1. Please describe at least 2 mechanisms of how oncogenic viruses transfrom or immortalize the cells and how you can design experiments to prove them. (15%)

2. Please describe, compare, and point out the differences in the replication of enterovirus 71 and human immunodeficiency virus with an emphasis on viral genome replication. (15%)

Please read the following article to answer questions 3 and 4.

Viroporins are present in tiny amounts in the virions of most enveloped animal RNA viruses. Examples include Sindbis virus 6K protein, influenza A virus M2 protein, poliovirus 2B and 3A proteins, mouse hepatitis virus E protein, human immunodeficiency virus (HIV) Vpu and severe acute respiratory syndrome coronavirus E protein. These small (around 100 amino acids), extremely hydrophobic proteins oligomerize to form pores in host-cell membranes through which viruses can bud. Viroporins contribute to the pathology of disease by altering membrane permeability and disrupting ion homeostasis in cells.

Inspection of the structural features and hydrophobicity profiles of small proteins encoded by hepatitis C virus (HCV) revealed two candidate viroporins, NS4A and p7. Using an expression system that was based on Sindbis virus to mimic the expression of viroporins during infection, the comparative effects of selected viroporins — Sindbis virus 6K protein, influenza A virus M2 protein, poliovirus 2B and 3A proteins, mouse hepatitis virus E protein and HCV p7 and NS4A proteins — on baby hamster kidney (BHK) cells was evaluated. On expression, each protein that was tested altered membrane permeability, which confirmed that these proteins are real viroporins.

Mouse hepatitis virus E protein and HIV Vpu had both previously been shown to induce apoptosis, so researchers looked for characteristic signatures of apoptosis in BHK cells that expressed viroporins. All the viroporins induced chromatin condensation, nuclear DNA fragmentation and activation of the key apoptosis enzyme caspase 3, but the strongest pro-apoptotic response was induced by HCV NS4A and poliovirus protein 2B. Another intriguing link between viroporins and apoptosis is the reported association of a fraction of HCV p7 and NS4A proteins with mitochondria. The authors showed that HCV NS4A and poliovirus 2B colocalized with mitochondria and that expression of other viroporins altered mitochondrial morphology and distribution. Notably, the expression of all viroporins led to the release of cytochrome *c* from mitochondria. Taken together, this evidence led the authors to propose that viroporins activate apoptosis by the mitochondrial pathway.

The induction of apoptosis in host cells by viruses is common and could aid virus spread. The next step in understanding the intriguing links between viroporins and apoptosis will be to unravel the mechanisms by which viroporins trigger apoptotic pathways and demonstrate that these findings are relevant during infection. (Viral pathogenesis: death by viroporin. S. Jones. *Nature Reviews Microbiology* 5, 907. 2007)

(背面仍有題目,請繼續作答)

3. Please describe the features, structure, and functions (in viral infection) of viroporin. (10%)

4. Based on this article, please describe the process of how viroporins induces death of infected cells and how the researchers prove it. (10%)

Read the following paragraphs and answer questions 5-9

The *Bacteroides fragilis* toxin (BFT) is the only known virulence factor of enterotoxigenic *B. fragilis*. BFT has previously been shown to act, at least in part, through cleavage of the intercellular adhesion protein Ecadherin. A specific cellular receptor for BFT has not been identified. The goal of this study was to determine if the initial interaction of BFT with intestinal epithelial cells was consistent with binding to a specific cellular receptor. Purified BFT was labeled with a fluorophore or iodide to assess specific cellular binding and the properties of BFT cellular binding. BFT binds specifically to intestinal epithelial cell lines in vitro in a polarized manner. However, specific binding occurs only at 37°C and requires BFT metalloprotease activity. The BFT receptor is predicted to be a membrane protein other than E-cadherin or a known protease-activated receptor (PAR1 to PAR4). BFT binding is resistant to acid washing, suggesting an irreversible interaction. Sugar or lipid residues do not appear to be involved in the mechanism of BFT cellular binding, but binding is sensitive to membrane cholesterol depletion. We conclude that intestinal epithelial cells in vitro possess a specific membrane BFT receptor that is distinct from E-cadherin. The data favor a model in which the metalloprotease domain of BFT processes its receptor protein, initiating cellular signal transduction that mediates the biological activity of BFT. However, activation of recognized protease-activated receptors does not mimic or block BFT biological activity or binding, suggesting that additional protease-activated receptors on intestinal epithelial cells remain to be identified. (Infection and Immunity, 2006, 74:5382–5390; abstract)

5. Please describe the characteristics of *Bacteroides fragilis*, including its morphology, growth properties, and pathogenicity. (10%)

6. Please name two other bacterial pathogens that require the same air condition as *B. fragilis*. Explain briefly why these bacteria need to be cultured in such air condition. (10%)

7. What are the characteristics of BFT (5%) and the putative BFT receptor (10%) according to the abstract above?

8. What are the features of the epithelial cells used in this study? (5%)

9. Design an experiment that would lead to the identification of the BFT receptors. (10%)