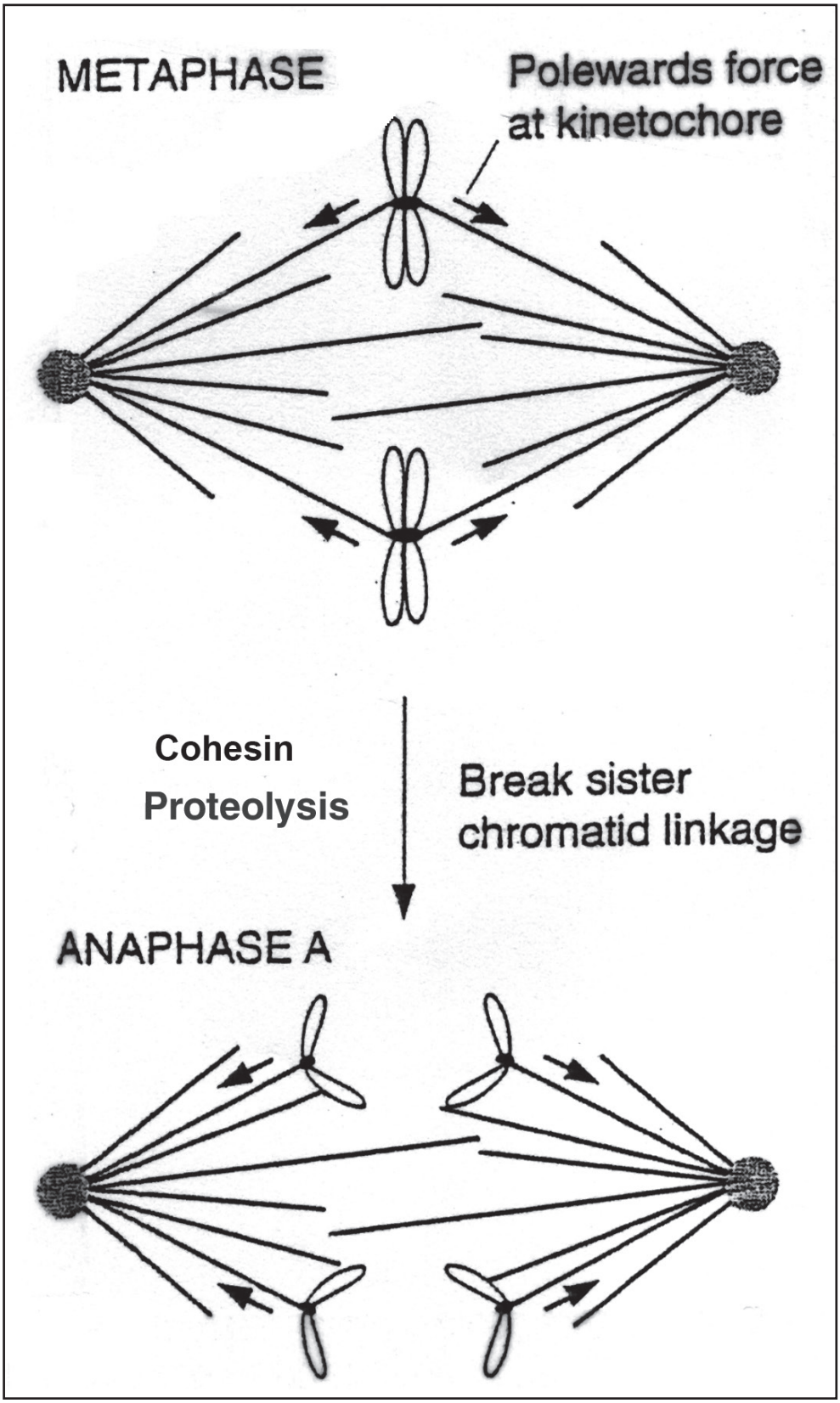


Mitosis

The purpose of mitosis is to segregate faithfully the 16 pairs of sister chromatids to opposite poles of the mitotic spindle. This is accomplished by attachment of a microtubule to each kinetochore, which is formed by the assembly on the centromere of a protein complex capable of capturing a microtubules. The other end of the kinetochore microtubule is attached to the centrosome (microtubule organizing centers called spindle pole bodies (SPBs) in yeast), which themselves are duplicated during the cell cycle. In fungi, SPBs are embedded in the nuclear envelope (which does not break down during mitos

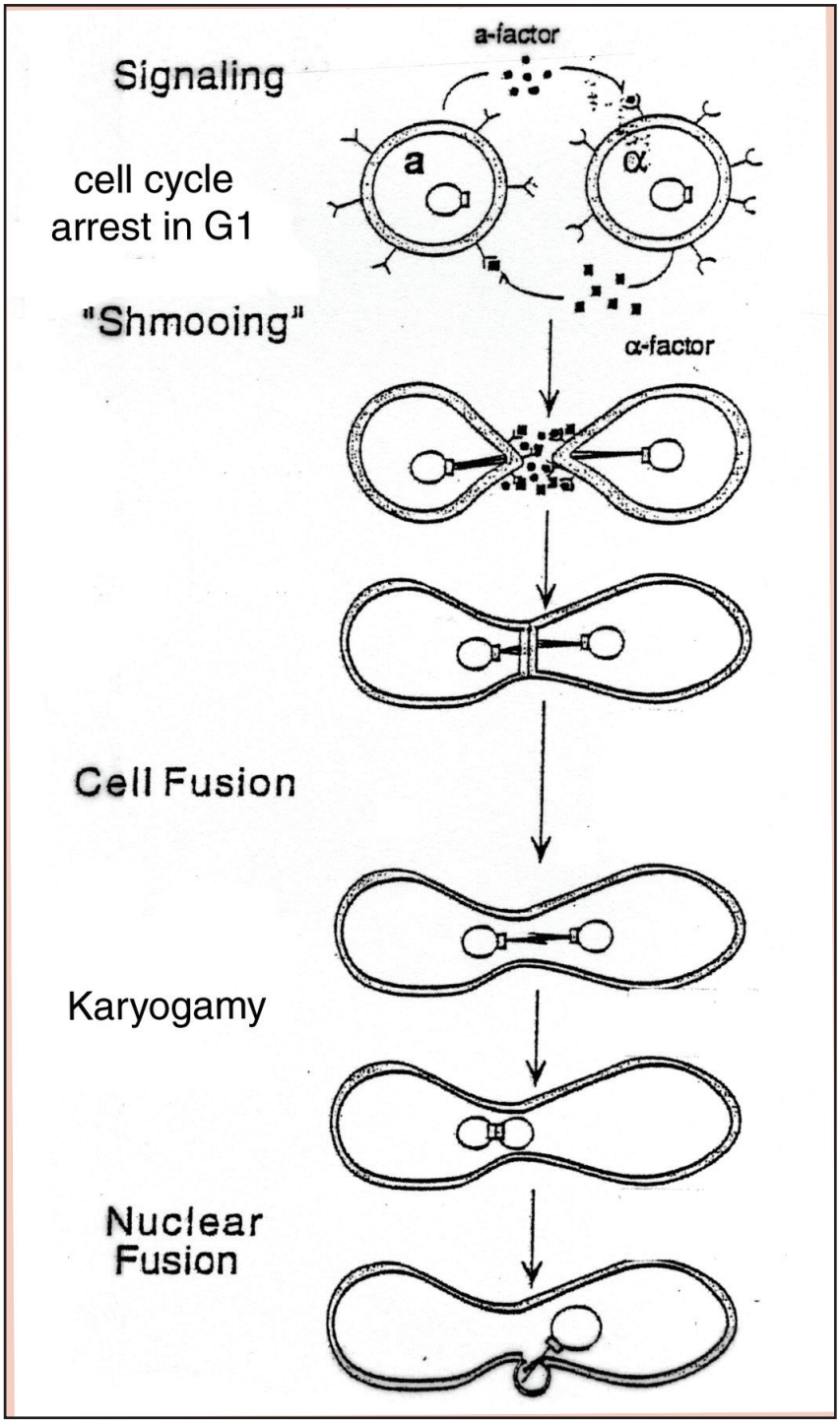


Anaphase is the precipitous loss of sister chromatid cohesion that occurs once the assembly of the mitotic spindle is completed. It is triggered in part by proteolysis of the cohesion proteins. Unlike metazoan mitoses, fungal mitoses do not involve breakdown of the nuclear envelope. Rather, **nuclear division** occurs by fission. The migration of one of the nuclei into the daughter bud triggers **mitotic exit** and **cytokinesis**. There has been spectacular progress in recent years on the regulatory mechanisms that ensure the orderly progression of these events in yeast.



Sex

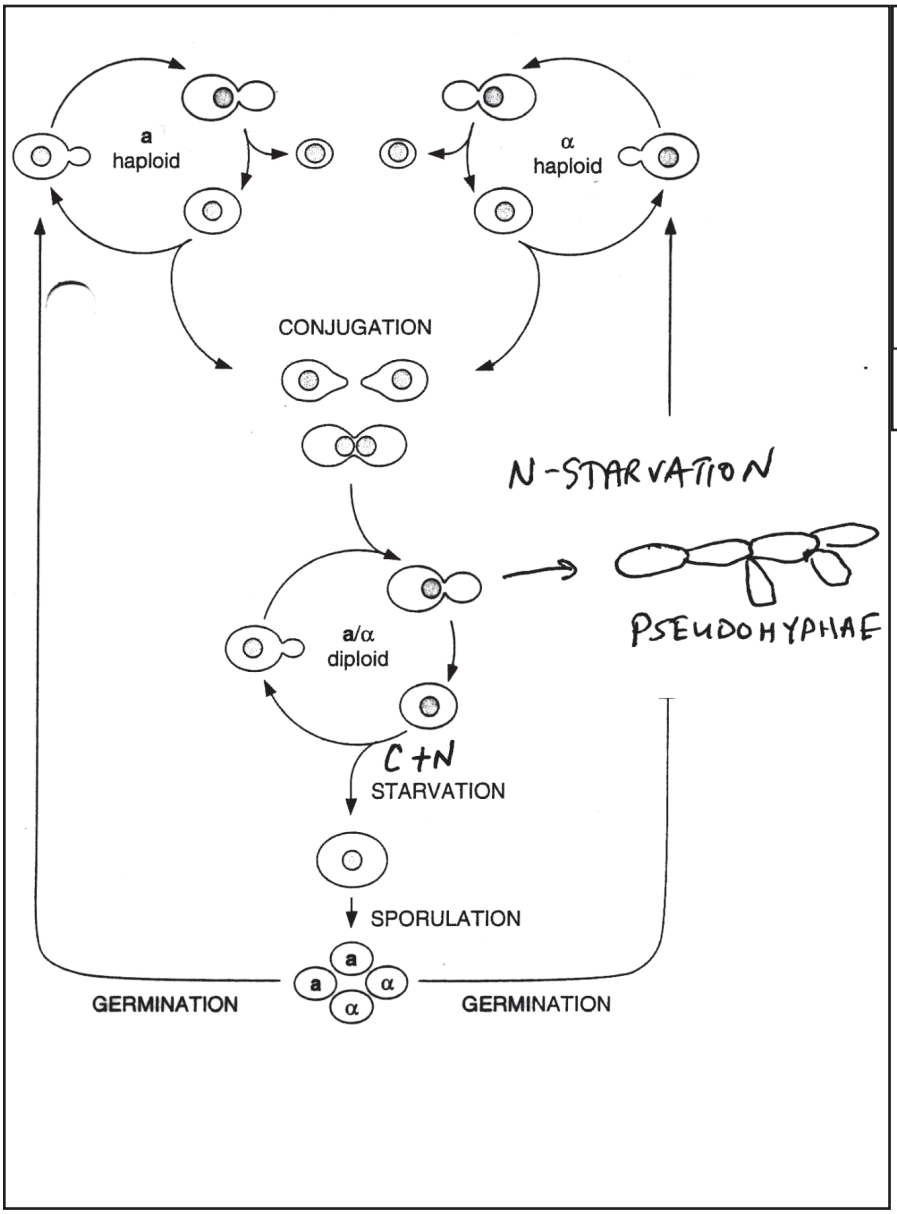
Yeast exists in two haploid mating types, **a** and α. Each cell type produces a different mating pheromone. **a** cells produce **a**-factor and α cells produce α-factor. In turn **a** cells express an α-factor receptor and α cells encode an **a**-factor receptor. Detection of pheromone results in arrest of the cell cycle in G1 followed by polarized growth towards the mating partner (chemotropism). Cell-cell attachment (agglutination), cell wall breakdown and membrane fusion cause the formation of a transient heterokaryon. Karyogamy is the process by which the two haploid nuclei move toward each other on microtubule tracks and culminates in nuclear fusion. The resulting **a**/α diploid (the third cell type) cell resumes its cell cycle and can be propagated stably.



Developmental potential of the three cell types

The three cell types of yeast have distinct developmental properties:

While **a** and α cells can mate, **a**/α diploids cannot. **a**/α cells form pseudohyphae in reponse to nitrogen starvation, but haploid cells do not. **a**/α cells (but not **a** or α cells) can undergo meiosis to produce four haploid progeny in response to combined nitrogen and carbon starvation. In yeast, this process is called sporulation and the haploid gametes are packaged in hardy spores. These are packaged in a spore sac called an ascus. Four spores in an ascus is called a tetrad. Two other processes are controlled by cell-type: budding pattern and mating type switching. **a** and α cells bud in the axial pattern, whereas **a**/α cells bud in the bipolar mode. Mating type switching is a process that will be discussed in lecture 5 that occurs in **a** and α cells, but not in **a**/α cells.



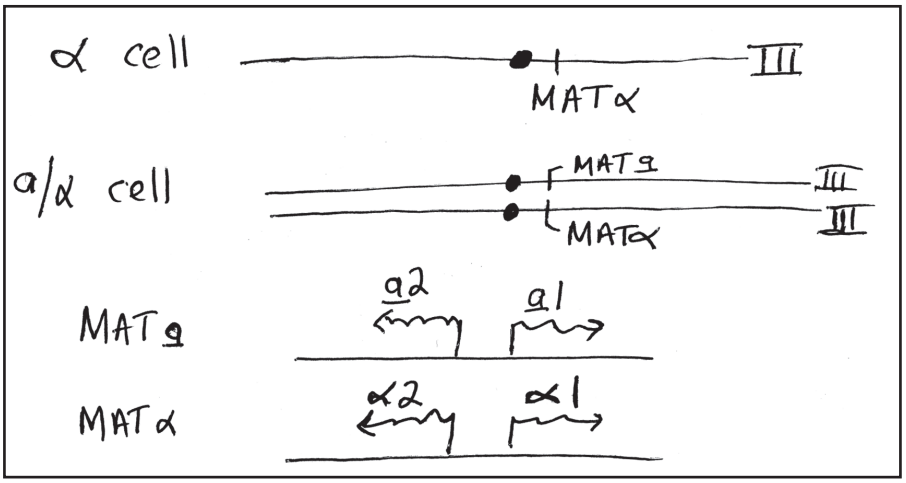
Process	Cell Type		
	a	α	**a**/α
mating	+	+	-
meiosis-sporulation	-	-	+
pseudohyphal growth	-	-	+
axial budding	+	+	-
bipolar budding	-	-	+
mating type switching	+	+	-

How the mating type locus, *MAT*, controls cell type

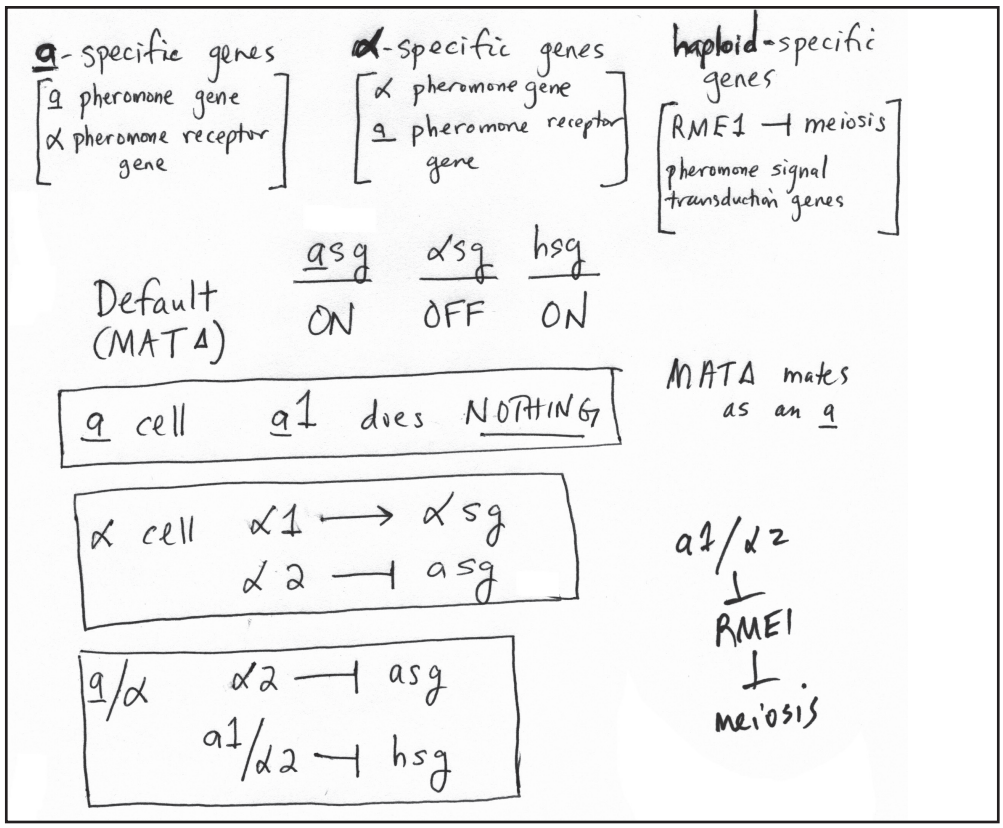
Mating type is controlled by a single locus on chromosome III: *MAT*. *MAT* has two alleles. **a** cells have the *MAT***a** allele, α cells have the *MAT*α alelle. This is the only genotypic difference between the two sexes. The **a**/α state is programmed by the combination of **a** and α information present in the heterozygous *MAT***a**/*MAT*α diploid.

The *MAT* locus encodes transcription factors of the homeodomain family. The *MAT*a allele encodes two proteins, **a**1 and **a**2. **a**2 has no known function.

*MAT*α encodes a transcriptional activator α1 and a repressor α2.



Of course neither α1 or α2 function in *MAT***a** cells. In α cells, α1 activates α-specific genes, such as the **a**-factor receptor gene and the α pheromone genes. α2 represses **a**-specific genes such as the α-factor receptor and the **a** pheromone gene. In the *MAT***a**/*MAT*α diploid, **a**1 and α2 form a heterodimer that represses haploid specific genes. One of the haploid-specific genes is *RME1*, which encodes a repressor of meiosis. Deletion of *RME1* allows haploid cells to sporulate.

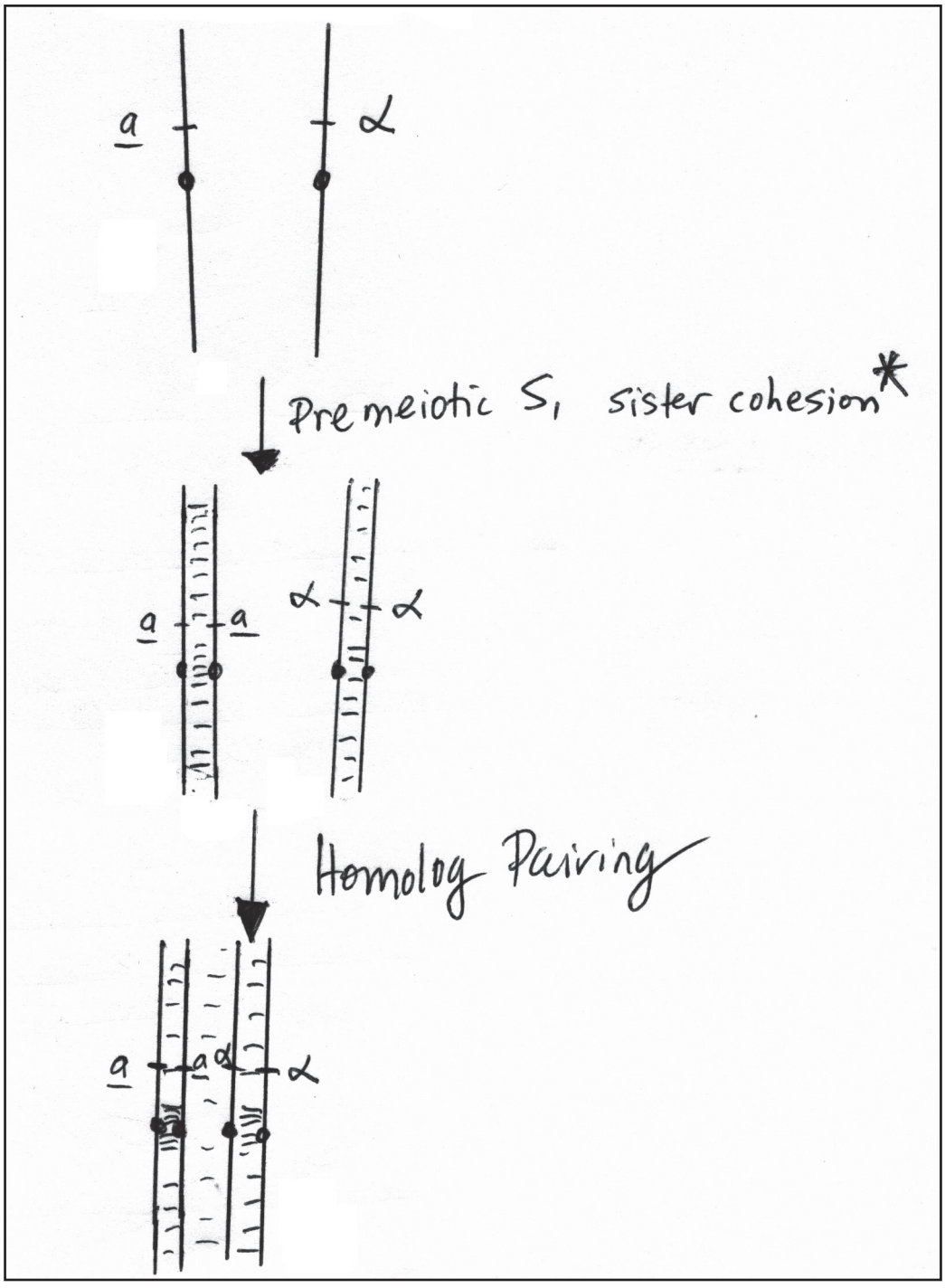


Meiosis

Diploids produce haploids through meiosis, which entails one round of DNA synthesis and two cell divisions (meiosis I and meiosis II). The key is understanding meiosis I (a.k.a. reductional division).

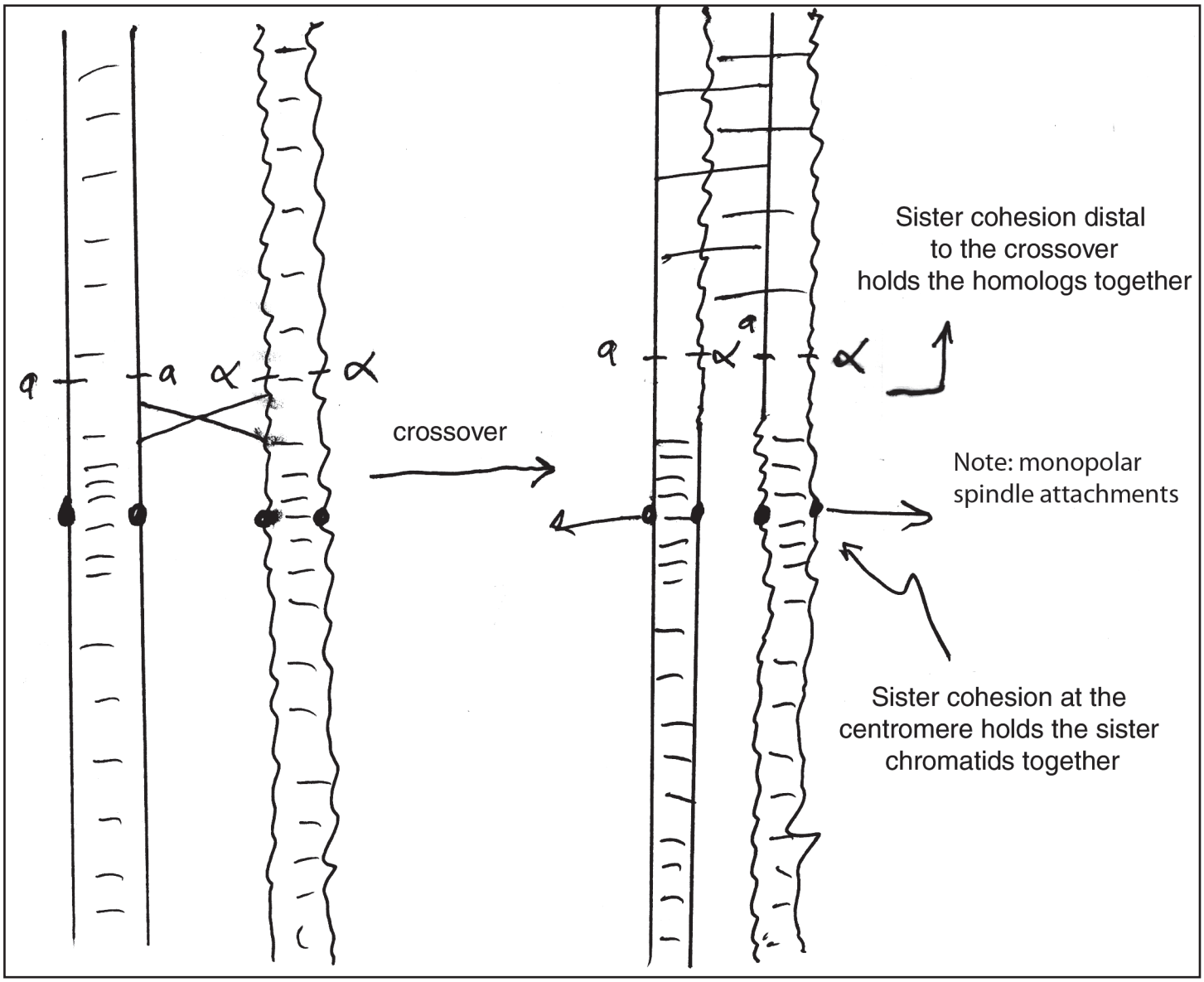
Premeiotic S phase, sister cohesion establishment and homolog pairing

Following carbon and nitrogen starvation, **a**/α cells (2C) undergo premeiotic DNA synthesis (4C). As in normal mitosis, sister chromatid cohesion is produced. This is accompanied by pairing of homologs (requires a homology search).



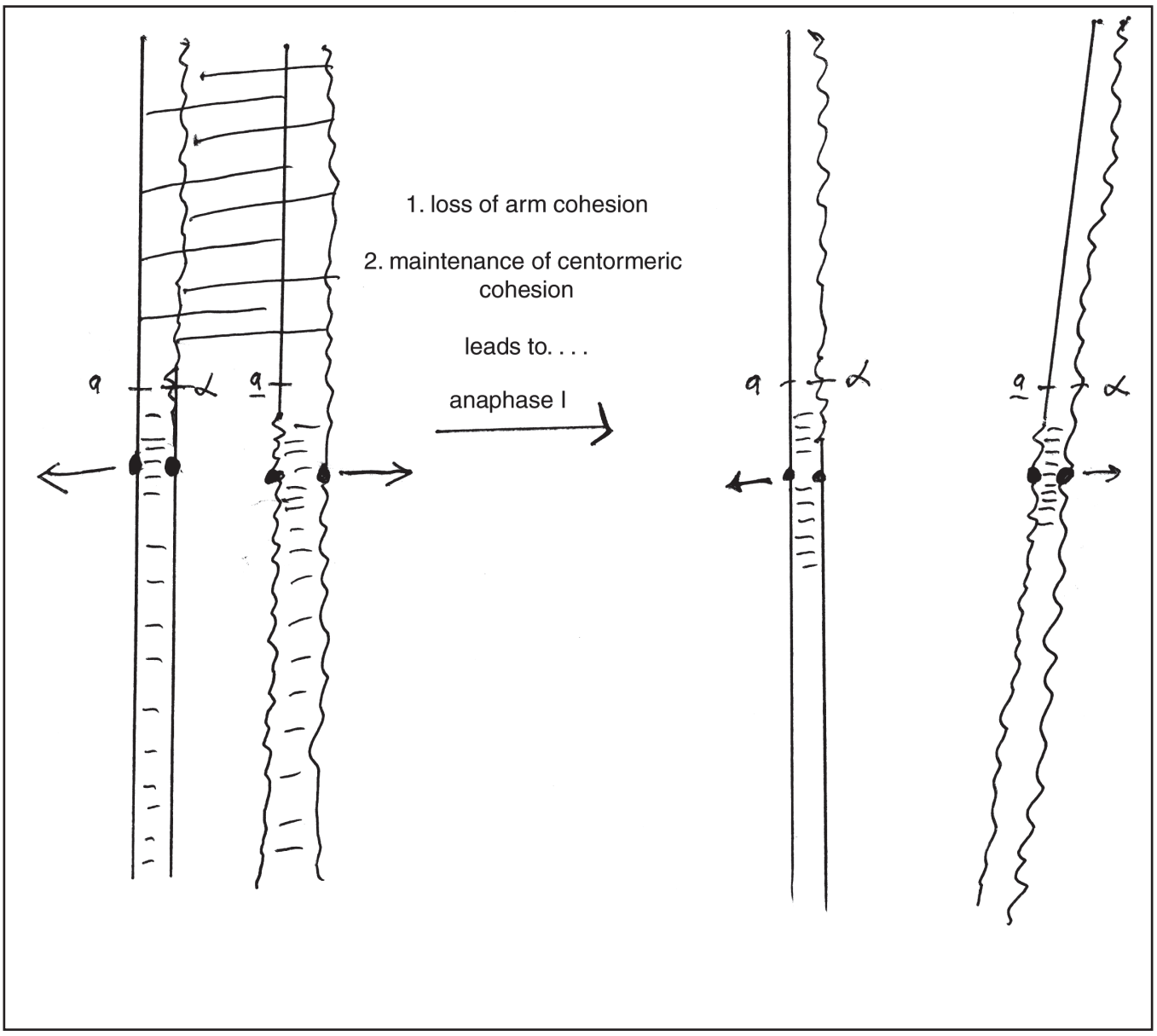
Crossovers: sites of genetic exchange required for proper chromosome segregation

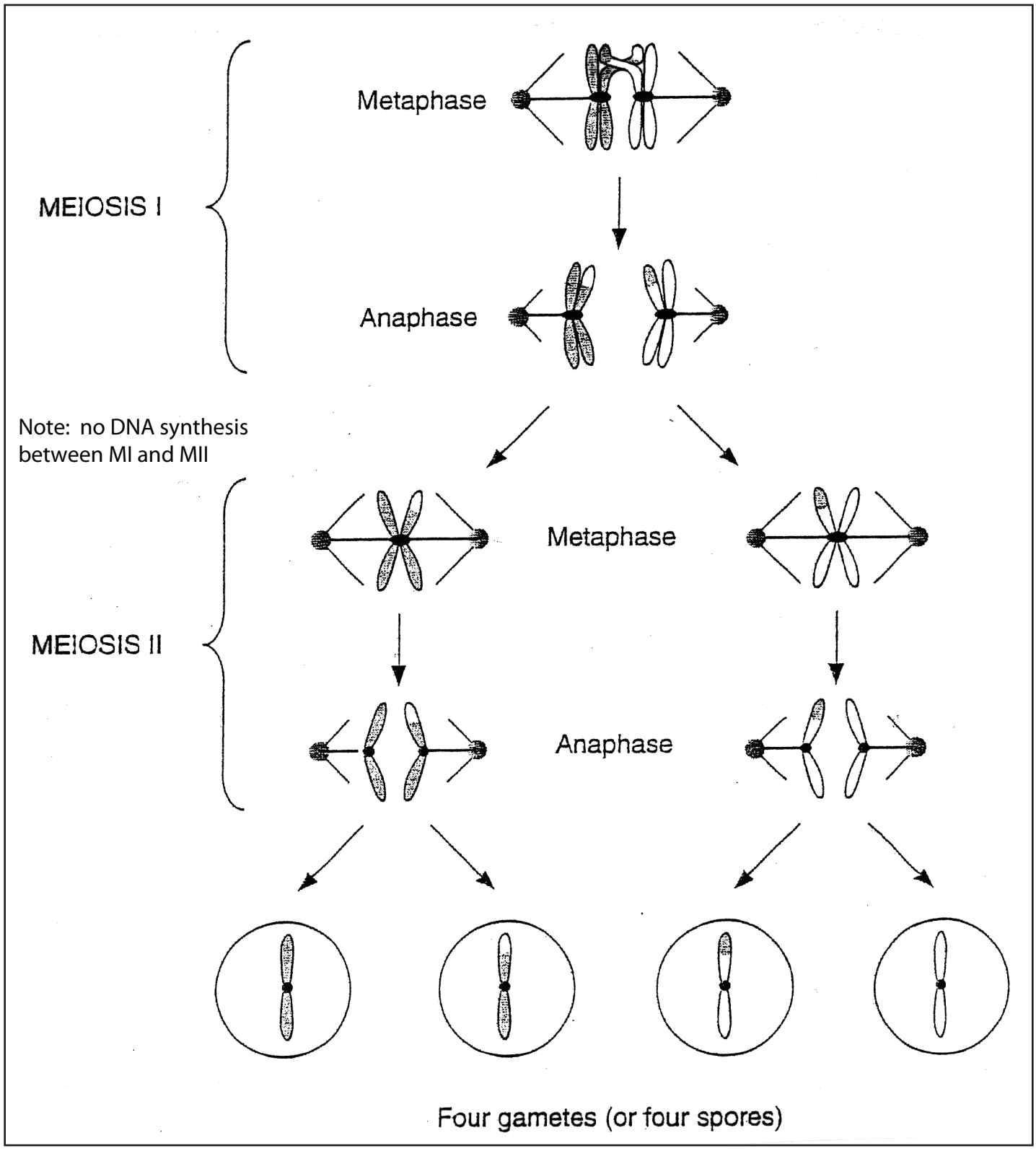
Genetic exchange between the parental chromosomes is acheived by **crossovers** which occur preferentially between homologs instead of sisters (mechanism of this preference is not well-understood). Crossovers have a second purpose: they are required for proper chromosome segregation (**disjunction**) at the ensuing meiosis I division. Without crossovers, the duplicated homologs segregate randomly to each pole (**nondisjunction**).



The second key difference with mitosis is that sister chromatid cohesion is not completely destroyed during meiosis I. Indeed it is essential to maintain it at the centromere so that sisters stay together during the reductional division. However, cohesion in the arms must be destroyed because the regions of chromatids distal to a cross-over would otherwise be pulled in two directions: toward the pole of their sister by cohesion and towards the other pole because they are now attached to their homolog. Thus, there are two distinct types of sister chromatid cohesion in meiosis: centromeric and arm cohesion, which are subject to differential regulation during meiosis I and meiosis II. Not surprisingly, the proteins involved in meiotic sister cohesion are different from those involved in mitotic sister cohesion. Once crossovers are resolved, homologs (one from each parent) segregate away from each other and nuclear division ensues.

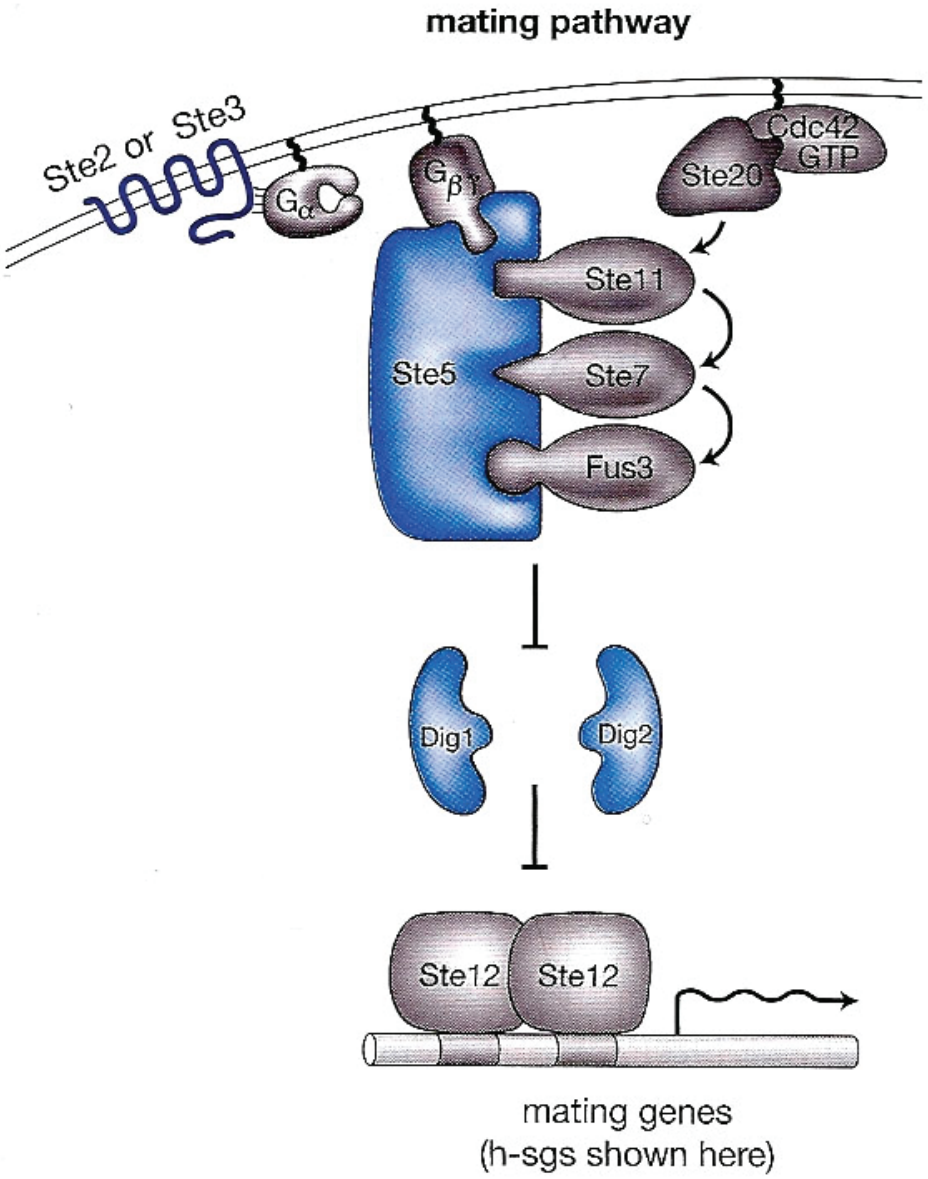
Meiosis II is a mitotic division in which sister cohesion (which remains at centromeres after meiosis I) is lost and sister chromatids segregate aways from each other, yielding four haploid products.




MEIOSIS I
Metaphase
Anaphase
Note: no DNA synthesis
between MI and MII
MEIOSIS II
Metaphase
Anaphase
Four gametes (or four spores)

Phereomone response requies a G-protein coupled receptor and a kinase cascade

The molecules by which *S. cerevisiae* sense mating pheromone from the opposite mating type were identified by first identifying mutants incapable of mating (“sterile” mutants), isolating the corresponding genes, and then defining the molecular function of the encoded proteins using biochemical approaches. This is arguably the most powerful, tried-and-tested approach in biology and is the means by which most of our knowledge of the molecules that control cell behavior has been obtained.



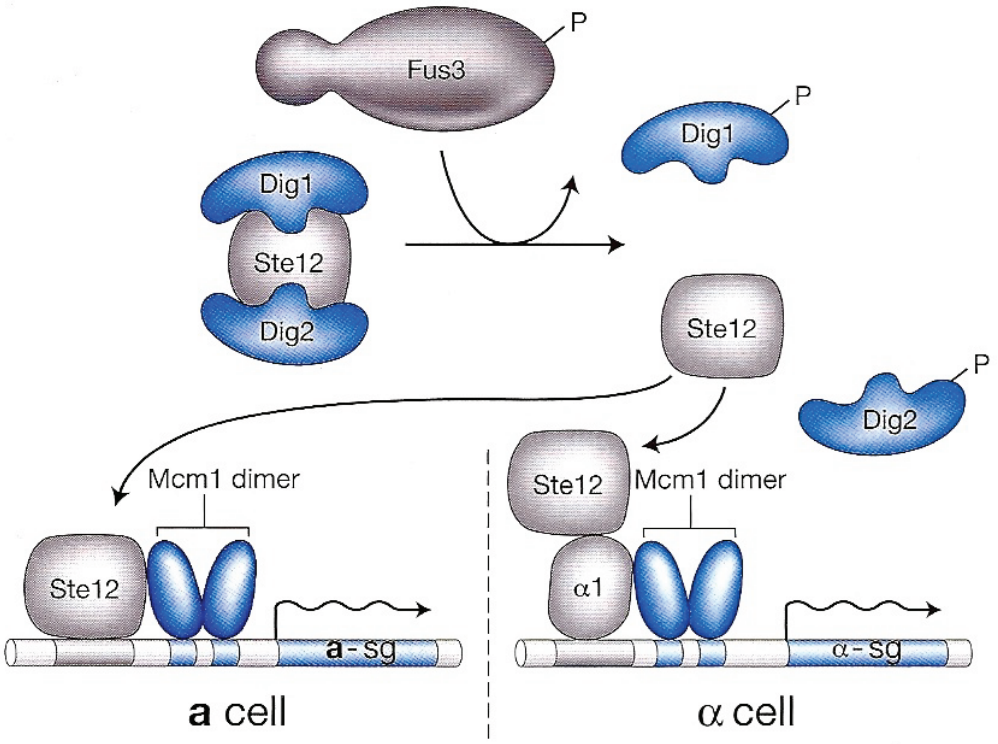


Figure 5-6. The Fus3 MAP kinase activates the transcription factor Ste12 by phosphorylating and inactivating inhibitors of Ste12.

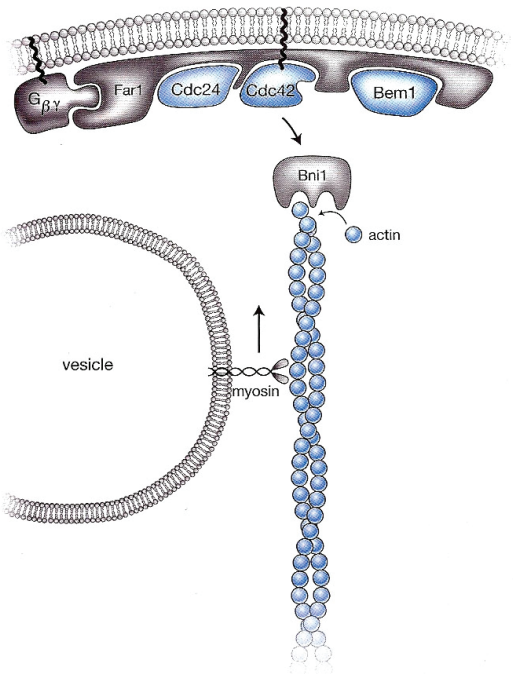
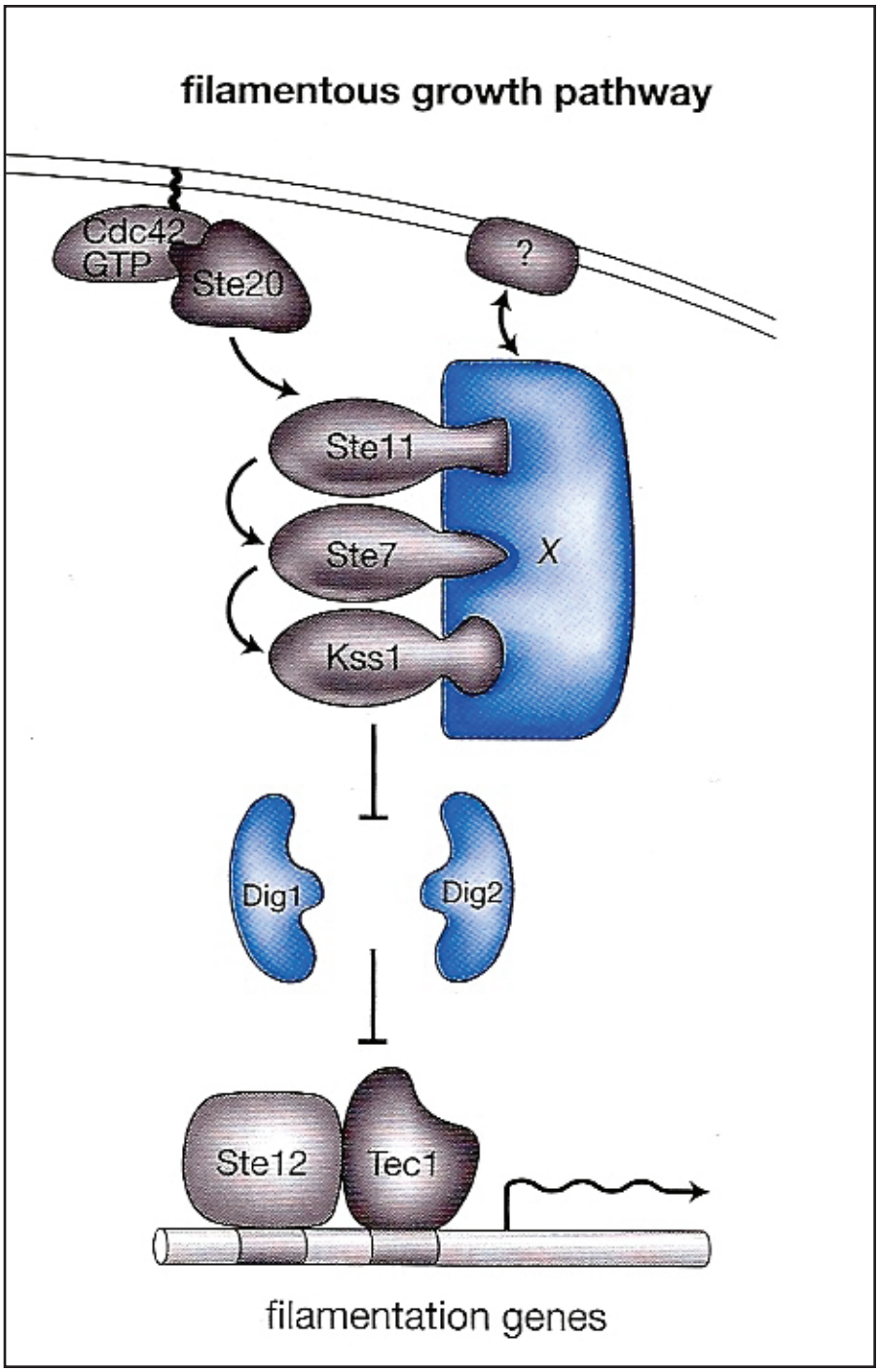


Figure 5-7. $G_{\beta\gamma}$ induces polarized cell growth by promoting actin polymerization. Far1 binds to the free $G_{\beta\gamma}$ complex as well as Cdc24, Cdc42, and Bem1. Cdc24 activates Cdc42 by promoting the exchange of GTP for GDP. Active Cdc42 binds to Bni1, inducing actin polymerization in the vicinity, which leads to the delivery of secretory vesicles. Movement of these vesicles to the plasma membrane requires a "myosin"—this is a so-called "motor protein" that uses the energy of ATP hydrolysis to walk long actin filaments. Not shown are two positive-feedback mechanisms that reinforce cell polarization at the site of highest $G_{\beta\gamma}$ concentration: (1) secretory vesicles deliver additional Cdc42 protein to the plasma membrane, increasing its concentration; and (2) GTP-bound Cdc42 binds Bem1 increasing the affinity of Bem1 for the complex; Bem1 in turn binds to Cdc24, thereby increasing the association of Cdc24 with the complex, which in turn promotes the activation of Cdc42.

The pheromone response pathway shares signaling components with other pathways: signaling specificity

A major surprise was the finding that many elements of the pheromone response pathway are also required for *S. cerevisiae* to undergo filamentous growth. How erroneous cross-talk is prevented between these two pathways remained a mystery until it was discovered that the MAP kinase for the pheromone respone pathway, Fus3, phosphorylates and triggers the ubiquitin-dependent degradation of the Tec1 transcription factor specific to the filamentous growth pathway.



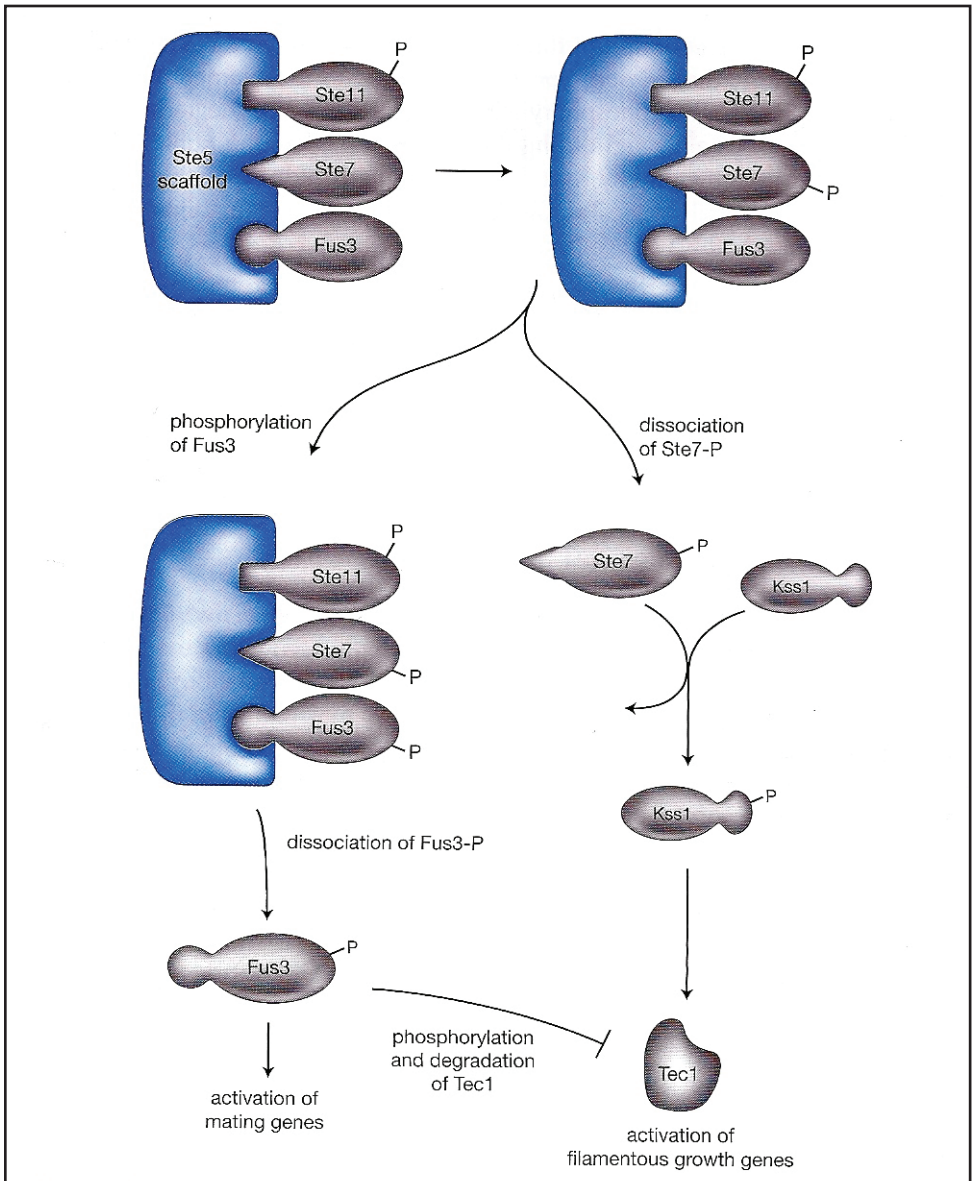


Figure 5-11. Pheromone-induced transcription of filamentous growth genes is prevented by Fus3-induced phosphorylation and degradation of the transcription factor Tec1.

Nomenclature and markers

Genetic nomenclature in *S. cerevisiae* is simple. Genetic names are italicized. Three letters and a number for each gene (e.g. *HIS3*). Wild type and dominant alleles are capitalized. Recessive mutant alleles are lower case and the allele is designated by a number (e.g. *his3-1*).

Because yeast can be grown in defined media, popular markers are mutations that produce auxotrophies (a nutrient requirement). These are assayed on synthetic media. "Drop-out" media lacks a particular small molecule, typically an amino acid or nutrient. For example, a *his3-1* mutant is defective in the biosynthesis of histidine. While it can grow on synthetic complete medium (medium containing all amino acids and nucleotides), it fails to grow on histidine drop-out media (abbreviated "SC-His" for "synthetic complete minus histidine"), and therefore is said to have a His - (as opposed to His+) phenotype.