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1. The food poisoning bacterium Clostridium perfringens makes a toxin that binds to members of the claudin family of proteins, which are the main constituents of tight junctions. When bound to a claudin, the C-terminus of the toxin allows the N-terminus to insert into the adjacent cell membrane, forming holes that kill the cell. The portion of the toxin that binds to the caudins has proven to be a valuable reagent for investigating the properties of tight junctions. MDCK cells are a common choice for studies of tight junctions because they can form an intact epithelial sheet with high transepithelial resistance. MDCK cells express two claudins: claudin-1, which is not bound by the toxin, and claudin-4, which is.

When an intact MDCK epithelial sheet is incubated with the C-terminal toxin fragment, claudin-4 disappears, becoming undetectable within 24 hours. In the absence of claudin-4, the cells remain healthy and the epithelial sheet appears intact. The mean number of strands in the tight junctions that link the cells also decreases over 24 hours from about four to about two, and they are less highly branched. A functional assay for the integrity of the tight junctions shows that transepithelial resistance decreases dramatically in the presence of the toxin, but the resistance can be restored by washing it out (figure 1A). Curiously, the toxin produces these effects only when it is added to the basolateral side of the sheet; it has no effect when added to the apical surface (figure 1B).

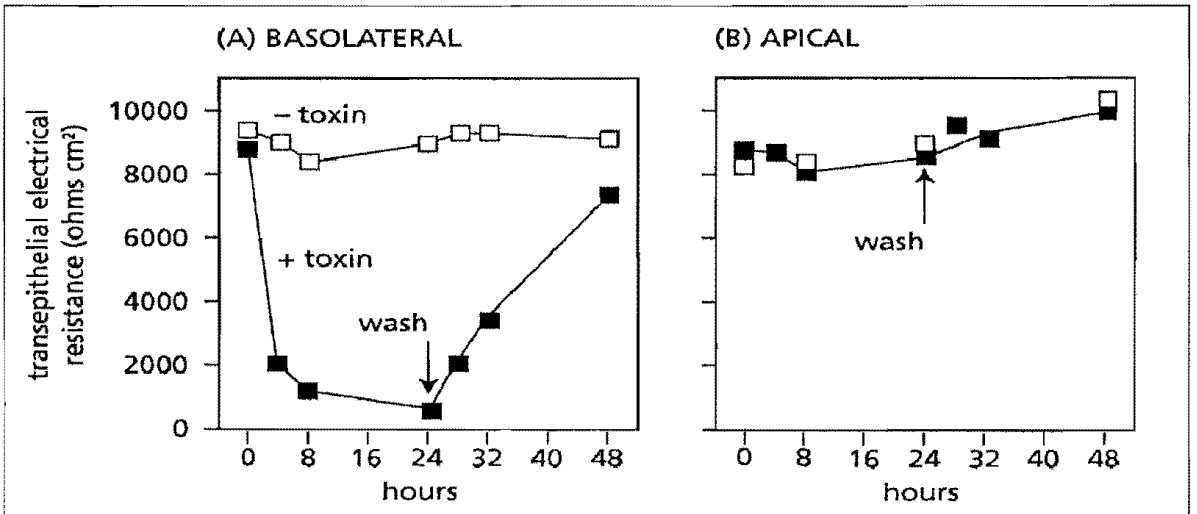

Figure 1 Effect of Clostridium toxin on the barrier function of MDCK cells. A. Addition of toxin from the basolateral side of the epithelial sheet. B. Addition of toxin from the apical side of the epithelial sheet. The higher the resistance the cells have, the less the paracellular current is for a given voltage.

a. How can it be that two tight junction strands remain, even though all of the claudin-4 has disappeared? (5%)
b. How do you suppose the toxin fragment causes the tight junction strands to disintegrate? (5%)
c. Why do you suppose the toxin works when it is added to the basolateral side of the epithelial sheet, but not when added to the apical side? (5%)

(背面仍有題目,請繼續作答)

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2. It is not an easy matter to assign particular functions to specific components of the basal lamina, since the overall structure is a complicated composite material with both mechanical and signaling properties. Nidogen, for example, cross-links two central components of the basal lamina by binding to the laminin-g1 chain and to type IV collagen. Given such a key role, it was surprising that mice with a homozygous knockout of the gene for nidogen-1 were entirely healthy with no abnormal phenotype. Similarly, mice homozygous for a knockout of the gene for nidogen-2 also appeared completely normal. By contrast, mice that were homozygous for a defined mutation in the gene for laminin-g1, which eliminated just the binding site for nidogen, died at birth with severe defects in lung and kidney formation. The mutant portion of the laminin-g1 chain is thought to have no other function than to bind nidogen, and does not affect laminin structure or its ability to assemble into basal lamina.

   a. How would you explain these genetic observations? (5%)
   b. What would you predict would be the phenotype of a mouse that was homozygous for knockouts of both nidogen genes? (5%)
   c. If the result of homozygous knockouts of both nidogen genes is opposite to what you predict, please give the alternative explanation. (5%)

3. Binding of fragments and competition for binding can be used to identify the portion of a larger ligand that is critical for binding. Fibronectin, which is a large glycoprotein component of the extracellular matrix, binds to fibronectin receptors on cell surfaces Fibronectin can stick cells to the surface of a plastic dish, to which they would otherwise not bind, forming the basis of a simple binding assay. By attaching small fragments of fibronectin to dishes, researchers identified the cell-binding domain as a 108-amino acid segment about three-quarters of the way from the N-terminus.

   Synthetic peptides corresponding to different portions of the 108-amino acid segment were then tested in the cell-binding assay to localize the active region precisely. Two experiments are conducted. In the first, peptides were linked covalently to plastic dishes via a disulfide bond to an attached carrier protein, and then tested for their ability to promote cell sticking (Table 1). In the second experiment, plastic dishes were coated with native fibronectin, and cells that stuck to the dishes in the presence of the synthetic peptides were counted (Table 2).

   a. The two experiments used different assays to detect the cell-binding segment of fibronectin. Does the sticking of cells to the dishes mean the same thing in both assays? Explain the difference between the assays. (6%)
   b. From the results in Tables 1 and 2, deduce the amino acid sequence in fibronectin that is recognized by the fibronectin receptor and explain why it is so. (8%)
   c. How might you make use of these results to design a method for isolating the fibronectin receptor? (6%)

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Table 1 Fibronectin-related peptides tested for their ability to promote cell sticking.

Fibronectin peptide	sequence	Concentration required for 50% cell attachment (nM)
Fibronectin		0.1
Peptide 1	YAVTGRGDSPASSKPISINYRTEIDKPSQM(C)*	0.25
2	VTGRGDSPASSKPI(C)	1.6
3	SINYRTEIDKPSQM(C)	>100
4	VTGRGDSPA (C)	2.5
5	SPASSKPIS(C)	>100
6	VTGRGD (C)	10
7	GRGDS (C)	3.0
8	RGDSPA (C)	6.0
9	RVDSPA (C)	>100

Table 2 Fibronectin-related peptides tested for their ability to block cell sticking.

Peptide	% of input cells sticking
GRGDSP (C)	2.0
GRGDAP (C)	1.9
GKGDSP(C)	48
GRADSP (C)	49
GRGESP (C)	44
None	47

4. a. Please briefly characterize the four types of cell adhesion molecules (CAMs). (4%)
   b. Among these CAMs, which type is mediating the hemidemosome and which is mediating the adherent belt? (2%)
   c. Also indicate the intracellular cytoskeletons and extracellular ligands that are attached to these two CAMs, respectively (4%).

(背面仍有題目，請繼續作答)

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5. An important cell-cell adhesion model in the blood stream involving at least two different adhesion events has been used to illustrate both leukocytes and cancer cells in response to inflammatory and metastatic stimuli, respectively. **Please** use ***leukocytes as an example*** to describe how this model works, including ***cellular and molecular events.*** (10%)

6. Please define the endosymbiosis for the origin of eukaryotic cell; and list five main differences between prokaryotic and eukaryotic cells. (10%)

7. Please define the signal hypothesis for protein sorting; and describe the molecular structure and function of signal recognition particle (SRP). (10%)

8. Please define the community effect on multicellular development; and describe the approaches to generate and select specific cell lineage from mouse embryonic stem (ES) cells. (10%)