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Student #: _____

# BCH4125 2nd Midterm Exam 2012

Professor: Dr. Kristin Baetz

Total Marks: 50 (24 in Section 1, 26 in Section 2)

## Guidelines:

**You must answer all questions directly on the exam paper in the space provided**

You may not consult your class notes, textbooks or other materials

Calculators are not permitted.

You can write this exam in either English or French – BUT ONLY ONE LANGUAGE PLEASE!!

**PLEASE WRITE YOUR NAME AND STUDENT # ON EVERYPAGE**
**You may remove the "description" pages.**

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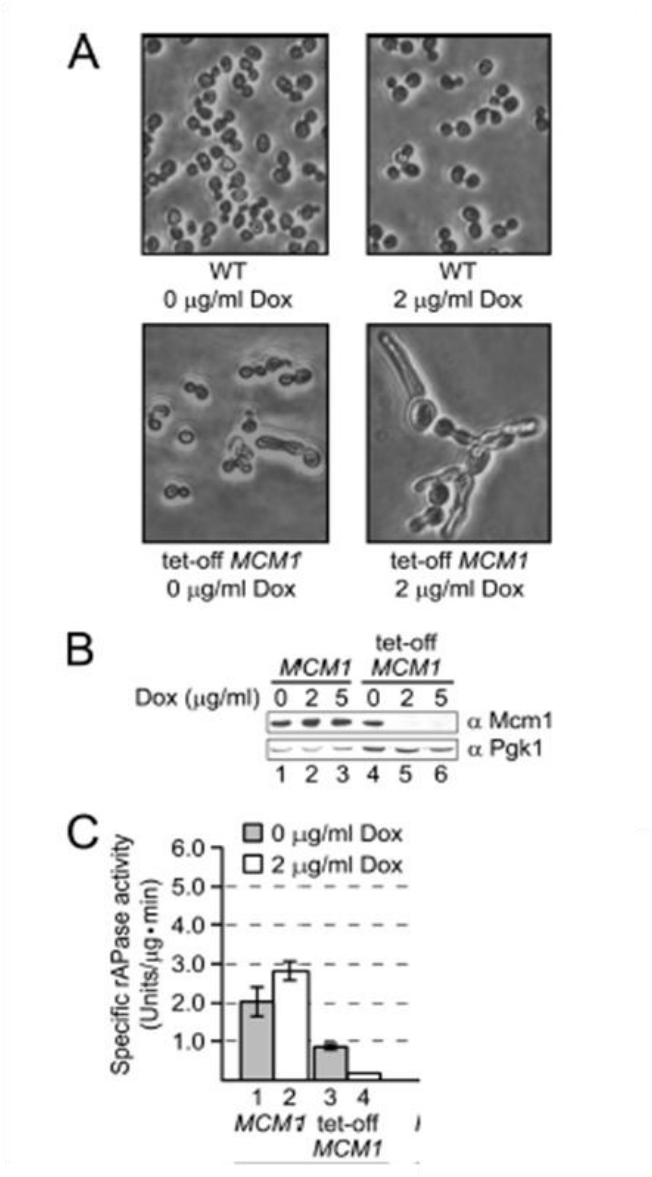
## Section 1 – Cell cycle regulated transcription

**1.** Many cell cycle regulated transcripts code for proteins implicated in micronutrient homeostasis exhibit peak periodic accumulation during M phase, including *PHO5*. Pho5 is a phosphatase that regulates the cellular levels of inorganic phosphate. When inorganic phosphate levels are low, *PHO5* mRNA is induced. As learnt in class, the transcription factor Mcm1 has established roles regulating genes in early G1 such as *CLN3* and *SWI4*, through ECB DNA elements. However, Mcm1 also regulates the transcription of ~80 genes in G2/M or the "CLB2 cluster". In addition to Mcm1, the "Clb2 cluster" genes are regulated by the functional redundant transcription factors Fkh1 and Fkh2, along with the co-activator Ndd1.




Things we know:

- Mcm1 and Fkh1/2 are constitutively expressed and binds DNA throughout the cell cycle
- Fkh1/2 bind DNA independently of Mcm1. Similarly, Mcm1 binding to DNA is independent of Fkhs.
- Neither the Fkhs or Mcm1 have transcriptional activation activity, rather they must recruit Ndd1in order to activate transcription.

The following experiments were performed by the Kladde lab to see if *PHO5* is regulated by Mcm1 and the Fkh proteins during G2/M.

**Figure1.** Because *MCM1* is an essential gene, they constructed a haploid strain, **tet-off *MCM1,*** in which the endogenous promoter of *MCM1* was replaced with *P*tetO7 in a strain containing TetR-Ssn6 that binds to DNA and represses transcription upon Doxcyclin (Dox) addition. A **tet-off *MCM1*** (*P*tetO7:*MCM1* TetR-Ssn6) was compared to a **WT** (*MCM1*) strain after growth in YPD with or without 2 g of Dox/ml and the following assessed:

**A)** Cells were then analyzed for budding morphology by light microscopy

**B)** Mcm1 protein levels were detected by immunoblotting using antibodies (α) against Mcm1 and Pgk1.

**C)** Pho5 activity levels were measured (rAPase activity is proportional to *PHO5* mRNA levels)

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**Question 1.** Draw two FAC profiles of **WT** and **tet off *MCM1*** cells treated with 2ug/mL Dox. Remember to label your axis. (**4 points)**

**Question 2**. From data presented in Figure1 is *PHO5* mRNA levels impacted by Mcm1? Explain in **maximum** 2-3 sentences your reasoning. (**4 points**)

**Question 3**. Is **the tet off *MCM1*** (Ptet07:MCM1) strain in the absence of DOX functioning like WT? From Figure 1 provide **three** specific examples/experiments to support your conclusion (**3 points**).

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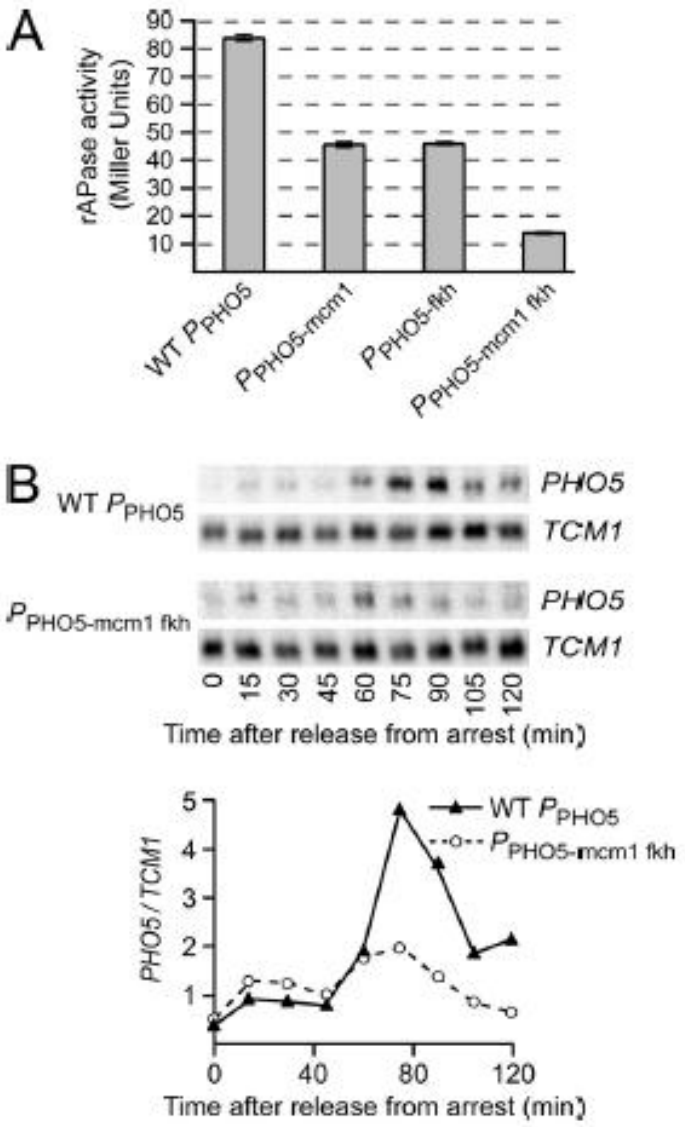


Similar to **tet off *MCM1*,** they determined that *fkh1Δfkh2Δ* displayed decreased rAPase activity. However, they did not know if this is direct or indirect. To address this, they made base substitutions in the candidate binding sites for Mcm1 ($P_{PHO5\text{-}mcm1}$), Fkh1/2 ($P_{PHO5\text{-}fkh}$) or both factors ($P_{PHO5\text{-}mcm1\ fkh}$) in the *PHO5* promoter at its native genomic location. The same mutations have been shown to disrupt essential protein-DNA contacts and thus abolish occupancy at other *CLB2* cluster targets in vitro and in vivo.

**Figure 2 A**. The impact of these mutations to rAPase activity in asynchronous cells

**Figure 2 B.** *PHO5* transcript levels in WT ($P_{PHO5}$) and mutant ($P_{PHO5\text{-}mcm1\ fkh}$) strains. Total RNA isolated at the indicated times after synchronous release from α-factor arrest. The top panel shows the results of RNA blotting for *PHO5* and *TCM1* mRNAs, and the **bottom panel** shows the quantified blotting results, normalizing *PHO5* to*TCM1* transcript levels for both strains.

*TCM1* is a housekeeping gene (Control).

**Question 4.** On the bottom panel of Figure 2B, draw your prediction of *PHO5/TCM1* transcript levels for $P_{Pho5\text{-}mcm1}$ strain. (**2 points**).

**Question 5**. Is the role of Mcm1 and Fkh proteins direct in regulating the G2/M cell cycle burst of *PHO5* transcription? In 2-3 sentences maximum, explain your reasoning by referring to specific experiment(s). (**4 points**)

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**Question 6**. When they performed the cell cycle experiment above (**Figure 2B**), we also collected cells to perform chromatin immunoprecipitations (CHIP) for Mcm1 and Ndd1 using antibodies specific for these two proteins. They looked at strains containing wild type promoter of *PHO5* (WT) or strains in which point mutations for the Fkh binding sites ($P_{Pho5\text{-}fkh1}$) or both Mcm1 and Fkh1 ($P_{Pho5\text{-}mcm1\ fkh1}$) at the endogenous *PHO5* promoter. Using all the information provided in section 1, draw in the ChIP results you anticipate for Mcm1 (TOP PANEL) and Ndd1 (BOTTOM PANEL).

Asyn = asynchronous.

- Make sure your results are unambiguous (ie clearly drawn) so we can differentiation between no IP, low/little IP, high/enriched IP.
- **11 marks.**