These exercises are based on former exam questions.

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.

Question 2:

In a strain of genotype *Mata*/*Matalpha* *his3*∆/*his3*∆ *leu2*∆/*leu2*∆ 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.

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?

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 Cells with old SPB

from previous mitosis from previous mitosis

*WT* 5% 5% 5%

*swe1∆* 26% 48% 5%

*CDC28 Y19F* 5% 5% 5%

*kin3∆* 19% 12% 26%

*yaf9∆* 23% 10% 35%

*swe1∆ CDC28 Y19F* 26% 48% 5%

*swe1∆ kin3∆* 41% 50% 31%

*swe1∆ yaf9∆* 42% 49% 35%

*kin3∆ yaf9∆* 32% 14% 50%

*swe1∆ kin3∆ yaf9∆* 50% 50% 49%

Table 1. The swe1∆, kin3∆ and yaf9∆ 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.

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 Cells with old SPB

from previous mitosis from previous mitosis

*nud1 S35A* 19% 12% 26%

*nud1 T70A* 26% 48% 5%

*nud1 K41R* 23% 10% 35%

*nud1 S35A swe1∆* 41% 50% 31%

*nud1 S35A kin3∆* 19% 12% 26%

*nud1 S35A yaf9∆* 32% 14% 50%

*nud1 T70A swe1∆* 26% 48% 5%

*nud1 T70A kin3∆* 41% 50% 31%

*nud1 T70A yaf9∆*  42% 49% 35%

*nud1 K41R swe1∆* 42% 49% 35%

*nud1 K41R yaf9∆* 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.

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.

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?

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∆* double mutant is dead at all temperature, whereas the *a d∆* double mutant shows growth phenotypes very similar to those of the *a* single mutant. Conversely, the *b c∆* double mutant is alive at low temperature and arrests at 37°C very much like the *b* single mutant cells. The *b d∆* 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?

Question 3:

A haploid strain carrying the *a* mutation was transformed with a mixture of about 108 different plasmids where each plasmid contains a different fragment of the wild type yeast genome as an insert. Two plates containing about 106 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 *c∆ e∆* double mutant to be? Provide a hypothesis about how the proteins encoded by the different genes identified here may function together.

Exercise 4

Question 1:

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Question 2:

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Question 3:

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Exercise 5

Question 1:

A strain of genotype *MATa cys3-1* is crossed with another strain of genotype *MATalpha pro3-∆2*. The mutation *cys3-1* makes the cells auxotroph for cysteine whereas the mutation *pro3-∆2* 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.

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 were 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?

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?

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?