

Problem Set #2 – Germ Cell Determinants
Zoology 470 – Spring 2009
20 Points Total

Problem Set Guidelines

1. Due date: This problem set is due by **2:10 pm on Monday, March 30, 2009. It must be submitted in class**, due to significant problems with submission of Problem Set 1 earlier in the semester.

2. Sources: You may use any sources at your disposal to answer the following questions. Legitimate sources include classmates, knowledgeable friends and colleagues, written documents, and any other scientific resources you find useful. **If you work with other classmates on this problem set, we ask that you list the other students with whom you worked to answer these questions.** Although you may discuss these questions as part of a group, **you are expected to answer the questions as an individual.** If you believe that published references will help you answer these questions, you may cite those references. However, **citation of additional references is not required, nor is it expected.**

3. Answering the questions: This problem set is designed to be answered concisely. **Brief but complete answers should be written in the space provided.** If you find it helpful, feel free to include diagrams in your answers. You need only turn in your answers. Necessary information: All of the information and techniques needed to answer the questions on p. 2-3 have been presented in class, or are to be found in Gilbert's *Developmental Biology*. This problem set requires you to answer questions about germ plasm and primordial germ cells, which are described in Chapter 19 of Gilbert, but other sections of Gilbert may be useful for some questions. Note that the lectures during which this material was covered were Wed. March 11 and Fri. March 13, which were the last two lectures before Spring Break.

Problem Statement

Primordial germ cell (PGC) differentiation and migration are well conserved processes in higher animal embryos. In particular, a great deal of progress has been made in mouse and zebrafish embryos toward the identification of key molecular events in PGC differentiation and migration. Answer the questions on the following pages, which relate to various events involved in PGC differentiation and migration. Although they should not be necessary for students who were present in class and who have access to the course textbook, you may also find the following references helpful. PDFs are available on Learn@UW.

- Bontems, F., Stein, A., Marlow, F., Lyautey, J., Gupta, T., Mullins, M. C., Dosch, R. (2009). Bucky ball organizes germ plasm assembly in zebrafish. *Curr Biol.* 19, 414-22.
- Doitsidou, M., Reichman-Fried, M., Stebler, J., Kopranner, M., Dorries, J., Meyer, D., Esguerra, C. V., Leung, T., and Raz, E. (2002). Guidance of primordial germ cell migration by the chemokine SDF-1. *Cell* 111, 647-59.
- Kedde, M. et al (2007). RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. *Cell.* 131, 1273-86.
- Knaut, H., Werz, C., Geisler, R., and Nusslein-Volhard, C. (2003). A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. *Nature* 421, 279-82.
- Molyneaux, K. A., Zinszner, H., Kunwar, P. S., Schaible, K., Stebler, J., Sunshine, M. J., O'Brien, W., Raz, E., Littman, D., Wylie, C., and Lehmann, R. (2003). The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. *Development* 130, 4279-86.
- Weidinger, G., Stebler, J., Slanchev, K., Dumstrei, K., Wise, C., Lovell-Badge, R., Thisse, C., Thisse, B., and Raz, E. (2003). dead end, a novel vertebrate germ plasm component, is required for zebrafish primordial germ cell migration and survival. *Curr Biol* 13, 1429-34.

Name: _____

Student Number: _____

If you worked in a group, other collaborators: _____

1. On March 10, 2009, Franck Bontems, Mary Mullins, Roland Dosch, and colleagues published a paper in which they identified a gene in zebrafish called *bucky ball* (This is not a joke: the phenotype of the oocytes/embryos resembles “buckyballs”, which are balls of molecular carbon, named for the architect Buckminster Fuller, not our own famous badger!).

a. In *bucky ball* mutant oocytes, mRNA for the germ plasm component *nanos1* fails to translocate to an organelle called the Balbiani body. What technique did Bontems et al. use to determine that *nanos* mRNA mislocalizes? (1 point)

Technique: *in situ hybridization*

b. Circle the correct answer (1 point). *bucky ball* is an example of a maternal effect mutant zygotic mutant

c. Bontems and colleagues showed that *bucky ball* mRNA is sufficient for production of primordial germ cells (PGCs). Describe an experiment that would show this in a zebrafish. **Clearly state the expected outcome that would support your hypothesis (3 points)**

Sufficiency requires that we show that, all of the rhtings being equal, addition of bucky ball alone will cause cells to differentiate as PGCs. The best way to do this (and the way that Bontems et al used) is mRNA overexpression. We then use a PGC marker to look for excess (i.e., ectopic) PGCs. We expect that overexpression of bucky ball will result in a greater number of PGCs in this case.

d. *bucky ball* mRNA is not maintained after the time when zebrafish switch from reliance on mRNAs placed into the oocyte to reliance on zygotic mRNAs. What is the equivalent “switching” event in frogs known as (1 point)?

Equivalent event in frogs: midblastula transition (oddly, though they have no blastula stage, zebrafish people actually call it the MBT in fish, too!)

e. Bontems et al. wanted to show that translation of *bucky ball* mRNA is required for it to exert its effects on germ plasm. Describe an experiment that would show this in a zebrafish. **Clearly state the expected outcome that would support your hypothesis (3 points)**

Morpholino antisense oligonucleotides typically block transltino of the targeted mRNA. If a morpholino were used, we would expect that the organization of germ plasm would be disrupted. This is what Bontems et al found.

2. The laboratory of Erez Raz, at the Max Planck Institute in Göttingen, discovered that a single-stranded RNA binding protein known as *dead end* localizes to germ plasm throughout all stages of PGC development. They found that all vertebrates have a *dead end* homologue.

a. In what structures within the gonads of *male* mouse embryos do you expect Raz and colleagues found *dead end* mRNA? **Assume that the gonads are no longer “indifferent”, and correspond to 8-week human embryos. (1 point)**

Structures where *dead end* mRNA is found in *male* mouse embryos: [Note: an email note corrected this from “6-week” to “8-week”. If a student said something about the gonads not being different, most of the points should be given]. Testis cords (or related ideas using the Latin equivalent names, which are found in Gilbert). You’ll have to decide about “testes”. Exterior tissues in the testis would be incorrect.

b. Normally, *nanos1* mRNA is not translated in somatic (non-germ) cells in zebrafish. In a subsequent paper, the Raz and Agami labs showed that *dead end* protein allows *nanos1* to be translated specifically in PGCs. It does so by preventing the binding of a molecule to the 3’ untranslated region (3’-UTR) of the *nanos1* mRNA that would otherwise prevent its translation. This molecule is not a protein. What type of molecule might it be? **Be as specific as possible. (1 point)**

Specific type of non-protein molecule: microRNA

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3. PGCs must migrate within the embryo to reach their final destination. Recall from class (or from outside reading) that Christiane Nüsslein-Volhard's lab in Tübingen showed that the *Cxcr4* gene encodes a chemokine receptor required for PGC migration in both zebrafish and mammals.

a. In zebrafish, it is possible to create “mosaic” embryos in which a few mutant cells are transplanted into a wildtype host, or vice versa. What result do you expect if wildtype PGCs are transplanted into a *Cxcr4* mutant embryo? **Clearly state your reasoning (3 points)**

In the mutant (which is defective for the receptor), the ligand should be made, and hence WT cells will move properly into the gonadal primordium.

b. Erez Raz's lab was competing with the Nüsslein-Volhard lab at the time, and published a similar paper, this time examining the ligand for *Cxcr4*, which you may recall is called *SDF-1*. They used mRNA overexpression to cause zebrafish embryos to produce excess *SDF-1* protein. Based on what you know, what effects on PGC migration would you expect them to obtain? **Explain your answer (3 points)**

Excess SDF-1 should “confuse” the PGCs by destroying the normal chemotactic gradient of protein. Thus PGCs should show defective migration (this is similar to the experiment with mice that we discussed in class).

c. *SDF-1* and *Cxcr4* have mammalian homologues. Chris Wylie's lab at the University of Cincinnati has examined the function of these proteins in the mouse. They found that mouse mutants lacking *Cxcr4* show failure of PGCs to migrate into their normal location. What technique did the Wylie lab and collaborators use to produce mutant mice that demonstrate the necessity of *Cxcr4* for PGC migration? **(1 point)**

Technique: ***They made knockout mice [note: yes, they needed to do a cross of the heterozygous mice to get homozygotes, but the way the mutant allele was made needs to be mentioned for full credit]***

d. The Wylie lab also examined where *SDF-1* is expressed in the mouse embryo. In what tissue/structure would you expect *SDF-1* to be expressed in mouse embryos, assuming that expression is assayed **before** the differentiation of morphologically different testes and ovaries? **(1 point)**

Tissue/structure expressing SDF-1: ***the gonadal ridge [please be generous in accepting answers similar to this]***

e. In order to track PGC migration in slices of living mouse embryonic tissue, the Wylie lab made transgenic mice into which they introduced DNA for the promoter of a gene known as *Oct4*, a gene normally expressed in PGCs, fused to the coding region of the green fluorescent protein (GFP). What is the name given to this type of construct? **(1 point)**

Technique: ***Reporter construct***

Extra credit! The original mutation in the *Cxcr4* gene in zebrafish was called *odysseus*. Why was the mutation given this name? **To get extra credit, you must address how the name of the mutation relates to the phenotype of the mutant. (1 point)**

Odysseus was a Greek hero of Homer's epic who wandered about, seemingly aimlessly, on his adventures. PGCs in the ody mutant are similar, in that they “wander aimlessly”, instead of migrating toward the gonad.