TE Biotechnology Sem. VI

Elective I

Stem Cell Biology - Solution

Q.1

A) Inductive signaling

Inductive Signals Can Create Orderly Differences Between Initially Identical Cells. The most straightforward way to make cells different is by exposing them to different environments, and the most important environmental cues acting on cells in an embryo are signals from neighboring cells. Thus, in what is probably the commonest mode of pattern formation, a group of cells start out all having the same developmental potential, and a signal from cells outside the group then drives one or more of the members of the group into a different developmental pathway, leading to a changed character. This process is called an inductive interaction. Generally, the signal is limited in time and space so that only a subset of the competent cells—those closest to the source of the signal—take on the induced character

b) Genesis of asymmetry through positive feedback

Positive Feedback Can Create Asymmetry Where There Was None Before. Inductive signaling and asymmetric cell division represent two distinct strategies for creating differences between cells. The answer lies in positive feedback: through positive feedback, a system that starts off homogeneous and symmetrical can pattern itself spontaneously, even where there is no organized external signal at all. And where, as very often happens, the environment or the starting conditions impose some weak but definite initial asymmetry, positive feedback provides the means to magnify the effect and create a full-blown pattern.

c) Role of morphogens in embryo development

Morphogens Are Long-Range Inducers That Exert Graded Effects. Signal molecules often seem to govern a simple yes–no choice: one outcome when their concentration is high, another when it is low. In many cases, however, responses are more finely graded: a high concentration may, for example, direct target cells into one developmental pathway, an intermediate concentration into another, and a low concentration into yet another. An important case is that in which the signal molecule diffuses out from a localized signaling center, creating a signal concentration gradient. Cells at different distances from the source are driven to behave in a variety of different ways, according to the signal concentration that they experience. A signal molecule that imposes a pattern on a whole field of cells in this way is called a morphogen. Vertebrate limbs provide a striking example

d) Maternal gene effects

Products of Maternal-Effect Genes Organize the Asymmetric Division of the Egg. In C. elegans,germ line is produced by a strict series of asymmetric cell divisions of the fertilized egg. The asymmetry originates with a cue from the egg's environment: the sperm entry point defines the future posterior pole of the elongated egg. The proteins in the egg then interact with one another and organize themselves in relation to this point so as to create a more elaborate asymmetry in the interior of the cell. The proteins involved are mainly translated from the accumulated mRNA products of the genes of the mother. Because this RNA is made before the egg is laid, it is only the mother's genotype that dictates what happens in the first steps of development. Genes acting in this way are called maternal-effect genes

Q.2 a) Heterochronic genes control the timing of development in Caenorhabditis elegans.

A cell does not have to receive an external cue in order to change: one set of regulatory molecules inside the cell can provoke the production of another, and the cell can thus step through a series of different states through its own internal mechanisms. These states differ not only in their responsiveness to external signals, but also in other aspects of their internal chemistry, including proteins that stop or start the cell-division cycle. In this way, the internal mechanisms of the cell, together with the past and present signals received, dictate both the sequence of biochemical changes in the cell and the timing of its cell divisions. Through genetic analyses, one can determine that the products of the heterochronic genes act in series, forming regulatory cascades. Curiously, two genes at the top of their respective cascades, called Lin4 and Let7, do not code for proteins but for microRNAs—short untranslated regulatory RNA molecules, 21 or 22 nucleotides long. These act by binding to complementary sequences in the noncoding regions of mRNA molecules transcribed from other heterochronic genes, thereby inhibiting their translation and promoting their degradation,

b) hematopoietic stem cell is called multipotent stem cell

Blood contains many types of cells, with functions that range from the transport of oxygen to the production of antibodies. Some of these cells stay within the vascular system, while others use the vascular system only as a means of transport and perform their function elsewhere. All blood cells, however, have certain similarities in their life history. They all have limited life spans and are produced throughout the life of the animal. Most remarkably, they are all generated ultimately from a common stem cell in the bone marrow. This hemopoietin (blood forming, also called hematopoietic) stem cell is thus multipotent, giving rise to all the types of terminally differentiated blood cells as well as some other types of cells, such as osteoclasts in bone.

Q.3 a) adult stem cells

Adult stem cells are undifferentiated cells, found throughout the body after development, that multiply by cell division to replenish dying cells and regenerate damaged tissuesWhen cells are removed from the body and maintained in culture or are transplanted from one site in the body to another, as in the procedures we have just described, they generally remain broadly faithful

to their origins. Keratinocytes continue to behave as keratinocytes, hemopoietic cells as hemopoietic cells, neural cells as neural cells, and so on. Placed in an abnormal environment, differentiated cells may, it is true, cease to display the full normal set of differentiated features, and stem cells may lose their stem-cell character and differentiate; but they do not switch to expressing the characteristics of another radically different cell type. Thus, each type of specialized cell has a memory of its developmental history and seems fixed in its specialized fate. Some limited transformations can certainly occur, as we saw in our account of the connective-tissue cell family, and some stem cells can generate a variety of differentiated cell types, but the possibilities are restricted. Each type of stem cell serves for the renewal of one particular type of tissue.

b) Regulatory DNA defines the program of development in Caenorhabditis elegans

While the first few differences between cells along the anteroposterior axis of C. elegans result from asymmetric divisions, further patterning, including the pattern of cell types along the other axes, depends on interactions between one cell and another. The cell lineages in the embryo are so reproducible that individual cells can be assigned names and identified in every animal (Figure 22–20); the cells at the four-cell stage, for example, are called ABa and ABp (the two anterior sister cells), and EMS and P2 (the two posterior sister cells). As a result of the asymmetric divisions we have just described, the P2 cell expresses a signal protein on its surface—a nematode homolog of the Notch ligand Delta— while the ABa and ABp cells express the corresponding transmembrane receptor—a homolog of Notch. The elongated shape of the eggshell forces these cells into an arrangement such that the most anterior cell, ABa, and the most posterior cell, P2, are no longer in contact with one another. Thus only the ABp cell receives the signal from P2, making ABp different from ABa and defining the future dorsal–ventral axis of the worm (Figure 22–21). At the same time, P2 also expresses another signal molecule, a Wnt protein, which acts on a Wnt receptor (a Frizzled protein) in the membrane of the EMS cell. This signal polarizes the EMS cell in relation to its site of contact with P2, controlling the orientation of the mitotic spindle. The EMS cell then divides to give two daughters that become committed to different fates as a result of the Wnt signal from P2. One daughter, the MS cell, will give rise to muscles and various other body parts; the other daughter, the E cell, is the founder cell for the gut, committed to give rise to all the cells of the gut and to no other tissues

Q4 a) regulatory mutations transform plant topology

The plant's pattern of branching is regulated through this choice of fate, and mutations that affect it can transform the structure of the plant. Maize provides a beautiful example. Maize represents one of mankind's most remarkable feats of genetic engineering. Native Americans created it by selective breeding, over a period of several centuries or perhaps millennia between 5000 and 10,000 years ago. They started from a wild grass known as teosinte, with highly branched leafy stems 1410 Chapter 22: Development of Multicellular Organisms L3 L2 L1 (A) (B) shoot apical meristem epidermis leaf primordium Clavata3 Clavata1 Wuschel Figure 22–

123 The feedback loops that are thought to maintain the shoot apical meristem. (A) The arrangement of cell layers constituting a shoot apical meristem. (B) The pattern of cell–cell communication that maintains the meristem. Artificial overexpression of Wuschel in the L3 region causes an increase in the number of cells in the L1 and L2 layers that behave as meristem cells and express Clavata3; artificial overexpression of Clavata3 in the L1 and L2 layers causes a reduction of Wuschel expression in the L3 region below and a decrease in the number of meristem cells. Clavata3 codes for a small signal protein, while Clavata1 codes for its receptor, a transmembrane protein kinase. Wuschel, which is expressed in the central part of the region that expresses the receptor Clavata1, codes for a gene regulatory protein of the homeodomain class. The size of the meristem is thought to be controlled by a self-regulating balance between a short-range stimulatory signal produced by cells expressing Wuschel (yellow arrow), and a longer-range inhibitory signal delivered by Clavata3 (red bars). and tiny ears bearing hard, inedible kernels. Detailed genetic analysis has identified a handful of genetic loci—about five—as the sites of the mutations that account for most of the difference between this unpromising ancestor and modern corn. One of these loci, with a particularly dramatic effect, corresponds to a gene called Teosinte branched-1 (Tb1). In maize with loss-of-function mutations in Tb1, the usual simple unbranched stem, with a few large leaves at intervals along it, is transformed into a dense, branching, leafy mass reminiscent of teosinte (Figure 22–125A). The pattern of branching in the mutant implies that axillary buds, originating in normal positions, have escaped from an inhibition that prevents them, in normal maize, from growing into branches.

b) association of flowering with climatic conditions

The switch from meristematic growth to flower formation is triggered by a combination of cues. The plant does not merely take account of the current temperature, light intensity, and nutritional conditions; it bases its decision to flower on past conditions as well. One important cue, for many plants, is day length. To sense this, the plant uses its circadian clock—an endogenous 24-hour rhythm of gene expression—to generate a signal for flowering only when there is light for the appropriate part of the day. The clock itself is influenced by light, and the plant in effect uses the clock to compare past to present lighting conditions. Important parts of the genetic circuitry underlying these phenomena have been identified, from the phytochromes and crypto chromes that act as light receptors (discussed in Chapter 15) to the Constans gene, whose expression in the leaves of the plant represents a signal for flowering. The signal is thought to be relayed from the leaves to the meristem via the vasculature by the product of another gene, Flowering locus T (Ft), that is regulated by Constans.

Q5 a) Genetically modified stem cells

Use of Genetically Modified Stem Cells in Experimental Gene Therapies. ... Gene therapy is still highly experimental, but has the potential to become an important treatment regimen. In principle, it allows the transfer of genetic information into patient tissues and organs.

Direct gene transfer is particularly attractive because of its relative simplicity. In this scenario, genes are delivered directly into a patient's tissues or bloodstream by packaging into liposomes (spherical vessels composed of the molecules that form the membranes of cells) or other biological micro particles. Alternately, the genes are packaged into genetically-engineered viruses, such as retroviruses or adenoviruses. Because of biosafety concerns, the viruses are typically altered so that they are not toxic or infectious (that is, they are replication incompetent). These basic tools of gene therapists have been extensively optimized over the past 10 years.

On the other hand, therapeutic genes can be delivered using living cells. This procedure is relatively complex in comparison to direct gene transfer, and can be divided into three major steps. In the first step, cells from the patient or other sources are isolated and propagated in the laboratory. Second, the therapeutic gene is introduced into these cells, applying methods similar to those used in direct gene transfer. Finally, the genetically-modified cells are returned to the patient. The use of cells as gene transfer vehicles has certain advantages. In the laboratory dish (*in vitro*), cells can be manipulated much more precisely than in the body (*in vivo*). Some of the cell types that continue to divide under laboratory conditions may be expanded significantly before reintroduction into the patient. Moreover, some cell types are able to localize to particular regions of the human body, such as hematopoietic (blood-forming) stem cells, which return to the bone marrow. This quot;homingquot; phenomenon may be useful for applying the therapeutic gene with regional specificity.

b) Angiogenesis

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels.[1][2][3] In precise usage this is distinct from vasculogenesis, which is the *de novo* formation of endothelial cells from mesoderm cell precursors,[4] and from neovascularization, although discussions are not always precise (especially in older texts). The first vessels in the developing embryo form through vasculogenesis, after which angiogenesis is responsible for most, if not all, blood vessel growth during development and in disease

Q.6 a) l Arabidopsis as a model organisms for plant molecular genetics.

To identify the genes that govern plant development and to discover how they function, plant biologists have selected a small weed, the common wall cress Arabidopsis thaliana (Figure 22–112) as their primary model organism. it is small, quick to reproduce, and convenient for genetics. It can be grown indoors in Petri dishes or tiny plant pots in large numbers and produces hundreds of seeds per plant after 8–10 weeks. It has, in common with C. elegans, a significant advantage over Drosophila or vertebrate animals for genetics: like many flowering plants, it can reproduce as a hermaphrodite because a single flower produces both eggs and the male gametes that can fertilize them. Therefore, when a flower that is heterozygous for a recessive lethal mutation is self-fertilized, one-fourth of its seeds will display the homozygous

embryonic phenotype. This makes it easy to perform genetic screens and so to obtain a catalog of the genes required for specific developmental processes.

b) Similarities and dissimilarities between adult stem cells and embryonic stem cells

Human embryonic and adult stem cells each have advantages and disadvantages regarding potential use for cell-based regenerative therapies. Of course, adult and embryonic stem cells differ in the number and type of differentiated cells types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are generally limited to differentiating into different cell types of their tissue of origin. However, some evidence suggests that adult stem cell plasticity may exist, increasing the number of cell types a given adult stem cell can become.

large numbers of embryonic stem cells can be relatively easily grown in culture, while adult stem cells are rare in mature tissues and methods for expanding their numbers in cell culture have not yet been worked out. This is an important distinction, as large numbers of cells are needed for stem cell replacement therapies.

A potential advantage of using stem cells from an adult is that the patient's own cells could be expanded in culture and then reintroduced into the patient. The use of the patient's own adult stem cells would mean that the cells would not be rejected by the immune system. This represents a significant advantage as immune rejection is a difficult problem that can only be circumvented with immunosuppressive drugs.

Embryonic stem cells from a donor introduced into a patient could cause transplant rejection. However, whether the recipient would reject donor embryonic stem cells has not been determined in human experiments.