Homework 3 for Cell Biology--- 2017

1. Cells communicate in ways that resemble human communication. Decide which of the following forms of human communication are analogous to autocrine, paracrine, endocrine, and synaptic signaling by cells.
   A. A telephone conversation
   B. Talking to people at a cocktail party
   C. A radio announcement
   D. Talking to yourself

腺苷

2. You are working with a hamster cell line that stops proliferating when intracellular cyclic AMP reaches high levels. By stimulating adenylyl cyclase with cholera toxin and by inhibiting cyclic AMP phosphodiesterase with theophylline, cyclic AMP can be artificially elevated. Under these conditions only cells that are resistant to the effects of cyclic AMP can grow.

   In this way you isolate several resistant colonies that grow under the selective conditions and assay them for PKA activity (test for cAMP, with cAMP PKA is active): they are all defective. About 10% of the resistant lines are completely missing PKA activity. The remainder possess PKA activity, but a very high level of cyclic AMP is required for activation. To characterize the resistant lines further, you fuse them with the parental cells and test the hybrids for resistance to cholera toxin. Hybrids between parental cells and PKA-negative cells are sensitive to cholera toxin, which indicates that the mutations in these cells are recessive. By contrast, hybrids between parental cells and resistant cells with altered PKA responsiveness are resistant to cholera toxin, which indicates that these mutations are dominant.

A. Is PKA an essential enzyme in these hamster cells?

B. PKA is a tetramer consisting of two catalytic (protein kinase) and two regulatory (cyclic-AMP-binding) subunits. Propose an explanation for why the mutations in some cyclic-AMP-resistant cell lines are recessive, while others are dominant.

C. Do these experiments support the notion that all cyclic AMP effects in hamster cells are mediated by PKA? Do they rule out the possibility that cyclic AMP-binding proteins play other critical roles in this hamster cell line?

3. Akt is a key protein kinase in the signaling pathway that leads to cell growth. Akt is activated by a phosphatidylinositol-dependent protein kinase (PDKI), which phosphorylates threonine 308. At the same time, serine 473 is phosphorylated. Your advisor has been unsuccessful in purifying the protein kinase responsible for the phosphorylation of serine 473, but you think you know what is going on. You construct genes encoding two mutant forms of Akt : one carries a point mutation in the kinase domain, Akt-K179M, which renders it kinase-dead, and the other carries a point mutation in the domain required to bind to PDK1 (Akt-T308A), which cannot be activated by PDK1. You transfect each of these constructs, and a construct for wild-type Akt, into cells that do not express their own Akt. You treat a portion of the cells with an insulin-like growth factor (IGF1), which activates PDK1, and analyze the phosphorylation state of the various forms of Akt using antibodies specific for Akt or for particular

phosphorylated amino acids (Figure 15-29). What is the identity of the enzyme that phosphorylates serine 473 on Akt?

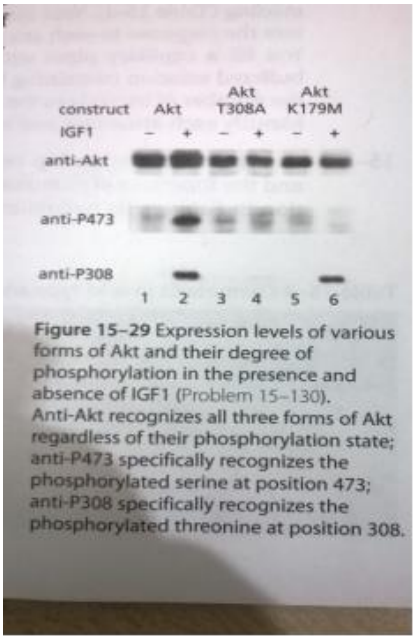

Figure 15–29 Expression levels of various forms of Akt and their degree of phosphorylation in the presence and absence of IGF1 (Problem 15–130). Anti-Akt recognizes all three forms of Akt regardless of their phosphorylation state; anti-P473 specifically recognizes the phosphorylated serine at position 473; anti-P308 specifically recognizes the phosphorylated threonine at position 308.

4. Yeast, and many other organisms, make a single type of clathrin heavy chain and a single type of clathrin light chains; thus, they make a single kind of clathrin coat. How is it then that a single clathrin coat can be used for three different transport pathways- Golgi to late endosomes, plasma membrane to early endosomes and immature secretory vesicle to Golgi-that each involves different specialized cargo proteins?

5. Cells have evolved a set of complicated pathways for addition of carbohydrates to proteins, implying that carbohydrates have important functions. List three functions that carbohydrates on proteins are known to carry out.

原骨胶原

6. Most exported proteins move across the Golgi apparatus in 5-15 minutes, but very large proteins, like procollagen type I can take more than an hour. How might you account for this observation_ different rates of protein movement –in the vesicle transport and cisternal maturation models for protein transport through the Golgi apparatus?

亚铁血红素　　细胞色色素　　血红蛋白

7. Iron is an essential trance metal that is needed by all cells. It is required, for example, for the synthesis of the heme groups that are part of cytochromes and hemoglobin. Iron is taken into cells via a two-component system. The soluble protein transferrin circulates in the bloodstream, and the transferrin receptor is a membrane protein that is continually endocytosed and recycled to the plasma membrane. Fe ions bind to transferrin at neutral pH but not at acidic pH. Transferrin binds to the transferrin receptor at neutral pH only when it has bound an Fe ion, but it binds to the receptor at acidic pH even in the absence of bound iron. From these properties, describe how iron is taken up and discuss the advantages of this elaborate scheme.

抗胰蛋白酶

8. Antitrypsin, which inhibits certain proteases, is normally secreted into the blood stream by liver cells. Antitrypsin is absent from the bloodstream of patients who carry a mutation that result in a single amino acid change in the protein. Antitrypsin deficiency causes a variety of severe problems. Particularly in lung tissue, because of uncontrolled protease activity. Surprisingly, when the mutant antitrypsin is synthesized in the lab, it is active as the normal antitrypsin at inhibiting protease. Why then does the mutation cause the disease? Think of more than one possibility and suggest ways in which you could distinguish among them.