Answer the following questions

1. Which of the following statements are correct? Explain your answer. (20%)
   (a) In bacteria the genes encoding ribosomal RNA. tRNA, and mRNA are transcribed by different RNA polymerases.
   (b) Most DNA binding proteins bind to the major groove of the double helix.
   (c) Of the major control points in gene expression (transcription, RNA processing, RNA transport, translation and control of protein's activity), transcription initiation is used for the vast majority of gene regulation events.
   (d) RNA polymerase II binds to TATA box sequences and distorts the DNA to allow the assembly of RNA start.

2. What cell adhesion molecules and extra cellular enzymes are involved in T-cell extravasation and how do they work? (10%)

3. Receptors in eukaryotes fall into several huge and diverse families. Please characterize the following receptor families (20%)
   (1) Ion channels receptors
   (2) Enzyme-linked receptors
   (3) G-protein coupled (GPC) receptors
   (4) Steroidal receptors

4. ATP is synthesized in mitochondria. (20%)
   (a) List two cytoplasmic molecules for the synthesis of ATP and describe the molecular pathways of their importing to mitochondria?
   (b) Where and how are the materials of (a) metabolized in mitochondria?
   (c) How ATP is synthesized from the metabolites from (b)?
   (d) Where do ATP synthases locate in mitochondria and what are their biological functions in mitochondria?
   (e) Describe the mechanisms that ATP is driven out of mitochondria through the inner and the outer membrane.

5. Read the article and answer the following questions. (30%)

Immune Cells Speed the Evolution of Novel Proteins

- Evolution isn't known for its quick work. In recent years, researchers have come up with numerous ways to give it a kick in order to evolve proteins with new functions. But most of these techniques are painfully slow, taking as long as a month to go through a single round of evolution. The immune cells of vertebrates long ago perfected a faster approach, which they use to generate the myriad antibody proteins that fight off infections. Now a team of California researchers has coaxed immune cells to apply their skill to other proteins, an ability that could speed the development of novel proteins for studies from catalysis to cell biology. "It's very elegant work," says David Liu, a protein evolution expert at Harvard University. The team hoped to improve the fluorescent properties of proteins that shine red when stimulated by green light.

【背面還有試題】

Molecular biologists link these and similar beacons to proteins of interest to reveal their location inside cells. In recent years, Roger Tsien, a biochemist at the University of California, San Diego (UCSD), has evolved fluorescent proteins to shine different colors of light, a trick that makes it possible to track more than one protein at a time. But because the new proteins still emit visible light, which body tissue absorbs, they are useless for following molecules in whole animals. Tsien's group has sought to improve matters by evolving proteins to shine infrared light, which penetrates tissue. Researchers typically start by isolating the gene for a fluorescent protein. Then they use an error-prone gene-copying method to introduce random mutations, splice the new gene variants into bacteria, and select out the microbes that shine the most interesting colors. Researchers must then clone the desired genes to identify how their sequences differ from the original. "Someone who is good at it can do about one round in 1 month," Tsien says. To speed up the process, Tsien and his colleagues-postdoc Lei Wang and technicians W. C. Jackson and P. Steinbach- turned to antibody-generating factories called B cells that mutate some genes 1 million times faster than other cells. Specifically, B cells generate antibody diversity with a built-in system that frequently mutates cytosine into one of the other three bases that make up DNA. Over the past 3 years, researchers in the United States and Switzerland have induced B cells to apply this process, called somatic hypermutation, to non-antibody proteins, in one case to restore an altered protein to its natural function. But little had been done to use the approach to evolve proteins with novel functions. Tsien's group started with the gene for red fluorescent protein (RFP), which they linked to a promoter DNA sequence that turns on production of RFP in response to an antibiotic called doxycycline. They then transfected this genetic tandem into millions of human B cells. When exposed to doxycycline, the cells started mutating the RFP gene and making variants of the original protein. The researchers then stimulated the cells with laser light and selected out those that showed a shift in fluorescence toward the infrared. After giving the cells time to multiply, the researchers treated them with doxycycline again and repeated a new round of evolution. Each round took only a few days. In the current issue of the *Proceedings of the National Academy of Sciences*, the UCSD team reports that after 23 such rounds of evolution, the wavelength at which the evolved proteins' emitted light shifted from 610 nanometers to 650 nanometers, about halfway from the red to the infrared. The effectiveness of this new technique shouldn't be limited to fluorescent proteins. As long as there is a good way to screen the resulting cells for the desired activity, "we think this can work on practically any protein," Tsien says. That should give a green light to the evolution of new catalysts and help molecular biologists who evolve proteins in order to study their function.

– ROBERT F. SERVICE. Science.

(a) What are traditional methods to introduce random mutations in genes.

(b) Describe the mechanisms that B-cells produce the specificity of immunoglobulins.

(c) How to apply your target proteins, such as glutathione transferase, to the somatic hypermutation system and how to induce this system to work?

(d) What advantages and disadvantages could you expect from the resulting cells using this system?