1. (15%) 試述 RNA Polymerase II 要進行轉錄時需那些 Basal transcription factors，其已知功能為何？

2. (10%) 試述真核細胞進行上述轉錄時，DNA 產生 Bending，其機轉為何？

3. (15%) 試舉出 E.coli 與 mammalian cells 各三種 DNA polymerases，並說明其個別之功能。

4. (10%) 假設 severe acute respiratory syndrome (SARS)為病毒引起，而且你手邊有此病毒，請舉兩種方法證明此病毒之基因體為 DNA 或 RNA。

5. (25%) After hepatectomy, there is a significant increase in the activity of ornithine decarboxylase in liver. From this experimental data, please propose five possible molecular mechanisms that can contribute to this change.

6. Drug X (DX) is an agonist for peroxisome proliferators activated receptor gamma (PPARγ, a nuclear receptor that is highly expressed in fat tissue. The activation of glyceroneogenesis by DX occurs mainly in visceral fat, the same fat depot that is specifically implicated in the progression of obesity to type II diabetes. The increase in glyceroneogenesis is a result of the induction of its key-enzyme, phosphoenolpyruvate carboxykinase, whose gene expression is PPARγ-dependent in adipocytes. The main role of this metabolic pathway is to allow the re-esterification of fatty acids via a futile cycle in adipocytes, thus lowering fatty acid release into the plasma. The importance of such a fatty acid re-esterification process in the control of lipid homeostasis is highlighted by the existence of a second DX-induced pathway involving glycerol kinase. It showed that glyceroneogenesis accounts for at least 75 % of the whole DX effect. Because elevated plasma fatty acids promote insulin resistance, these results suggest that the glyceroneogenesis-dependent fatty acid regulation by DX could be an essential aspect of the anti-diabetic action of these drugs.

a. (5%) The effects of Drug X (DX) is to:

接背面

(1) increase plasma triglyceride level
(2) increase plasma fatty acid level
(3) increase hormone-sensitive lipase activity
(4) decrease plasma glucose level
(5) decrease glycerol kinase activity
(6) decrease glucose metabolism

b. (5%) The peroxisome proliferators activated receptor gamma is located in:

(1) cell membrane
(2) cytosol
(3) mitochondria
(4) lysosomes
(5) Golgi complex
(6) nucleus

7. Ceramidase is a key enzyme involved in regulating cellular levels of ceramide, sphingosine, and possibly sphigosine 1-phosphate and thus could modulate sphingolipid signaling. The deduced amino acid sequences of the mammalian ceramidase contain a serine-threonine-rich domain (mucin box), which follows the signal/anchor sequence, whereas those of bacterial and invertebrate enzymes completely lack a mucin box, suggesting that the specific domain has been acquired during evolution. In HEK293 kidney cells overexpressing ceramidase, the enzyme was not only secreted into the medium after cleavage of the NH_2-terminal signal/anchor sequence but also localized at the plasma membrane as a type II integral membrane protein. Lectin blot analysis using peanut agglutinin revealed that the mucin box of the enzyme is highly glycosylated with *O*-glycans. Interestingly, a mutant lacking the mucin box or possible *O*-glycosylation sites in the mucin box was secreted into the medium but not localized at the surface of the cells. In addition, it is also found that the 112-kDa membrane-bound enzyme from mouse kidney is *O*-glycosylated, whereas the 94-kDa soluble enzyme from liver is not. Furthermore, a mucin box-fused chimera green fluorescent protein (GFP), but not GFP itself, with the signal/anchor sequence was distributed on the surface of the cells. These results suggest that *O*-glycosylation of the mucin box retains proteins on the plasma membranes.

a. (5%) These results indicate that post-translational modification of

接次頁

ceramidase:

(1) is depended on the specificity of glycan transferase.
(2) is required for the localization of ceramidase to the liver membrane.
(3) is depended on the mucin protein in the membrane.
(4) is depended on the level of gene expression.
(5) is not happened in the kidney tissue.
(6) is depended on the chemical treatment of the culture cells.

b. (5%) In order to artificially retain ceramidase in the cytosol, which gene manipulation is required?

(1) Construct the ceramidase-GFP chimera.
(2) Mutate all the serine and threonine residues in the mucin box.
(3) Delete the signal peptide sequence from ceramidase cDNA.
(4) Delete the mucin box sequence from the ceramidase cDNA.
(5) Add agglutinin to the cell culture.
(6) Add tunicamycin to the cell culture.

c. (5%) Which of the following statement is incorrect?

(1). Ceramide is synthesized from palmitoyl-CoA and one amino acid.
(2). Sphingomyelin is synthesized from ceramide and phosphatidylcholine.
(3). Cerebroside is synthesized from ceramide and a sugar moiety.
(4). GTP is the precursor that involves in the biosynthesis of cerebrosides.
(5). Ceramide may act as a lipid mediator (second messenger), activating a protein kinase.
(6). Lysophosphatidic acid and sphingosine 1-phosphate are first messengers that can activate G protein-coupled receptors (GPCR) on the cell surface.

試題隨卷繳回