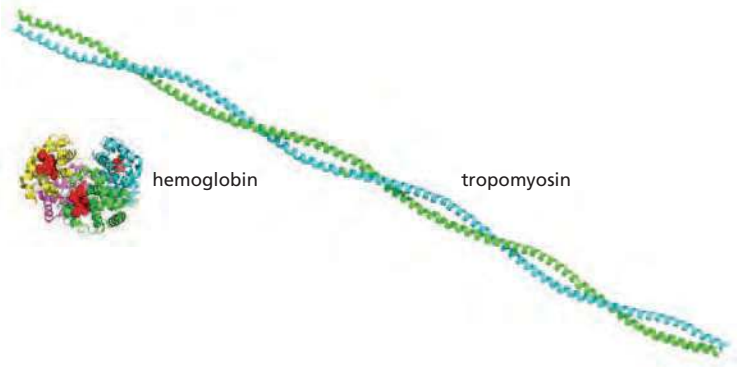
BIO-205-9

Discuss this in pairs

Isolation of cells from tissues, luorescence-activated cell sorting, and laser-capture microdissection are just a few of the ways for generating homogeneous cell populations. Why do you suppose it is important to have a homogeneous cell population for many experiments?

It is the general goal of cell biology research to discover how individual cells work, but it is very difficult to study most processes in single cells. Analysis of a population of identical cells can yield valid conclusions about the workings of the individual cells. By contrast, if the population is a mixture of diferent cell types, its analysis will give properties of the mixture, which may or may not accurately describe the individual cells. Consider an analogy. We know from looking at individual human eyes that they are various shades of brown or blue or green. Yet if we could tell eye color only by looking at 1000 at a time, and if we started with a random population, we might conclude that eyes were a bluish brown—a color that doesn't apply to any single individual.

Tropomyosin, at 93 kd, sediments at 2.6S, whereas the 65-kd protein, hemoglobin, sediments at 4.3S. (The sedimentation coefficient S is a linear measure of the rate of sedimentation: both increase or decrease in parallel.) How is it that the bigger protein sediments more slowly than the smaller one? Can you think of an analogy from everyday experience that might help you with this problem?



The rate of sedimentation of a protein is based on size *and* shape. The nearly spherical hemoglobin will sediment faster than the more rod- shaped tropomyosin, even though tropomyosin is the larger protein. Shape comes into play because molecules that are driven through a solution by centrifugal force experience the equivalent of frictional drag. A spherical protein, with its smaller surface-to-volume ratio, will experience less drag than a rod, and therefore will sediment faster. You can demonstrate this diference using two sheets of paper. Crumple one into a sphere and roll the other into a tube. Now drop them. The crumpled ball will hit the ground faster than the tube. In this demonstration, the centrifugal force is replaced by gravity and the friction with molecules in solution is replaced by friction with air. The underlying principles are the same.

Distinguish among ion-exchange chromatography, hydrophobic chromatography, gel-iltration chromatography, and affinity chromatography in terms of the column material and the basis for separation of a mixture of proteins.

All these methods employ small beads that are packed into columns to which a solution of proteins is applied.

Ion-exchange chromatography uses beads that carry positive charges (anion exchangers) or negative charges (cation exchangers). Proteins spend more or less time associated with the beads, depending on the arrangement of charged groups on their surfaces. Weaker-binding proteins elute from the column earlier and tighter-binding proteins elute later. Because the strength of association varies with pH and ionic strength of the solution that is passing down the column, the association between proteins and the beads can be varied systematically to find the best conditions for puriication of a particular protein.

Hydrophobic chromatography uses beads that have hydrophobic groups protruding from their surfaces. These hydrophobic groups can interact with hydrophobic regions on the surfaces of proteins and delay their progress through the column. Once again, the stronger the interaction with the beads the longer the protein remains on the column.

Gel-filtration chromatography (also known as size-exclusion chromatography) uses beads with pores. Proteins that are too large to it into the beads pass unretarded through the column, whereas proteins that can enter the beads are retarded by the time they spend inside the bead. For proteins that can enter the beads, larger proteins come of the column earlier than smaller proteins. Beads with a variety of pore sizes are available so that a gel-filtration column can be tailored to purification of a particular protein.

Affinity chromatography uses beads to which specific molecules, small or large, have been attached. The choice of molecule depends on the particular protein whose purification is desired. One common application, for example, uses glutathione Sepharose® (the small molecule glutathione attached to Sepharose beads) to capture proteins fused to GST (glutathione-*S*-transferase). Passing glutathione through the column, which displaces the GST-tagged protein, elutes the bound protein. Another common example attaches antibodies that are specific for a particular protein to the beads, allowing a specific protein to be bound to the column; these interactions can be disrupted with high salt or changes in pH to allow the protein to be eluted.

Multiple choices

1. Which statement(s) are correct for *in vitro* studies?
   a. Transformed cell lines can be used only for short period of time, e.g. 10 passages max.
   b. Immortalized cell lines require to have overexpressed telomerase enzyme.

c. Proteolytic enzymes facilitate the detachment and singularization of the cells.
d. All cells require tissue culture dishes coated with extracellular matrix components.

Answer: The correct answer is b and c. Transformed cell lines can be used longer than 10 passages. They have indefinite replication cycles in the culture because they are isolated from the tumors. They don't lose their properties rapidly. Not every cell type require extracellular matrix on the surface of the tissue culture dishes (for example cancer cell lines). Also, some cell can be grown as suspension culture rather than adherent culture, thus no need for any attachment components.

2. Which of the following is the correct order to purify and identify your protein of interest from the cells?

    a. Harvest the cells by using proteolytic enzymes, lyse the cells, centrifugation, column chromatography, mass spectrometry
    b. Lyse the cells, column chromatography, harvest the cells by using proteolytic enzymes, mass spectrometry, centrifugation
    c. Centrifugation, mass spectrometry, column chromatography, lyse the cells, harvest the cells by using proteolytic enzymes
    d. Harvest the cells by using proteolytic enzymes, lyse the cells, column chromatography, centrifugation, mass spectrometry

Answer: A. You need to collect your cells from the culture conditions by using proteolytic enzymes like trypsin. Then you need to lyse your cells to break the cell membranes and release the cytoplasmic content by osmotic shock, ultrasonic vibrations or detergents. You need to apply centrifugation to the lysate/homogenate to separate the elements by size and density. After you collect the protein content of the homogenate, you need to subject them to the column chromatography to separate them due to the charge, hydrophobicity, molecular weight or affinity and you need to identify the protein of interest by mass spectrometry method according to its mass-to-charge ratios.

3. Researchers use model organisms…

    a. To understand the structure and functions of a gene/protein
    b. To decipher the mechanisms of fundamental questions in biology, e.g. cell cycle.
    c. To mimic human disease and discover or develop pharmaceutical applications
    d. To reduce the amount of time and cost for the experiments

Answer: All of them.

4. Which of the followings are INCORRECT about the methods to resolve the protein structures?

    a. NMR spectroscopy is a suitable technique for the small proteins.

b. X-ray crystallography offers high resolution atomic position for larger protein structures.
c. Proteins should be crystallized for nuclear magnetic resonance (NMR) spectroscopy.
d. Cryo-EM makes it possible to work with proteins which are hard to crystallize.

Answer: C. NMR doesn't depend on crystallization, it can analyze the molecules in solution, reflecting their natural environments, aqueous solution.

5. You want to know the interaction partners of your protein of interest, let's call it "SHINE" and the size of your protein is 70 kDa. You have 1 possible candidate protein (45kDa in size), which you hypothesized that it might be interacting with SHINE. You want to test whether your candidate protein interacts with SHINE. You tagged your candidate with a green fluorescent protein (GFP) (25kDa) because you only have GFP antibody to detect your protein. Which method would you use to reveal the interaction of SHINE and GFP-tagged candidate protein? And, which size you expect to see your protein complex, if they interact with each other?

A. Immunoprecipitation and southern blot. Expected size of protein complex would be 70 kDa.
B. HPLC and western blot. Expected size of protein complex would be 140 kDa.
C. HPLC and SDS-PAGE. Expected size of the protein would be 45 kDa.
D. Immunoprecipitation and western blot. Expected size of the protein complex would be 140 kDa.

Answer: D. Immunoprecipitation enables to detect interacting protein. We can detect the proteins in the precipitates by western blot. As you expect to see SHINE and GFP-tagged candidate is interacted, you should see a band around 140 kDa (sum of the size of the proteins altogether because they are interacting and form a complex).

6. What are the differences between primary and secondary cell cultures?

a. Primary cultures have infinite number of subculturing, while secondary cultures go through senescence much faster.
b. Primary cultures are more heterogeneous than secondary cultures.
c. Primary cultures resemble the *in vivo* conditions more than secondary cultures.
d. Primary cultures are isolated directly from a tissue or organ, secondary cultures are derived from the primary culture by subculturing.

Answer: B, C and D.

7. What is the primary application of hybridoma cell lines?

a. Production of vaccines for viral infections
b. Synthesis of recombinant proteins
c. Large-scale production of monoclonal antibodies

d. Generation of pluripotent stem cells

Answer: C. Hybridoma cell lines are extensively utilized for producing monoclonal antibodies, which target a single specific antigen. These antibodies play a crucial role in diagnostics, therapeutic applications such as treating cancer and autoimmune diseases, and scientific research.

True or False

1. Monoclonal antibodies recognize different epitopes of a specific protein.

FALSE. Polyclonal antibodies recognize distinct antigenic sites of the protein, while monoclonal antibodies are specific to only one antigenic site on a protein.

2. Basic Local Alignment Tool (BLAST) allows us to compare the protein sequences across different species and it gives information about protein structure and function across different species.

FALSE. Only comparison of the protein sequences across different species. No information about the protein functionality.

3. X-ray crystallography is one of the methods that we can utilize to reveal the structure of a protein of interest.

TRUE.

4. Sodium dodecyl sulfate and B-mercaptoethanol help to denature the protein structure for them to run through the PAGE properly.

TRUE

5. Southern blot is used to detect proteins of interest, whereas western blot allows the researchers to visualize RNA.

FALSE. Western blots for protein detection, southern blot is to target DNA molecules. RNA molecules can be detected by northern blot technique.

6. Affinity chromatography is based on the ability of a protein to bind small molecules.

TRUE

7. The cells can grow indefinitely on a tissue culture dish coated with extracellular matrix components.

FALSE. They stop dividing after a certain number of divisions, they go through senescence because of the shortening telomeres. They also die when they become over confluent on a dish due to cell contact inhibition of proliferation.