The exercise practiced in the lecture, with their solution

haploid yeast will have hsg active whic supress dsg. also depending on the mating type. yeast will express pheromones of its mating type and have receptors of the opposite mating type. MF-alpha supresses asg genes while MFA1,2 suppress alpha-sg. After mating, the heterdimer a1/alpha1 will supress hsg and MFA2 which supresses totally the asg genes. Also alpha-sg are suppressed since MF-alpha1 changes its function in the heterodimer, so it's simply not present to activate alpha-sg.

Exercise 1

Question 1:
The mating pheromone a-factor is a peptide encoded by two genes, identical in sequence, *MFA1* and *MFA2*. In contrast, the alpha-factor pheromone is encoded in a single gene, *MFalpha1*. Explain how yeast cells ensure that they express these genes and others as a function of their mating type and ploidy, such as to be able to detect and mate with partners of opposite mating type.

Response: As in the script.

Question 2:
In a strain of genotype *Mata*/*Matalpha his3*Δ/*his3*Δ *leu2*Δ/*leu2*Δ the *MFA1* coding sequence is replaced by the *HIS3* coding sequence on one chromosome and a second construct composed of the promoter sequence of the *MFalpha1* gene placed upstream of the *LEU2* coding sequence is inserted heterozygously at the *leu2* locus. What auxotrophy phenotypes do you expect for this strain? This strain is induced to sporulate, and the tetrads are dissected. Explain what types of tetrads you expect to observe and how you expect the different spores to grow on rich medium, synthetic medium lacking histidine, and synthetic medium lacking leucine.

Response:
The diploid would not grow on –HIS and –LEU media since the pheromone genes are haploid specific genes and repressed in the diploid.

After sporulation, each of the MFA1p:HIS3 and MFalpha1p:LEU2 loci segregate 2:2, independently of each other and of the MATa loci. As a consequence, half of the MATa cells are [HIS+] and half are [his-]; all of them are [leu-] since even those that inherited the MFalpha1p:LEU2 construct fail to express it. Similarly, half of the MATalpha cells are [LEU+] and half are [leu-]; all of them are [his-] since even those that inherited the MFA1p:HIS3 construct fail to express it.

Question 3:
A researcher realized that this strain is ideal to look for additional karyogamy mutants. Can you suggest how s/he could proceed to do so?

Response:
He could randomly mutagenize a MATalpha rho0 strain carrying the MFalpha1:LEU2 construct, mate it with a MATa, RHO+ wild type haploid and select immediately after for cells growing on medium lacking leucine and having glycerol as sole carbon source.

Or vice versa mutagenized, he could randomly mutagenize a MATa MFA1:HIS3 Rho- strain and mate it to a WT MATalpha Rho+ and select for Rho+ [HIS3+]

The growth on glycerol shows that the cells have received mitochondria from their partner, while the growth on medium lacking either leucine (in the first case) or histidine (in the second one) shows that they remained haploid and hence, failed to undergo karyogamy with the nucleus of their partner. Hence, these growing clones must have acquired a mutation affecting their ability to undergo karyogamy upon mating.

Exercise 2:

Budding yeast form their spindle pole bodies (SPBs, the microtubule-organizing centers – MTOCs – and centrosome equivalent) by duplication, such that each dividing cell has one newly synthesized SPB at one end of their mitotic spindle and an old SPB (inherited from the previous division cycle) at the opposite spindle end. These SPBs then segregate in a non-random manner upon cell division: The mother cell keeps the new SPB and the old SPB segregates to the bud. The biological relevance of this conserved pattern of MTOC inheritance is still completely mysterious.

Experimental approach: To study this process, a graduate student decided to search for yeast mutants that segregate SPBs in a more random manner. These studies identified two kinases, called Swe1 and Kin3, and an acetyltransferase, called Yaf9. The Swe1 kinase is known to phosphorylate Cdc28, a cyclin-dependent kinase and the main driver of the cell cycle, on tyrosine 19 (Y19) and to thereby inhibit it. Kin3 has no known function and Yaf9 has no known role in cell cycle events. The effects of the following mutations and combinations thereof are summarized in table 1 below.

Mutant genotype	% of divisions with new SPB in the bud		
	All cells	Cells with young SPB from previous mitosis	Cells with old SPB from previous mitosis
WT	5%	5%	5%
swe1Δ	26%	48%	5%
CDC28 Y19F	5%	5%	5%
kin3Δ	19%	12%	26%
yaf9Δ	23%	10%	35%
swe1Δ CDC28 Y19F	26%	48%	5%
swe1Δ kin3Δ	41%	50%	31%
swe1Δ yaf9Δ	42%	49%	35%
kin3Δ yaf9Δ	32%	14%	50%
swe1Δ kin3Δ yaf9Δ	50%	50%	49%

Table 1. The swe1Δ, kin3Δ and yaf9Δ alleles are complete null alleles, where the entire open reading frame of the gene is deleted. The *CDC28 Y19F* allele is a dominant active form of the *CDC28* gene, which encodes a form of the kinase where the tyrosine in position 19 is replaced by a phenylalanine (similar amino acid but that cannot by phosphorylated).

Question 1:
Please, indicate the epistasis relationship between these genes and propose an order of action.

Response:
SWE1 acts on new SPBs but only little on old ones, KIN3 and YAF9 act mainly on old SPBs and a little bit on new ones. CDC28 is not involved. On old SPBs, KIN3 and YAF9 act in parallel of each other. On old SPBs, Swe1 is in the YAF9 pathway and therefore acts somewhat redundantly with KIN3.

In parallel, Nud1, also called centriolin in mammals, is a conserved protein of MTOCs the inactivation of which fully randomizes SPB inheritance in yeast. Interestingly, mass-spectrometry data indicate that it is phosphorylated on two residues in its N-terminus (serine 35 -S35- and threonine 70 -T70) and acetylated on lysine 41 (K41). Mutations of these residues lead to the observations reported in the table 2 (below).

Question 2:
What do you conclude from these results? What prediction and what measurements would like to make to confirm your model? Please, draw what you would expect to happen on the SPBs over the division cycle and upon SPB inheritance.

Mutant genotype	% of divisions with new SPB in the bud		
	All cells	Cells with young SPB from previous mitosis	Cells with old SPB from previous mitosis
nud1 S35A	19%	12%	26%
nud1 T70A	26%	48%	5%
nud1 K41R	23%	10%	35%
nud1 S35A swe1Δ	41%	50%	31%
nud1 S35A kin3Δ	19%	12%	26%
nud1 S35A yaf9Δ	32%	14%	50%
nud1 T70A swe1Δ	26%	48%	5%
nud1 T70A kin3Δ	41%	50%	31%
nud1 T70A yaf9Δ	42%	49%	35%
nud1 K41R swe1Δ	42%	49%	35%
nud1 K41R yaf9Δ	23%	10%	35%
nud1 S35A K41R	32%	14%	50%
nud1 T70A K41R	42%	49%	35%

Table 2. The nud1 S35A, nud1 T70A and nud1 K41R alleles are mutations where the corresponding serine, threonine and lysine have been replaced either by an alanine (A) or by an arginine (R) in order to prevent modification without changing much the structure and charges on the protein. The other alleles are as in the table 1.

Response:
All three genes act on Nud1. Swe1 is in the same pathway as T70, Kin3 is in the pathway of S35, and Yaf9 on that of K41. The most parsimonious hypothesis is that Swe1 phosphorylates T70, Kin3 phosphorylates S35 and Yaf9 acetylates K41. To test this hypothesis, the simplest is to use the same mass-spectromtry method as that mentioned above on the Nud1 protein prepared from extracts of the swe1Δ mutant cells, from cells lacking Kin3, and from cells lacking Yaf9. If our hypothesis is correct, the first batch of cells should no-longer show phosphorylation of T70, the second batch should lack phosphorylation of S35, and the third one should specifically lack acetylation on K41.

Question 3:
Knowing that SPB duplication starts upon activation of Cdc28 and based on the results above, please propose a model for how cells distinguish between preexisting and newly synthesized SPBs.

Response:
When a new SPB is present in the cell the mark of Swe1 phosphorylation on T70 is necessary to allow wt segregation. –Swe1 gives the "old mark"
If there is an old SPB in the cell, the joint marking of Nud1 by Yaf9 (on K41) and Kin3 (on S35) is necessary to allow a wt segregation. Yaf9 and Kin3 therefore keep the old mark active… In support of this last model, the young SPB needs phosphorylation on T70 by Swe1 in order to allow acetylation of K41 by Yaf9, as suggested by the fact that Yaf9 and Swe1 act in the same pathway for old SPBs.

Exercise 3

The temperature sensitive mutations *a* and *b* were both identified in the same genetic screen for cell cycle mutants. At the restrictive temperature (37°C), the *a* mutation causes the cells to arrest as unbudded cells with a single nucleus and a 1C DNA content. Under the same condition, the *b* mutation causes the cells to arrest with a large, elongated bud, a 2C DNA content and a single nucleus. Thus, both mutations affect indeed cell cycle progression, yet at distinct points in the cell cycle. We want now to understand how these mutations affect cell cycle progression, and what is the function of the corresponding gene.

Question 1:
Haploid cells carrying either one of these two mutations are crossed with a wild-type haploid strain of the opposite mating type. The diploids obtained behaved in both cases like wild type cells, showing no cell cycle arrest when grown at the restrictive temperature. Explain what this means.
The *a*/+ and the *b*/+ diploids were induced to sporulate. Upon dissection, two types of spores were identified in both cases and in identical proportions: 50% of the spores give raise to colonies that behave like wild type, whereas the other 50% of the spores lead to colonies that grow at low temperature but stop dividing when grown at 37°C. Finally, when haploid cells carrying the *a* mutation were crossed with haploid cells of opposite mating type and carrying the *b* mutation, the resulting diploid cells were temperature sensitive. When these cells were grown at 37°C, they all arrested as unbudded cells with a single nucleus and 1C DNA content. When these diploid cells were induced to sporulate at permissive temperature, all spores were temperature-sensitive. When grown at 37°C, progenies from 50% of the spores arrested as unbudded cells, while the progenies from the other 50% of the spores arrested as large budded cells. What do you conclude from these observations?

Response:
If the diploids are temperature sensitive this means that there is no additional wt copy to complement the mutation, or that these mutations are in two genes but are in a case of non-allelic non-complementation.

To distinguish between these two possibilities, we look at the segregation of the traits after sporulation. And since all haploid spores, or segregants, of this cross are temperature sensitive, the mutations are in the same gene.

The fact that wt crosses of mutants behave like wt show that these mutations are recessive.

Together, these results show that the same gene (now called AB), is involved in two different processes in the cell cycle: promoting the G1/S transition and promoting mitotic entry. These two functions can be separated by separation of function alleles, such as the alleles a and b. AB is probably an essential gene (ts). We will now note the mutant a as ab-a (allele a of the mutant gene ab) and the mutant b: ab-b.

Question 2:
The gene *C* is not essential. Upon its deletion, the cells are larger and stay longer in G1 prior to entry into the cell cycle, but form colonies as quickly as wild type (only with less but bigger cells). Likewise, the *D* gene is also not essential. However, cells lacking it form longer buds and delay the onset of mitosis, still making colonies as large as wild type colonies. Remarkably, the *a* *c*Δ double mutant is dead at all temperature, whereas the *a* *d*Δ double mutant shows growth phenotypes very similar to those of the *a* single mutant. Conversely, the *b* *c*Δ double mutant is alive at low

temperature and arrests at 37°C very much like the *b* single mutant cells. The *b dΔ* double mutant cells are not viable under any condition. What hypotheses can you make to explain these results? Are you surprised by the fact that the *C* and *D* genes are not essential? How would you possibly explain this finding?

Response:
Mutations in the genes AB and C are synthetic lethal (epistatic). As well as mutations in genes AB and D. The gene ab-a and C are on one genetic pathway and D and ab-b are on another. Indeed: you can conclude about synthetic lethality indicating that two genes are in parallel pathways only if you are working with complete loss of function mutations. The ab-a and ab-b mutations are ts, and therefore not full deletions…

In such a case, a synthetic lethality between two mutations generally indicate that they act in the same pathway. For example, C activates AB for the G1/S transition, and D activates AB for the G2/M transition. If you lose C, the a allele is not stimulated enough at the permissive temperature to overcome its mutation and promote the G1/S transition. Similar scenario for the relationships between D and the b allele. All in all, we now say that C acts together with AB for the G1/S transition and D with AB in the G2/M transition. The fact that C and D are not essential is strange. Perhaps, there is another gene acting in parallel to C?

Question 3:
A haploid strain carrying the *a* mutation was transformed with a mixture of about 10^8 different plasmids where each plasmid contains a different fragment of the wild type yeast genome as an insert. Two plates containing about 10^6 transformants each where then incubated at 37°C for three days. On each of these plates about 300 colonies formed over this time. Explain why they grew, and what could explain their number.
When 50 clones were analyzed, 32 contained the *A* gene in the insert, 7 contained gene *C*, and 11 contained a gene *E*. Formulate hypotheses about why this is the case. What would you expect the phenotype of the *cΔ eΔ* double mutant to be? Provide a hypothesis about how the proteins encoded by the different genes identified here may function together.

Response:
The gene AB is essential.
E and C can both "rescue by increased dosage the A gene". It is generally correct to conclude in such as case that the multicopy suppressors stabilize the point-mutated, essential protein AB upon their overexpression. Thus, a simple and likely possibility is that the gene products of C and E interact with AB such as to promote the G1/S transition. Interestingly, E seems to do something similar to what C does. Thus, E could be the gene we were looking for that might act in parallel to C. If we are right, we expect the cΔ eΔ strain to be non-viable.

Actually, this entire story tells us how Cdk1 (AB) was identified in cerevisiae, and how the cyclins (C, D, E) were subsequently identified. C and E are the G1 cyclins Cln1 and Cln2, and D is the mitotic cyclin Clb2 (or Clb1, 4, 5… which were all identified the same way).

Exercise 5

Question 1:
A strain of genotype *MATa cys3-1* is crossed with another strain of genotype *MATalpha pro3-Δ2*. The mutation *cys3-1* makes the cells auxotroph for cysteine whereas the mutation *pro3-Δ2* makes them auxotroph for proline. The diploid was induced to sporulate and 100 tetrads were dissected and for each of them the genotype of each of the four spores was determined. All spores were viable on rich medium. For what concerns the ability to grow on medium without cysteine or on medium without proline these 100 tetrads contained 42 parental ditypes (PD), 43 non-parental ditypes (NPD) and 15 tetratypes (TT).

Explain what PD, NPD and TT correspond to. What do you conclude from these results.

Response:
One of these genes is close to the centromere and the other a bit further away (d=15/200). They are on different chromosomes.

Question 2:
A Swiss scientist mutagenized his strain (strain SW-A), derivative from a wild type cell isolated on a grape in Höngg. From this mutagenesis he recovered a point mutation that conferred a temperature sensitive (noted [*ts*]) phenotype to the cells: the cells grew at wild type speed when incubated at room temperature, whereas at the restrictive temperature (37°C) they stopped proliferating at the G1/S transition and made no colony. The mutation is noted *cdc4-1*. Our scientist wants to cross this mutation into a wild type strain isolated in the USA (strain USA1). Both strains are fully prototroph and of opposite mating type.

After mating them, he sporulated the diploid and dissected 100 tetrads at room temperature. Out of these tetrads he observes that 25 % of the spores grow very slowly already at room temperature. These spores are always [*ts*] when incubated at 37°C, showing the G1/S arrest of the *cdc4-1* mutant cells. This new phenotype is noted [*its*] for increased temperature sensitive. The distribution of this phenotype in tetrads indicates that the 100 tetrads fall into three categories. The first category (17 tetrads) has no [*its*] spore. In each of these tetrads, two spores are [*ts*] whereas the two others grow at all temperatures ([*WT*]). In the second category (67 tetrads), the tetrads contain one [*its*] spore, one [*ts*] spore and two [*WT*] spores. The last category (16 tetrads) corresponds to tetrads that contain two [*its*] spores and two [*WT*] spores.

What do you conclude from these results?

Response:
There is one more mutation (different gene, suggested by the segregation pattern) in the USA1 strain that has an epistatic relation with cdc4-1. Namely, that additional "mutation" is synthetic lethal with the cdc14-1 mutation. However, since we are not talking of a mutatnt but of wild type cells, we should rather say that the USA1 strain contains a variant allele at a second locus that is fully unlinked from the CDC4 gene. This is probably a wild type variant in the context of the USA1 genome, but not in the SW-A genome. Let's call that locus the locus ICF1, for interacts with Cdc four. This locus exists in two variants: as $ICF1^{sw\text{-}a}$ in the SW-A background and $ICF1^{usa1}$ in the USA1 background.

Question 3:

One [*its*] spore is crossed back to the original [*ts*] strain (SW-A background) and 100 tetrads are isolated and analyzed again. Here, all tetrads are ditypes, containing two spores [*ts*] and two spores [*its*]. When one of the [*its*] spores is crossed back to the strain USA1, what do you expect to observe?

Response:
You should expect that the $ICF1^{usa1}$ locus is now homozygous in that cross. Therefore, 50% of the spores will be [its] and the rest 50% will be [WT].

Question 4:
In reality, the scientist observes that this last cross gives him three types of tetrads again: 17% of the tetrads contain two [*its*] and two [*WT*] spores. The next 17% of the tetrads contain two [*ts*] and two [*WT*] spores. The last 66 % of the tetrads contain one [*ts*], one [*its*] and two [*WT*] spores.

What can you suggest to explain this observation?

Response:
The first reaction would be: There is a suppressor mutation in the USA1 strain that functions as a suppressor of the temperature sensitivty. But let's think about it more broadly and hence a little differently: What you have to conclude here is that there is a second locus, which we could call ICF2 and which exist as $ICF2^{usa1}$ in USA1 and $ICF2^{sw-a}$ in SW-A. This locus is such that the $ICF1^{usa1}$ $ICF2^{usa1}$ cdc4-1 cells have the phenotype [ts], the $ICF1^{sw-a}$ $ICF2^{sw-a}$ cdc4-1 cells as well, and that the $ICF1^{usa1}$ $ICF2^{sw-a}$ cdc14-1 cells are [its], as well as the $ICF1^{sw-a}$ $ICF2^{usa1}$ cdc14-1. In summary, what this says is that the protein Cdc14 is probably activated or activates a complex made of at least two proteins, Icf1 and Icf2. This complex requires the two proteins to fit to each another. This is the case in both wild type background but the interface between these proteins has evolved, such that the tow proteins no-longer recognize each other correctly between the two backgrounds. In that sense, there are no mutants here, only wild type variants…