Questions

1 Your laboratory colleague detaches cells from a plastic dish on which they grow, mechanically with a cell scraper, to prepare single cell suspensions, in order to be able to count them. Unfortunately, these cells, when detached from the plastic, fail to form single cells and instead adhere one to the others. He tells you that he wants to add ethylenediaminetetraacetic acid (EDTA), a Ca^{++} chelating agent, because he heard this might help. However, he is not sure it will help, and he does not have a scientific explanation for the effect of EDTA.

A) Do you think adding EDTA will help to obtain single cells?
B) Can you explain to your colleague how EDTA will help, or why it will not help?

2 Which of the following possibilities has no immediate negative impact on cell-cell adhesion. Explain each proposal.

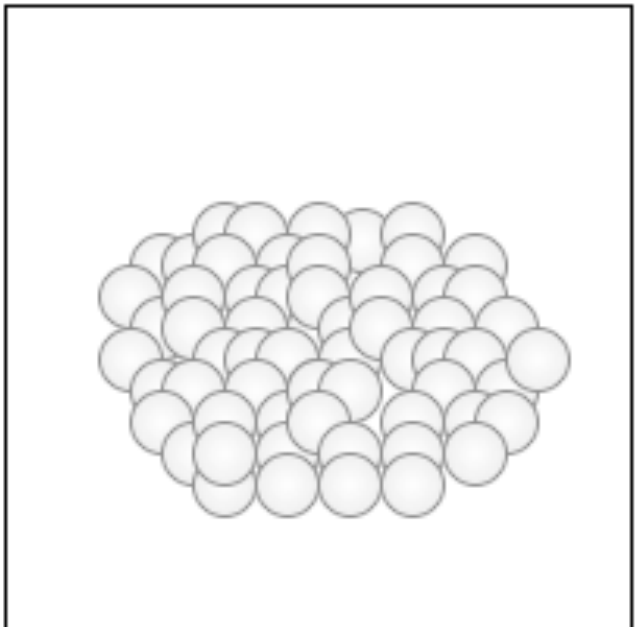
A) Diminish the activity of a transcription factor whose role is to boost the expression of cadherin mRNAs.
B) Incubate cells with trypsin.
C) Diminish the expression of cadherins at the membrane.
D) Mutate cadherins such that they are devoid of the cytoplasmic part.
E) Increasing extracellular [Ca^{++}] to 5 mM.

3 Consider group A epithelial cells that express a unique cadherin, E-cadherin. Through this E- cadherin, these cells make cell-cell adhesions with another group of epithelial cells (group B) that also express E-cadherin. Predict, and explain your reasoning, what will happen at adherens junctions between group A and B cells if:

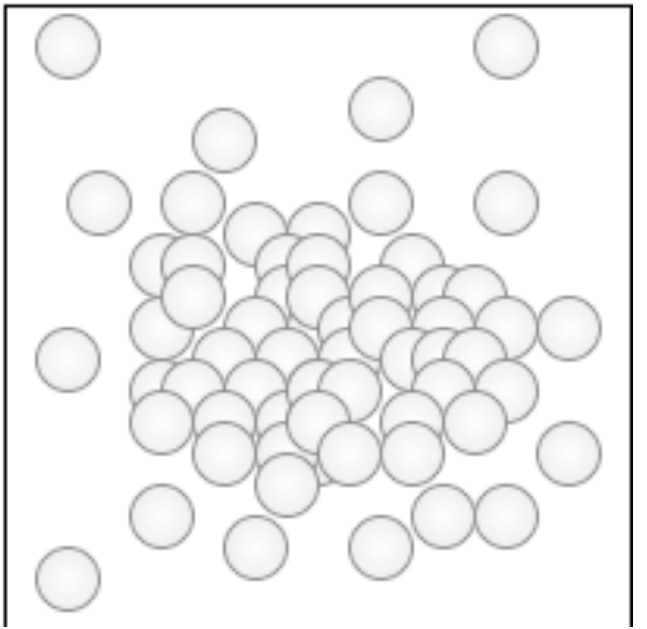
A) All E-cadherin proteins of group A cells are mutated, and lack their transmembrane + cytoplasmic part; instead, they are bound to the membrane by a glycosylphosphatidylinositol (GPI) anchor.
B) All extracellular portions of E-cadherin of group A cells are replaced by that of another cadherin superfamily member, Flamingo.

4 You have isolated epithelial cells from mouse mammary glands and have grown them in a three- dimensional gelatinous matrix in the presence or absence of a specific inhibitor that targets a transmembrane metalloprotease. The protease is capable of cleaving cadherin near the base of its extracellular domain to release an N-terminal fragment containing the extracellular cadherin domains. The distribution of the cells in the matrix, as seen under a light microscope, one day after the transplantation of the isolated tissue is presented in the following schematic drawings. Which condition (1 or 2) do you think corresponds to the presence of the protease inhibitor? Write down 1 or 2 as your answer.

1



2



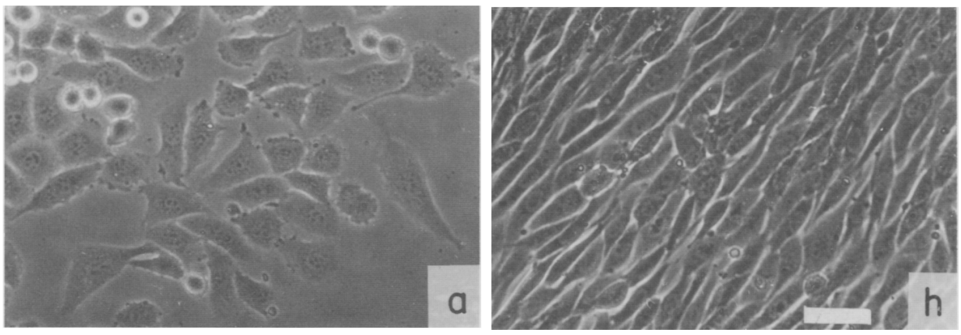
5 Why can we consider the protein b-catenin important in cell-cell junctions and in intracellular signaling?

6 Two cells are next to each other and in physical contact. One cell expresses a GPCR that is activated by an extracellular ligand. The other cell does not express the GPCR. When you add the extracellular ligand, you see that in both cells the transcription factor CREB is activated and driving transcription. How can this be?

7 You are designing an extracellular matrix that needs to be very stiff. What kind of collagen would you use and why?

8: Your friend is designing two new extracellular matrices, both contain fibronectin, laminin and nidogens. In one he uses collagen IV, in the other he uses collagen I. Which one resembles the basal lamina better? What do you predict will be the main difference between the different ECMs?

9: Look at the cells in experiment below. In panel H these cells are incubated with fibronectin, in panel A they are not. What will happen if you incubate the cells with a mutant fibronectin where the RGD amino acid sequence is changed to an LGL amino acid sequence? How will they look, explain why.



10 A: You want to create a cell where the integrins cannot interact with the actin cytoskeleton anymore. Which protein will you mutate so it will not function anymore?

10B: Would vinculin be a good choice? Explain why or why not?

10C: Will your cells be able to move across the basal lamina? Explain why.

11: Why are the integrins in hemidesmosomes not involved cellular migration?

12: Which protein structure do integrins have to cross the plasma membrane?

13: Why do a lot of ECM proteins have protein domains that allow for protein-protein interaction between the different ECM proteins?