

National University of Ireland, Galway
 Óllscoil na hÉireann, Gaillimh
 Fourth Science Anatomy: December 2001
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Answer **QUESTION 1** (50 marks) and **TWO** others (25 marks each)

Question 1. Please read this question fully and carefully before attempting to answer.

Background.

During the course of development a particular cell type, BW, migrates from one site in the embryo to another, where it changes phenotype from a relatively undifferentiated state (pre-migration) to a differentiated state (post-migration). This process involves BW cells detaching from their original basement membrane (called BM1), migrating in the embryo, and attaching to a different basement membrane (called BM2) at the site to which they migrate.

You are working in a lab that is studying BW cells, and the changes they undergo during development. You have identified a transmembrane receptor protein that BW cells use to attach to the basement membrane. You have named this protein **Attachin**. You have also identified a soluble protein signal, named **Detachin**, that is produced in embryos at the time BW cells begin migration.

Assumptions.

You may assume that you have all the equipment and reagents (including antibodies, DNA probes, primers etc.) that you require. In particular you have a plentiful source of undifferentiated BW cells that can be maintained in culture, and the basement membrane (BM1) to which they attach. You have purified basement membrane from the site to which BW cells migrate (BM2). You also have pure sources of the proteins, Detachin and Attachin.

You do not have to describe how each technique works. Concentrate on the design of the experiments and the specific techniques that are most appropriate to answer the questions asked below.

Investigations.

- A.** You hypothesize that once levels of Detachin reach a certain critical concentration, BW cells detach from their basement membrane, BM1.
 - i) What experiment(s) would you perform to test this hypothesis?
- B.** Having established that a critical level of Detachin does indeed cause cells to detach from the basement membrane, you are now going to investigate a potential mechanism by which this occurs. You believe that Detachin acts to **reduce** the amount of Attachin found on BW cells, causing them to lose their attachment to BM1.
 - ii) What experiment(s) would you perform to test this hypothesis?
 - iii) Can you suggest **TWO** mechanisms by which Detachin might reduce the levels of Attachin?
 - iv) Outline an experiment which would demonstrate that one of the mechanisms you have proposed actually occurs.
 - v)

After migration, BW cells undergo a significant change in phenotype. The change in phenotype results in the cells assuming a tall columnar appearance with well-developed junctional complexes between them. They also start to express an elaborate cytoskeleton consisting of the intermediate filament protein vimentin, and expressing a cell surface marker protein, CS121, which is not found in the undifferentiated state.

- C.** You now wish to examine how this phenotypic change occurs. Your hypothesis is that it is the attachment of BW cells to the new basement membrane (BM2) which causes this change to occur.
 - v) Having regard to the specific phenotypic characteristics of differentiated BW cells (outlined above), what experiment(s) would you perform to test your hypothesis?

Question 2

Compare transmission and scanning electron microscopy with respect to the microscopes, specimen preparation methods, and the specific circumstances in which you would utilize these techniques.

Question 3

Describe **THREE** light microscopic techniques with which you are familiar, including notes on the specific uses/value of these techniques.

Question 4

Write an essay on techniques of protein electrophoresis.