系所組別： 生物化學暨分子生物學研究所乙組

考試科目： 細胞生物學概論

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1. **Please elucidate how** an injured cell uses a specialized cell junction to prevent the damage from spreading to its neighbors. Also, **please *briefly* design an experiment** to prove the existence of this type of cell junction regulation. (6%)

2. 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***. (6%)

3. The integrins serve as the main receptors in mediating the interactions between cells and the matrix, lacking of which leads to a cell disaster. What is ***the specific terminology*** to describe this phenomenon? Please draw 2 simple pictures to demonstrate an embryonic organogenesis that represents the outcome of this phenomenon. By what kind of signaling regulation (also answer by ***using a specific terminology***) do intergrins translate the signals to prevent cells from a disaster? (5%)

4. In the abstract of a Cell paper, Scott D. Emr et. al. summarized that "The diversity of plasma membrane (PM) proteins presents a challenge for the achievement of cargospecific regulation of endocytosis. Here, we describe a family of proteins in yeast (ARTs, for arrestin-related trafficking adaptors) that function by targeting specific PM proteins to the endocytic system. Two members (Art1 and Art2) of the family were discovered in chemical-genetic screens, and they direct downregulation of distinct amino acid transporters triggered by specific stimuli. Sequence analysis revealed a total of nine ART family members in yeast. In addition to similarity to arrestins, the ARTs each contain multiple PY motifs. These motifs are required for recruitment of the Rsp5/Nedd4-like ubiquitin ligase, which modifies the cargoes as well as the ARTs. As a result, ubiquitinated cargoes are internalized and targeted to the vacuole (lysosome) for degradation. We propose that ARTs provide a cargo-specific quality-control pathway that mediates endocytic downregulation by coupling Rsp5/Nedd4 to diverse plasma membrane proteins."

   (1) What would be the paper's title best addressing what authors found? (4%)
   (2) When chimeric mutants were constructed so that multiple PY motifs were inserted into C-terminal of PM proteins, the PM proteins were continuously endocytosed without any stimulation. However, the PA motif insertion failed to do so. What is your conclusion from this result? (3%)
   (3) According to the abstract, what cellular interaction is the key to degradation of the specifically internalized proteins? (2%)
   (4) In this study, there were **four different protein-protein bindings** leading to the endocytic downregulation of diverse plasma membrane proteins. What are they? (4%)

(背面仍有題目，請繼續作答)

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5. "Epithelial cells contain noncentrosomal microtubules (MTs), whose minus ends are oriented apically. In contrast with the well-known interactions of the minus ends with the centrosome, little is known about the termination site of the noncentrosomal minus ends. Here we show that a population of MT minus ends is anchored at the zonula adherens (ZA), the apical-most part of the cadherin-based adherens junction, via a protein that we have termed Nezha. We initially identified PLEKHA7 as a ZA component and subsequently detected Nezha as a partner for PLEKHA7. Nezha bound MTs at their minus ends and tethered them to the ZA. Furthermore, we found that a minus end-directed motor, KIFC3, was concentrated at the ZA in a PLEKHA7/Nezha/MTdependent manner; and depletion of any of these proteins resulted in disorganization of the ZA." The above statement came from the abstract of a Cell paper.

   (1) Please give **an appropriate title** for this paper. (4%)
   (2) In addition to adherens junction, please **name another three types of cell-cell junctions** belonging to epithelial cells. What **cytoskeleton filament(s),** if any, is(are) associated with the respective junctions? (5%)
   (3) What are **the three main functions** for centrosomal MT? (3%)
   (4) What **protein/protein pairs** in the following protein pool do you think having direct stable binding relationhip? Noncentrosomal MTs, centrosomal MTs, Noncentrosomal MT minus ends, Noncentrosomal MT plus ends, centrosome MT minus ends, centrosome MT plus ends, centrosome, ZA, KIFC3, Nezha, PLEKHA 7. (3%)

6. In a recent paper in Nature Cell Biology, the abstract was written as follows: "A large number of macrophages and haematopoietic progenitor cells accumulate in pre-metastatic lungs in which chemoattractants, such as S100A8 and S100A9, are produced by distant primary tumours serving as metastatic soil. The exact mechanism by which these chemoattractants elicit cell accumulation is not known. Here, we show that serum amyloid A (SAA), which is induced in pre-metastatic lungs by S100A8 and S100A9, has a role in the accumulation of myeloid cells and acts as a positive-feedback regulator for chemoattractant secretion. We also show that in lung endothelial cells and macrophages, Toll-like receptor (TLR) acts as a functional receptor for SAA3 in the pre-metastatic phase. In our study, SAA3 stimulated NF-κB signalling in a TLR4-dependent manner and facilitated metastasis. This inflammation-like state accelerated the migration of primary tumour cells to lung tissues, but this was suppressed by the inhibition of either TLR4 or SAA3. Thus, blocking SAA3–TLR4 function in the pre-metastatic phase could prove to be an effective strategy for the prevention of pulmonary metastasis."

   (1) Please write down **the sequential signaling cascade** for pre-metastatic lungs to facilitate the final metastasis of cancer cells from the distant primary tumor tissues. Included please also indicate the cells that express every signaling component. (5%)
   (2) What are **the four types of in vivo induction**? Which type of induction do you think best

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illustrating the metastatic induction in this paper? (3%)

(3) What activities does "positive feedback" regulate? (3%)

(4) The bacteria product LPS often exists in purified bacteria recombinant proteins. It appears that LPS is a strong stimulator for TLR-mediated cell migration activities. To rule out the possibility that the TLR-mediated endothelial and macrophage cell migration triggered by bacteria recombinant GST-SAA3 was due to LPS contamination, what experiment did authors possibly design (controls must be included for the specificity)? (4%)

7. Please compare the main differences between prokaryotic and eukaryotic cells, as well as describe the major common features in both prokaryotic and eukaryotic cells. (10%)

8. Please design and describe the step-by-step experiments that can be used to identify and dissect the mitochondrial targeting sequence and mechanism in a novel mitochondrial protein. (10%)

9. Please describe the detailed processes of mammalian fertilization and the biological functions of cytosolic calcium wave and cortical reaction in fertilized egg. (10%)

10. Please compare the difference between embryonic stem (ES) cells and tissue- or organ-specific stem cells, as well as describe the methods or protocols that can be used to generate or establish the induced pluripotent stem (iPS) cells. (10%)