Reverse Genetics

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Problem 1

Cultured embryonic stem cells (ESC) can be used to create specific sequence alterations in the murine genome and introduce them into a host strain (e.g. knock-ins and knock-outs).

a) How are such rare homologous recombination events selected for? Describe the selection methods applied in mouse ESC.

b) Can this ESC-based system also be used in Drosophila? Explain your answer.

c) What are the benefits of using this technique to injecting sequences into the pronucleus of a one-cell mouse zygote?

d) Give one example of the use of knock-ins in biomedical research.

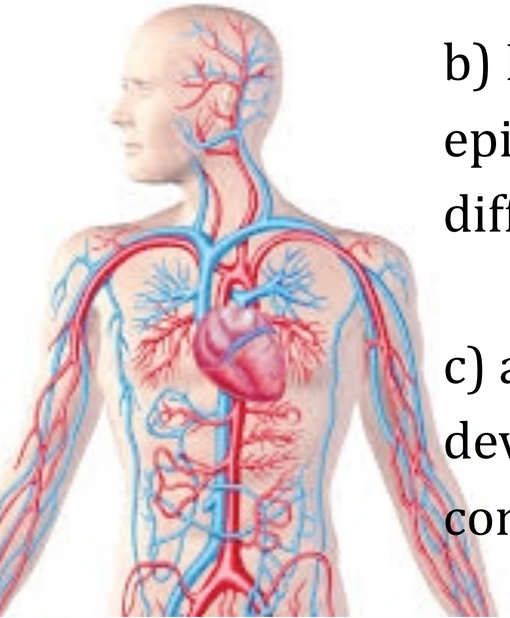
a) -

b) no it cant, because there are no known embryonic cell lines in drosophila, but the idea can stilll be applied

Problem 2

In Drosophila the gene *branchless* (*bnl*) plays an important role in the development of the tracheal (airing) system. It is required for the proper branching of tracheal tubes. The lungs in the mouse also consist of a complex branched tubular system. You are interested in studying whether there is a common genetic basis for the development of branched tubes in invertebrates and vertebrates.

a) How do you find the mouse homolog of *bnl* (*m-bnl*)? If there are *bnl* homologous sequences present in the mouse genome, which would be the next question(s) to be addressed before you start a functional study?



b) How can you experimentally test whether *m-bnl* is required for branching of the epithelial tubes in the lungs when no mutations were available before? Describe two different experimental approaches.

c) as b) but assuming that *m-bnl* is pleiotropic and has vital functions in early development preceding its ¨late¨ function in lung development? What do you have to consider and how may this change your experimental design?

Problem 3

In Drosophila you discovered a new recessive mutation which, when homozygous, is lethal at the pupal stage. The developing imago is stuck inside the pupal case and dies. Therefore you name the affected gene *trapped* (*trp*). Based on its interesting phenotype you decide to analyse the *trp* gene in more details.

A) A main question you want to address is in which tissue *trp* needs to be active to allow normal eclosion from the pupal case. Since TRP protein has structural similarity to secreted neuronal hormones, a likely production site is the brain. How can you test this hypothesis using the available recessive mutation?

B) How would you test this hypothesis when only the gene sequence is available but no mutation. Which reverse genetic tools can be used? Describe one approach in detail. Which possible outcomes may you expect?

Problem 4

What are maternal-effect genes? Give an example. How can you test their requirement in the germline for normal development of the progeny when their function is also essential for somatic development of the mother? Describe an experimental approach.

Problem 5

The *Mesp* transcription factor plays an important role in the development of the heart in the primitive chordate *Ciona intestinalis* (sea squirt). It is required for specification of cells forming the single cardiac tube. The heart of the mouse is multi-chambered and distinctly more complex. Nevertheless, you want to find out whether there is some overlap in the genetic circuits which are used to specify the heart field in primitive chordates and mammals.

a) Describe a method of how the function of *Mesp* can be investigated even in a non-classical model system such as *Ciona intestinalis*.

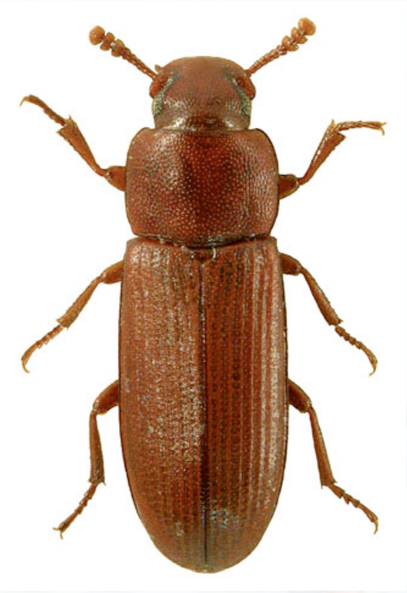
b) How do you find the homolog of *Mesp* in the mouse and how can you test a function of this homolog in heart development of this classical model system? Describe one method in detail.

c) The mouse heart is built of cells migrating from different locations to the heart field during embryonic development. What strategy can be used to find out in which of these cells the *Mesp* homolog is required for correct heart development? Explain the rationale behind your experimental approach

dpnt worry too much aboiut the sex-determination thing, because we did not learn it (lecturer taught it in a previous semester though and they had to know it)

Problem 6

In the red flour beetle *Tribolium castaneum* the homolog of the Drosophila gene *transformer* (*tra*) is believed to have a conserved female determining function. Functional products of this autosomal gene can be only detected in XX female beetles, but not in XY male beetles. To test whether it is indeed involved in sex determination, the gene was silenced by dsRNA injections into embryos. As a result, XX individuals develop male structures, but die at larval stages. XY individuals, on the other hand, show normal male differentiation and are fully viable.



a) Is this outcome in line with a role in sex determination? Explain your answer.

b) How can you experimentally test whether this *tra* homolog is <u>sufficient</u> to impose female development?

c) How can the XX-specific lethality be explained and what process may be affected?

d) Can sex-limited lethality also occur in other XX /XY animal systems? If yes, explain how and give an example

Problem 7

In the parasitic wasp *Nasonia vitripennis* sex is determined by the key switch gene *transformer* (*tra*). When ON it directs female development, when OFF male development follows. In unfertilized eggs zygotic *tra* remains OFF and haploid males develop, fertilization activates zygotic *tra* and diploid females develop. If it is not differences in gene dosage what else may be the basis of differential regulation of *tra*? How is this phenomenon called? Describe a possible scenario of how *tra* is regulated

hint: epigenetics

Does this type of parental gene regulation also occur in mammals? If yes, give an example of a gene regulated in this manner. Give also an example of a human syndrome which is based on a malfunction of this mechanism

Problem 8

A recessive loss-of-function mutation in the Drosophila gene *tegamino* causes female germ cells to arrest at an early stage during oocyte maturation. In Drosophila ovaries, the developing oocyte is surrounded by somatic (follicle) cells which are needed for the proper differentiation of the oocyte.

Design a genetic experiment to test in which tissue - somatic cells or germ cells - *tegamino* must be active to support normal egg differentiation. What are the possible outcomes?

Problem 9

Hox genes play an important role in assigning different identities along the main body axis. In vertebrates, for instance, the transition from cervical to thoracic vertebrae in the vertebral column is defined by the anterior border of expression of the *Hox6c* gene. Compared to other vertebrates snakes have an increased number of thoracic vertebrae at the expense of cervical vertebrae suggesting that expression of the snake's *Hoxc6* gene shifted anteriorly. You work with pythons and would like to investigate this interesting problem.

- You find that expression of python's *Hoxc6* indeed extends into the anterior region. What could be the reason for this difference in expression of *Hoxc6* genes in snakes and other vertebrates e.g. mouse? Design an experiment with which your hypothesis can be tested. (**2 points**)