Schriftliche Sessionsprüfung

Concepts in Modern Genetics

(WS 06/07)

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Donnerstag 23.8.2007
HPV G5
9:00 - 11:30

Hilfsmittel: keine / Dauer: 150 Minuten

Name, Vorname: _____

Email: _____

Eingeschrieben: ETH UZH

Unterschrift _____

Remarks

- Maximum number of points in total: 24
- Maximum number of points for every question is stated.
- The exam is diveded into **4 parts (I - IV)**. The answers for the different parts **must be on separate sheets**!
- Please indicate on the question sheet if you don't answer a question.
- Label all **extra sheets with your name, first name and page number / total page number (e.g. 3/12).**
- We accept answers in **English and/or German.**
- **Unreadable and unclear writing** and **drawings** will not be interpreted by the examiners, and will not be considered when assigning points.

Part I

Question 1 (2 points)
(A) Which is the predominant force in histone-DNA interactions? Why?
(B) List the different histones according to their binding strength to DNA (weak < strong).
(C) Describe briefly one experiment that supports your statements in (A) and (B).

Question 2 (2 points)
What is the significance of differential binding of histones for chromatin structure, transcription and replication?

Question 3 (2 points)
(A) Why does UV-C light (100 - 290 nm) damage DNA? What are the most frequent DNA-lesions generated by UV-C?
(B) What is the most prominent pathway to remove UV-lesions from chromatin? Schematically illustrate the individual steps and indicate the enzymatic activities required in the individual steps (you don't have to provide the names of the proteins).

Part II

Question 4 (2.5 points)
Unlike in animal cells, disruption of type II myosin, encoded by the *MYO1* gene, is not lethal in yeast. Cytokinesis still takes place in the *myo1Δ* cells, although slower. Disruption of a second gene, *HOF1*, has the same consequences. However, if *MYO1* is co-deleted with *HOF1* in the same cells, the double mutant cells completely fail cytokinesis and die.

(A) What conclusion can you draw from these observations about the genetic control of cytokinesis? (1 point)
(B) What approaches could you use to identify other genes involved in cytokinesis? (1.5 points)

Question 5 (3.5 points)
A multicopy suppressor screen was carried out to identify genes that, when overexpressed, restore the full growth rate of *myo1Δ* cells back to wild type levels. In short, a genomic library of 2micron plasmids, each containing a random fragment of the yeast genome, was transformed into *myo1Δ* cells. After two days, several large colonies were picked among the many small colonies normally produced by the *myo1Δ* cells within that time frame. The plasmids were recovered from the fast growing cells, and sequenced. Upon verification that the fast growing character was linked to the plasmid, this approach identified two genes: *IQG1* and *CYK3*.

(A) The *IQG1* and *CYK3* genes were disrupted in a *ura3-Δ52*/*ura3-Δ52 TRP1*/*trp1-Δ1* diploid strain by replacement of one of the loci with the *URA3* gene. Upon sporulation and tetrad analysis of the two disrupted diploids, the following results were obtained.

iqg1Δ::URA3 strain: All obtained tetrads contained two viable and two dead spores. Scoring the viable spores, the three following types of tetrads were obtained:

ura$^-$ *trp*$^-$ *ura*$^-$ *trp*$^-$	*ura*$^-$ *TRP*$^+$ *ura*$^-$ *TRP*$^+$	*ura*$^-$ *TRP*$^+$ *ura*$^-$ *trp*$^-$
53 tetrads	49 tetrads	4 tetrads

cyk3Δ::URA3 strain: All spores were viable. The three following types of tetrads were obtained:

URA$^+$ *TRP*$^+$ *URA*$^+$ *TRP*$^+$ *ura*$^-$ *trp*$^-$ *ura*$^-$ *trp*$^-$	*URA*$^+$ *trp*$^-$ *URA*$^+$ *trp*$^-$ *ura*$^-$ *TRP*$^+$ *ura*$^-$ *TRP*$^+$	*URA*$^+$ *TRP*$^+$ *URA*$^+$ *trp*$^-$ *ura*$^-$ *TRP*$^+$ *ura*$^-$ *trp*$^-$
19 tetrads	20 tetrads	78 tetrads

What do you conclude from these results? (2 points)

(B) *myo1Δ*::KAN^R cells and *hof1Δ*::KAN^R cells were crossed with the *cyk3Δ::URA3* strain, and upon sporulation 120 tetrades were dissected for each cross. The following results were observed:

In both crosses, only 75% of the spores germinated. Scoring only the full tetrads (4 spores viable), only one class of full tetrads was obtained for each cross:

myo1Δ cyk3Δ cross	*hof1Δ cyk3Δ* cross
URA$^+$ *kan*^{S}	*URA*$^+$ *kan*^{S}
URA$^+$ *kan*^{S}	*URA*$^+$ *kan*^{S}
ura$^-$ *KAN*^{R}	*ura*$^-$ *KAN*^{R}
ura$^-$ *KAN*^{R}	*ura*$^-$ *KAN*^{R}
21 tetrades	18 tetrades

What do you conclude from these results about the genetic control of cytokinesis in budding yeast? (1.5 points)

Part III

Question 6 (2 points)
You found a recessive mutation *a* in Drosophila, which is lethal at the pupal stage when homozygous. You suspect that there is a maternal contribution of the wild-type allele that prevents death at an earlier stage. Design an experiment to test this.

Question 7 (2 points)
Describe how the positive-negative selection works in generating mouse embryonic stem cells with targeted gene replacements.

Question 8 (2 points)
In 1995 Wilikins proposed a model for the divergent evolution of sex determining pathways: the ¨moving up the hierarchy¨ hypothesis. Explain the principle of this hypothesis and the pathways found in insects that support this theory.

Part IV

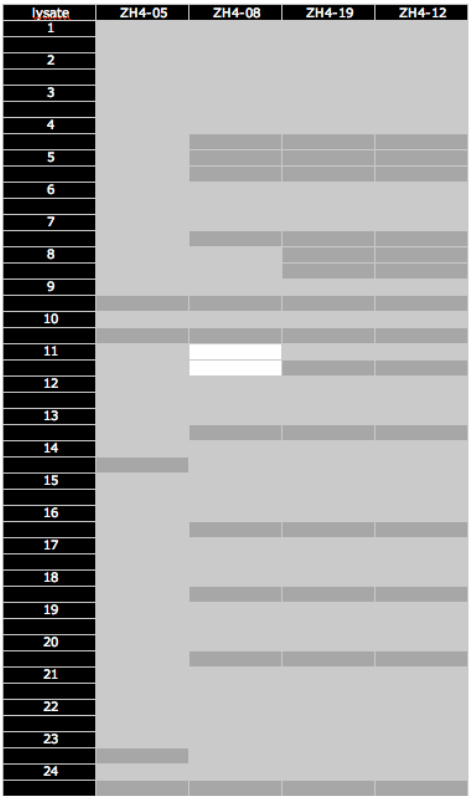
Question 9 (3 points)
Wild-type *Drosophila melanogaster* fruit flies have one R7 cell in each omatidium of their compound eyes. Fruit flies that are homozygous for the *sev* mutation have no R7 cells. Fruit flies that are homozygous for the *yan* mutation have more than one R7 cell per omatidium. Describe an experiment by which you could determine the order of the functions of *sev* and *yan* relative to each other, and discuss the different results you might obtain from your experiment.

Question 10 (3 points)
You have isolated in a forward genetic screen in *C. elegans* a mutation that causes a recessive larval lethal phenotype. You have generated your mutation in the *C. elegans* variety Bristol background, and use the polymorphic strain *C. elegans* variety Hawaii for mapping. (On average, there is one polymorphism every 1,5 kb between *C. elegans* Bristol and Hawaii)

a) Write down the cross you have to do in order to map the mutated gene to a specific chromosome and chromosomal subregion.

b) Using the cross described under (a), you have found linkage to chromosome 4 and are now testing four polymorphic markers covering chromosome 4 in the following order:
left end- zh4-05, zh4-08, zh4-19 and zh4-12- right end of chr. 4

After genotyping 24 recombinants obtained from the cross described under (a), you observe the following distribution of the markers (light gray= Bristol genotype, dark gray= Hawaii genotype, white= no or unclear result)



Where on chromosome 4 does the mutation map? (indicate this in the table)
How can you increase the resolution of your mapping experiment?